WYDZIAŁ BIOLOGII i OCHRONY ŚRODOWISKA

Uniwersytet Łódzki

Stacjonarne Studia Doktoranckie
Genetyki Molekularnej, Cytogenetyki
i Biofizyki Medycznej

Katarzyna Weronika Białek

Rola stanu zapalnego w patogenezie zaburzeń depresyjnych

The role of inflammation in the pathogenesis of depressive disorders

Praca doktorska

wykonana w Katedrze Genetyki
Molekularnej Instytutu Biochemii

pod kierunkiem
prof. dr hab. Tomasza Śliwińskiego

Łódź, 2021

Spis treści

Informacje wprowadzające

Streszczenie

Summary

Publikacje będące podstawą rozprawy doktorskiej

Oświadczenia współautorów

Informacje wprowadzające

Źródła finansowania

Dorobek naukowy

Publikacje wchodzące w skład rozprawy doktorskiej

W skład rozprawy doktorskiej wchodzą cztery publikacje: jedna praca przeglądowa i trzy prace doświadczalne.

Praca przeglądowa:

1. **Bialek K**, Czarny P, Strycharz J, Sliwinski T. Major depressive disorders accompanying autoimmune diseases - Response to treatment. Prog Neuropsychopharmacol Biol Psychiatry. 2019;95:109678. **100pkt MNiSW** (punktacja z dnia 31.07.2019 r.); **IF = 4.361, IF 5-letni = 4.198**

Prace doświadczalne:

1. **Bialek K,** Czarny P, Watala C, Synowiec E, Wigner P, Bijak M, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Preliminary Study of the Impact of Single-Nucleotide Polymorphisms of IL-1α, IL-1β and TNF-α Genes on the Occurrence, Severity and Treatment Effectiveness of the Major Depressive Disorder. Cell Mol Neurobiol. 2020; 40(6):1049-1056. **100pkt MNiSW** (punktacja z dnia 31.07.2019 r.); **IF = 3.606, IF 5 letni = 3.644**

2. **Bialek K,** Czarny P, Watala C, Wigner P, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Novel association between TGFA, TGFB1, IRF1, PTGS2 and IKBKB single-nucleotide polymorphisms and occurrence, severity and treatment response of major depressive disorder. PeerJ. 2020; 8:8676. **100pkt MNiSW** (punktacja z dnia 31.07.2019 r.); **IF = 2.380, IF 5-letni = 2.749**

3. **Bialek K**, Czarny P, Wigner P, Synowiec E, Barszczewska G, Bijak M, Szemraj J, Niemczyk M, Tota-Glowczyk K, Papp M, Sliwinski T. Chronic Mild Stress and Venlafaxine Treatment Were Associated with Altered Expression Level and Methylation Status of New Candidate Inflammatory Genes in PBMCs and Brain Structures of Wistar Rats. Genes (Basel). 2021; 12(5):667. **100pkt MNiSW** (punktacja z dnia 31.07.2019 r.); **IF = 3.759, IF 5-letni = 3.822**

Suma punktów MNiSW wynosi: 400; IF = 14.106; IF 5-letni = 14.413

Pozostały dorobek naukowy

Publikacje:

1. Sliwinska A, Sitarek P, Toma M, Czarny P, Synowiec E, Krupa R, Wigner P, **Bialek K**, Kwiatkowski D, Korycinska A, Majsterek I, Szemraj J, Galecki P, Sliwinski T. Decreased expression level of BER genes in Alzheimer's disease patients is not derivative of their DNA methylation status. Prog Neuropsychopharmacol Biol Psychiatry. 2017;7:311-316. **35pkt MNiSW** (punktacja z dnia 09.12.2016 r.); **IF = 4.361, IF 5-letni = 4.198**

2. Wigner P, Czarny P, Synowiec E, Bijak M, **Białek K**, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Variation of genes involved in oxidative and nitrosative stresses in depression. Eur Psychiatry. 2018;48:38-48. **35pkt MNiSW** (punktacja z dnia 09.12.2016 r.); **IF = 4.464, IF 5-letni = 4.462**

3. Wigner P, Czarny P, Synowiec E, Bijak M, **Białek K**, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Association between single nucleotide polymorphisms of TPH1 and TPH2 genes, and depressive disorders. J Cell Mol Med. 2018;22(3):1778-1791. **35pkt MNiSW** (punktacja z dnia 09.12.2016 r.); **IF = 4.486, IF 5-letni = 4.626**

4. Czarny P, **Bialek K**, Ziolkowska S, Strycharz J, Sliwinski T. DNA damage and repair in neuropsychiatric disorders. What do we know and what are the future perspectives? Mutagenesis. 2020;35(1):79-106. **70pkt MNiSW** (punktacja z dnia 31.07.2019 r.); **IF = 3.379, IF 5-letni = 2.994**

5. Wigner P, Synowiec E, Jóźwiak P, Czarny P, Bijak M, Barszczewska G, **Białek K**, Szemraj J, Gruca P, Papp M, Śliwiński T. The Changes of Expression and Methylation of Genes Involved in Oxidative Stress in Course of Chronic Mild Stress and Antidepressant Therapy with Agomelatine. Genes (Basel). 2020;11(6):644. **100pkt MNiSW** (punktacja z dnia 31.07.2019 r.); **IF = 3.759, IF 5-letni = 3.822**

6. Wigner P, Synowiec E, Jóźwiak P, Czarny P, Bijak M, **Białek K**, Szemraj J, Gruca P, Papp M, Śliwiński T. The Effect of Chronic Mild Stress and Venlafaxine on the Expression and Methylation Levels of Genes Involved in the Tryptophan Catabolites Pathway in the Blood and Brain Structures of Rats. J Mol Neurosci. 2020

Sep;70(9):1425-1436. **70pkt MNiSW** (punktacja z dnia 31.07.2019 r.); **IF = 2.678, IF 5-letni = 2.496**

7. Wigner P, Synowiec E, Jóźwiak P, Czarny P, Bijak M, **Białek K**, Szemraj J, Gruca P, Papp M, Śliwiński T. The Effect of Chronic Mild Stress and Escitalopram on the Expression and Methylation Levels of Genes Involved in the Oxidative and Nitrosative Stresses as Well as Tryptophan Catabolites Pathway in the Blood and Brain Structures. Int J Mol Sci. 2020;22(1):10. **100pkt MNiSW** (punktacja z dnia 31.07.2019 r.); **IF = 4.556, IF 5-letni = 4.653**

8. Wigner P, Synowiec E, Jóźwiak P, Czarny P, **Białek K**, Bijak M, Szemraj J, Gruca P, Papp M, Sliwinski T. The Impact of Chronic Mild Stress and Agomelatine Treatment on the Expression Level and Methylation Status of Genes Involved in Tryptophan Catabolic Pathway in PBMCs and Brain Structures. Genes (Basel). 2020 Sep 18;11(9):1093. **100pkt MNiSW** (punktacja z dnia 31.07.2019 r.); **IF = 3.759, IF 5-letni = 3.822**

9. Czarny P, **Białek K**, Ziółkowska S, Strycharz J, Barszczewska G, Sliwinski T. The Importance of Epigenetics in Diagnostics and Treatment of Major Depressive Disorder. J Pers Med. 2021;11(3):167. **70pkt MNiSW** (punktacja z dnia 31.07.2019 r.); **IF = 4.433, IF 5-letni = 4.726**

Sumaryczny dorobek naukowy: 105 pkt MNiSW według punktacji z dnia 09.12.2016r oraz 510 pkt według punktacji z dnia 31.07.2019 r. Łączna suma punktów MNiSW wynosi: 615; IF = 35.875; IF 5-letni = 35.635

Rozdziały w zagranicznych monografiach:

1. Czarny P, **Bialek K**, Ziolkowska S, Talarowska M, Śliwiński T, Chapter 1 - Epigenetics in depression, The Neuroscience of Depression, Academic Press. Elsevier 2021, 3-13, ISBN 9780128179352

Komunikaty zjazdowe:

1. **Bialek K**, Czarny P, Barszczewska G, Sliwinski T, Novel association between *PTGS2* single nucleotide polymorphisms and major depressive disorder, National Scientific Conference „Science and Young Researchers" – 4th edition, Łódź, 06.06.2020

2. Barszczewska G, **Bialek K**, Sliwinski T, Crucial players in gut-brain interaction, National Scientific Conference „Science and Young Researchers" – 4th edition, Łódź, 06.06.2020

3. **Białek K**, Czarny P, T. Śliwiński, Wpływ polimorfizmu pojedynczego nukleotydu genu *IRF1* na ryzyko rozwoju Depresji Dokonania naukowe doktorantów, Kraków, 6.04.2019

4. **Białek K**, Czarny P, Wróblewski A, Wigner P, Synowiec E, Szemraj J, Papp M, Śliwiński T, Expression of IL10 in peripheral blood and six brain regions of rats subjected to chronic mild stress and during Venlafaxine treatment The FEBS Congress 2019, Krakow, 6-11.07.2019

5. Czarny P, **Białek K,** Wróblewski A, Wigner P, Synowiec E, Strycharz J, Szemraj J, Papp M, Śliwiński T, Hippocampal expression of genes involved in DNA repair, and replication and degradation of mitochondrial DNA in the chronic mild stress model of depression and during administration of venlafaxine, The FEBS Congress 2019, Krakow, 6-11.07.2019

6. **Białek K**, Wigner P, Czarny P, Śliwiński T, Wpływ polimorfizmu pojedynczego nukleotydu genów *IRF1, IKBKB* oraz *IL1B* na efektywność działania leków przeciwdepresyjnych, V Ogólnopolska konferencja doktorantów nauk o życiu „BioOpen", Uniwersytet Łódzki, 30-31.05.2019

7. Wróblewski A, Strycharz J, Świderska E, Zieleniak A, Matyjas T, Rucińska M, Kumoniewski P, Pomorski L, **Białek K**, Szemraj J, Drzewoski J, Śliwińska A, Diabetes and prediabetes similarly affect expression of miR-708-5p, miR-10a-5p and miR-99a-5p, The FEBS Congress 2019, Krakow, 6-11.07.2019

8. Wigner P, **Białek K**, Synowiec E, Śliwiński T, Wpływ wenlafaksyny na poziom ekspresji i stopień metylacji promotorów genów kodujących katalazę i dysmutazę ponadtlenkową 1 w zwierzęcym modelu chronicznego łagodnego stresu, V Ogólnopolska konferencja doktorantów nauk o życiu „BioOpen", Uniwersytey Łódzki, 30-31.05.2019

9. **Białek K**, Wigner P, Śliwiński T, Polimorfizmy pojedynczego nukleotydu genu IL-B w rozwoju depresji, VII Konferencja Biologii Molekularnej, Uniwersytet Łódzki, Wydział Biologii i Ochrony Środowiska, 12-14.04.2018

10. **Białek K**, Wigner P, Śliwiński T,Polimorfizmy pojedynczego nukleotydu genu IL-1a oraz TNF-a w zaburzeniach depresyjnych, X Interdyscyplinarna Konferencja Naukowa TYGIEL 2018 „Interdyscyplinarność kluczem do rozwoju", Uniwersytet Marii Curie-Skłodowskiej, 17-18.03.2018

Streszczenie

Wstęp

Depresja (ZD, zaburzenia depresyjne) będąc jedną z najczęściej diagnozowanych chorób psychicznych, dotyka ponad 260 milionów ludzi na całym świecie oraz stanowi główny czynnik przyczyniający się do globalnego obciążenia chorobami. Ze względu na stale rosnącą liczbę zachorowań szacuje się, że depresja jest drugą najczęstszą przyczyną niepełnosprawności społecznej. Choroba ogranicza prawidłowe funkcjonowanie ludzi, wywołując uporczywy smutek, brak zainteresowania i niepokój [1]. Te oraz inne objawy obejmujące zachowania psychotyczne i wycofanie społeczno-behawioralne często stają się przewlekłe lub nawracające, w skrajnych przypadkach prowadząc do samobójstwa. Ponadto jednym z głównych problemów związanych z depresją jest wysoki wskaźnik nawrotów, a także fakt, że ponad jedna trzecia pacjentów nie reaguje na tradycyjne leczenie przeciwdepresyjne [2,3]. Dlatego też, ważne jest, aby zidentyfikować nie w pełni poznaną patogenezę i mechanizmy molekularne leżące u podstaw depresji.

Pomimo wieloletnich badań, nie udało się dogłębnie uchwycić molekularnych aspektów leżących u podstaw rozwoju ZD. Niewątpliwie chorobę charakteryzuje wieloczynnikowa etiologia, a ryzyko jej rozwoju często warunkowane są przez czynniki genetyczne, środowiskowe oraz osobnicze cechy biologiczne [4]. Coraz częściej jednak, wśród wielu procesów przyczyniających się do rozwoju choroby upatruje się roli stanu zapalnego jako czynnika odgrywającego ważną rolę w etiologii depresji [5]. Jednym z mechanizmów jest „hipoteza cytokinowa", której potwierdzeniem jest fakt, że u pacjentów z depresją obserwuje się wyższy poziom cytokin prozapalnych we krwi, takich jak interleukina-1α (IL-1α, ang. *Interleukin 1α*), interleukina-1β (IL-1α, ang. *Interleukin 1β*), interleukina 6 (IL-6, ang. *Interleukin 6*) oraz czynnik martwicy guza (TNF-α, ang. *Tumor necrosis factor α*). Według licznych doniesień, pacjenci z ZD wykazują także zwiększone stężenie innych markerów stanu zapalnego, w tym białek fazy ostrej, prostaglandyn, cząsteczek adhezyjnych i chemokin [6–11]. Przykładowo, zarówno prostaglandyny jak i enzym PTGS2 (ang. *Prostaglandin-endoperoxide synthase 2,* inaczej COX-2) ogrywają znaczną rolę w wyzwalaniu kaskady zapalnej w ZD [12,13]. Pacjentów z depresją charakteryzuje wzmożona ekspresji genu *PTGS2* [14]. Doniesienia te znajdują potwierdzenie również w badaniach z wykorzystaniem zwierzęcego modelu depresji, sugerujące wyższy poziom ekspresji *PTGS2* w mózgu chorych osobników [15]. Co więcej, podanie selektywnego inhibitora COX-2 spowodowało zmniejszenie zachowań depresyjnych u szczurów jak również obniżyło poziom cytokin w

podwzgórzu zwierząt [16]. Podobnie u pacjentów z ciężką depresją, terapia selektywnym inhibitorem może nie tylko złagodzić zachowania depresyjne, ale także zredukować poziom cytokin prozapalnych w surowicy [17]. Przedłużająca się aktywacja układu odpornościowego i chroniczny stany zapalny są silnie związane z rozwojem i postępem choroby. Białka uwalniane podczas aktywacji układu immunologicznego działają jako neuromodulatory i mogą wyzwalać kaskadę zmian neurochemicznych, neuroendokrynnych i behawioralnych modulując wiele funkcji biologicznych, takich jak aktywacja osi podwzgórze-przysadka-nadnercza (HPA, ang. *hypothalamic-pituitary-adrenal axis*), metabolizm neurotransmiterów czy neuroplastyczność, których zaburzenia obserwuje się w rozwoju i progresji depresji [6,18,19]. Nadmierne pobudzenie układu odpornościowego wpływa również na metabolizm tryptofanu poprzez zdolność cytokin prozapalnych do regulacji enzymu 2,3-dioksygenazy indoloaminowej (IDO). Stymulowana przez cytokiny zwiększona aktywność enzymu IDO, a co za tym idzie wzmożona konwersja tryptofanu do kinureniny, powoduje ograniczenie ilości tego aminokwasu jako prekursora serotoniny [20,21]. Cytokiny mogą również zakłócać neurotransmisję serotoninergiczną poprzez wpływ na aktywność enzymu SERT (*sodium-dependent serotonin transporter*) [22]. Ponadto, przewlekły stan zapalny znajduje odzwierciedlenie w ośrodkowym układzie nerwowym (OUN), charakteryzujące się mobilizacją komórek odpornościowych, zwanych mikroglejem [23,24]. Stymulowany mikroglej odpowiedzialny jest za produkcję cytokin w OUN, co może przyczynić się do rozwoju zapalenia układu nerwowego i zaburzeń pracy mózgu [25]. Proces ten jest ściśle połączony z aktywacją jądrowego czynnika transkrypcyjnego NF-kappa β (NF-κβ, ang. *nuclear factor-κβ*), odpowiedzialnego za produkcję cytokin prozapalnych [26]. Jednakże komórki odpornościowe rezydujące w OUN, w celu antagonizowania uszkodzeń wywołanych stanem zapalnym, stymulują również cząsteczki wykazujące działanie neuroprotekcyjne. Przykład stanowi transformujący czynnik wzrostu (TGF β, ang. transforming growth factor β), którego poziom ulega rozregulowaniu w trakcie depresji [25,27]. Odnotowuje się niższą ekspresję TGF-β u pacjentów z depresją w porównaniu do osób zdrowych [28–31]. Natomiast w badaniach z wykorzystaniem zwierzęcego modelu depresji, stwierdzono, że poziom TGF-β jest podwyższony u osobników z objawami choroby [32]. Co więcej, potwierdzono, że stres wywołuje stan zapalny w obszarach mózgu, takich jak kora czołowa, podwzgórze i hipokamp [33,34]. Ponadto, przypuszcza się, że podawanie leków przeciwdepresyjnych może skutecznie zmniejszyć prozapalne cytokiny u osób z depresją [35]. Obecnie w pierwszej linii leczenia ZD stosuje się leki z grupy inhibitorów zwrotnego wychwytu serotoniny (SSRIs, ang. *selective serotonin reuptake inhibitors*), inhibitorów zwrotnego wychwytu serotoniny i noradrenaliny

(SNRIs, ang. *serotonin norepinephrine reuptake inhibitors*) oraz trójcykliczne leki przeciwdepresyjne (TCA, ang. *tricyclic antidepressants*) [36,37]. Jednakże wpływ leków przeciwdepresyjnych na poziom cząsteczek zapalnych w obwodowym i ośrodkowym układzie nerwowym nie jest w pełni poznany. Z powodu możliwości prowadzenia badań na ludzkiej tkance mózgowej jedynie *post mortem*, aby poznać procesy zachodzące w układzie nerwowym podczas choroby niezbędne jest wykorzystanie zwierzęcych modeli depresji takich jak procedura chronicznego łagodnego stresu (CMS, ang. *chronic mild stress*) [38]. Co interesujące, badania wskazują na zaburzoną równowagę pomiędzy poziomem cytokin pro- i przeciwzapalnych u zwierząt z objawami depresji wywołanymi procedurą CMS [39].

Podsumowując, aktywacja układu odpornościowego w depresji opiera się na złożonej sieci ściśle połączonych ze sobą szlaków, dlatego też wystąpienie jakiejkolwiek zmienności czy zaburzeń w jednym z nich może spowodować rozregulowanie powiązanych czynników i wyzwolić kaskadę zapalną. Ponadto pomimo potwierdzonego zaangażowania układu odpornościowego w depresję, wiedza o innych niż cytokiny cząsteczkach zapalnych, w szczególności mających swój udział w zapaleniu układu nerwowego i funkcjonowaniu mózgu w patogenezie choroby, jest niepełna.

W niniejszej pracy zbadane zostały polimorfizmy genów zaangażowanych w procesy zapalne, mogące wpływać na aktywność kodowanych białek. Poddany ocenie został wpływ procedury chronicznego łagodnego stresu, stanowiącej zwierzęcy model depresji oraz terapii wenlafaksyną na poziom ekspresji genów związanych ze stanem zapalnym. Co więcej zbadano czy wyżej wymienione czynniki wpływają na modyfikacje epigenetyczne, takie jak zmiana stopnia metylacji regionów promotorowych badanych genów.

Cel pracy

Celem pracy było określenie udziału stanu zapalnego w rozwoju depresji oraz mechanizmie działania leków przeciwdepresyjnych. Cel został osiągnięty poprzez:

- Określenie związku genotypów i alleli polimorfizmów pojedynczego nukleotydu (SNP ang. Single nucleotide polimorphism) genów *IL1A, IL1B, TNFA, TGFA, TGFB, PTGS2, IRF1, IKBKB* z ryzykiem wystąpienia depresji, skutecznością leczenia przeciwdepresyjnego, wiekiem chorego podczas pierwszego epizodu oraz ciężkością nasilenia objawów

- Określenie wpływu procedury chronicznego łagodnego stresu, stanowiącej zwierzęcy model depresji oraz terapii wenlafaksyną na poziom ekspresji genów *TGFA, TGFB, PTGS2, IRF1, IKBKB* w komórkach jednojądrzastych krwi obwodowej oraz wybranych strukturach mózgu

- Określenie wpływu procedury chronicznego łagodnego stresu, stanowiącej zwierzęcy model depresji oraz terapii wenlafaksyną na stopień metylacji regionu promotorowego genów *TGFA, TGFB, PTGS2, IRF1, IKBKB* w komórkach jednojądrzastych krwi obwodowej oraz wybranych strukturach mózgu

Materiały i Metody

Badania na materiale klinicznym

Materiał do badań stanowiły próbki krwi obwodowej pobrane od pacjentów ze zdiagnozowaną depresją oraz od zdrowych osób stanowiących grupę kontrolną. Próbki zostały pozyskane od pacjentów hospitalizowanych w Klinice Psychiatrii Dorosłych Uniwersytetu Medycznego w Łodzi. Grupa badana była włączona do badania na podstawie kryteriów zawartych w Międzynarodowej Statystycznej Klasyfikacji Chorób i Problemów Zdrowotnych klasyfikacji ICD-10 (ang. *International Statistical Classification of Diseases and Related Health Problems*) (F32.0-F.32.2, F33.0-F33.8). Kryteria wyłączenia z eksperymentu obejmowały: zaburzenia osi I i II, chroniczne lub ostre zaburzenia somatyczne, stan zapalny, choroby nowotworowe, choroby autoimmunologiczne oraz zaburzenia centralnego układu nerwowego. Stopień nasilenia objawów epizodu depresyjnego został oceniony na podstawie skali Hamiltona (HDRS, ang. *Hamilton Depression Rating Scale*) przed rozpoczęciem leczenia oraz po terapii lekami antydepresyjnymi z grupy SSRI. Pacjenci oraz grupa kontrolna zostali dopasowani pod względem wieku oraz płci. Uczestnicy złożyli pisemną zgodę na uczestnictwo w projekcie po zapoznaniu się z przebiegiem i celem badania zgodnie z protokołem zaaprobowanym przez Komisję Bioetyczną Uniwersytetu Medycznego w Łodzi (Nr. RNN/70/14/KE).

DNA z krwi pełnej zostało wyizolowane za pomocą komercyjnie dostępnego zestawu Blood Mini Kit (A&A Biotechnology, Gdynia, Poland). Czystość DNA oraz stężenia zostały zmierzone spektrofotometrycznie przez obliczenie stosunku między absorbancją przy 260 nm i 280 nm. Badanie rozkładu genotypów i alleli wybranych polimorfizmów zostało przeprowadzone z użyciem sond TaqMan za pomocą łańcuchowej reakcji polimerazy z detekcją w czasie rzeczywistym (Real-Time PCR) (Bio-Rad CFX96 Real-Time PCR). Analizie poddano łącznie 11 polimorfizmów (Tabela 1).

Tabela 1. Charakterystyka analizowanych polimorfizmów pojedynczego nukleotydu.

Gen	Numer rs	Nazwa	Lokalizacja
TGFA	rs2166975	g.70677994G>A	Ekson
TGFB1	rs1800469	g.41354391A>G	Koniec 5'
IRF1	rs2070729	g.132484229C>A	Intron
IKBKB	rs5029748	g.42140549G>T	Intron
PTGS2	rs5275	g.186643058A>G	3' UTR
	rs4648308	g.186640617C>T	Koniec 3'
IL1A	rs17561	g.10749G>T	Ekson
IL1B	rs1143623	g.113595829C>G	Koniec 5'
	rs1143627	g.113594387G>A	Koniec 5'
TNFA	rs1799964	g.4970C>T	Koniec 5'
	rs1800629	g.31543031G>A	Koniec 5'

Analizę statystyczną rozkładu genotypów i alleli badanych polimorfizmów przeprowadzono za pomocą modelu regresji logistycznej w celu otrzymania ilorazu szans (ang. odds ratio, OR) przy przedziale ufności równym 95%. Ponadto, wyniki istotne statystycznie zostały dodatkowo potwierdzone przy użyciu dwóch podejść: metody bootstrap (wielokrotne losowanie ze zwracaniem z próby, 10 000 interakcji) oraz techniki *d*-jackknife. Dobroć dopasowania modeli regresji logistycznej została oszacowana za pomocą testu Hosmera-Lemeshowa. Rozkład normalny został oceniony w teście Shapiro-Wilka.

Badania *in vivo*

Badania z wykorzystaniem modelu chronicznego stresu łagodnego przeprowadzono na dorosłych samcach szczurów rasy Wistar (Charles River, Niemcy). Pierwszym etapem eksperymentu było przystosowanie zwierząt do warunków laboratoryjnych oraz spożywania 1% roztworu sacharozy. Spożycie roztworu sacharozy jest najczęstszym sposobem określenia behawioralnego wpływu procedury CMS poprzez pomiar zdolności reagowania na bodźce nagradzające. Spożycie sacharozy weryfikowano raz w tygodniu, w kontrolowanych warunkach, do momentu zakończenia eksperymentu. Zwierzęta były poddawane standardowej procedurze CMS (Tabela 2), polegającej na długotrwałej ekspozycji zwierząt na bodźce stresowe o łagodnym nasileniu. Procedura stresowania powoduje pojawienie się szeregu zmian behawioralnych oraz biochemicznych, odzwierciedlających objawy depresji u ludzi.

Po 2 tygodniach, zarówno grupy kontrolne, jak i grupy stresowane zostały dalej podzielone na dopasowane podgrupy, a następnie otrzymywały placebo (1 mL/kg, IP) lub wenlafaksynę (10 mg/kg, IP) z grupy leków SSRI przez kolejne 5 tygodni. Po zakończeniu procedury szczury poddano dekapitacji i pobrano od nich próbki krwi (jednojądrzaste komórki krwi obwodowej, PBMCs) oraz tkanki mózgowej z wypreparowanymi częściami mózgu (hipokamp, ciało migdałowate, podwzgórze, śródmózgowie, kora przedczołowa i jądra zwojów podstawy).

Tabela 2. Harmonogram procedury CMS i szczegółowy opis wszystkich zastosowanych bodźców stresowych

Start eksperymentu		
5 tygodni adaptacji do spożycia 1% roztworu sacharozy		
2 tygodnie bez stresu	2 tygodnie początkowego stresu	
5 tygodni bez stresu oraz administracja	5 tygodni stresu oraz administracja placebo	5 tygodni stresu oraz administracja wenlafaksyny

Procedura CMS		
Bodziec stresowy	**Czas trwania**	**Liczba okresów**
Pozbawienie żywności i wody	10 – 14 godzin	2 okresy
Odchylanie klatki pod kątem 45°	10 – 14 godzin	2 okresy
Zabrudzanie klatki (250 ml wody w ściółce z trocin)	10 – 14 godzin	2 okresy
Parowanie osobników	10 – 14 godzin	1 okres
Oświetlenie stroboskopowe o niskiej intensywności (150 błysków /min)	10 – 14 godzin	2 okresy
Przerywane oświetlenie (światło włączane i wyłączane co 2 godziny)	10 – 14 godzin	2 okresy
Brak bodźców stresowych	10 – 14 godzin	3 okresy

Końcowy test spożycia sacharozy oraz dekapitacja zwierząt

DNA oraz RNA z krwi pełnej zostało wyizolowane za pomocą komercyjnie dostępnych zestawów, odpowiednio QIAamp DNA mini kit (Qiagen, Hilden, Germany) oraz GenElute mammalian total RNA miniprep kit (Sigma-Aldrich, St. Louis, MO, USA). W przypadku

wybranych regionów mózgu zastosowany został zestaw ISOLATE II RNA/DNA/protein kit (Bioline). Przed przystąpieniem do procedury izolacji, tkanki zostały poddane homogenizacji oraz sonikacji. Czystość RNA i DNA oraz ich stężenia zostały zmierzone spektrofotometrycznie przez obliczenie stosunku między absorbancją przy 260 nm i 280 nm.

Profil ekspresji badanych genów został określony za pomocą techniki Real-Time PCR, z wykorzystaniem sond TaqMan Gene Expression Assay (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA). W tym celu RNA zostało poddane odwrotnej transkrypcji w celu otrzymania komplementarnego DNA (cDNA) za pomocą zestawu zestawu High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). We wszystkich reakcjach Real-Time PCR użyto genu metabolizmu podstawowego kodującego podjednostkę 18S rybosomalnego RNA jako kontroli wewnętrznej. Dla każdego genu ekspresję mRNA obliczono w stosunku do genu referencyjnego, korzystając z formuły $2^{-\Delta ct}$, gdzie $\Delta Ct = Ct_{genu\ badanego} - Ct_{18S}$.

Analiza stopnia metylacji sekwencji promotorowych wybranych genów została przeprowadzona za pomocą metody denaturacji DNA z wysoką rozdzielczością wrażliwej na metylację (MS-HRM, ang. *methylation sensitive – high resolution melting*). W tym celu DNA zostało poddane reakcji bisulfidacji z wykorzystaniem CiTi Converter DNA Methylation KiT (A&A Biotechnology). Do oceny poziomu metylacji zastosowano komercyjnie dostępne standardy metylowanego (CpGenome™ Rat Methylated Genomic DNA Standard; Merck Millipore) i niemetylowanego DNA szczura (CpGenome™ Rat Unmethylated Genomic DNA Standard; Merck Millipore). Startery dla regionów promotorowych genów zawierających wyspy CpG zostały zaprojektowane przy użyciu oprogramowania Methyl Primer Express ™ Software v 1.0 (Tabela 3).

Tabela 3. Charakterystyka starterów wykorzystanych do oznaczenia poziomu metylacji regionów promotorowych badanych genów

Gen	Sekwencja startera (5'->3')	Tm [C°]	Rozmiar produktu [pz]	Liczba wysp CpG
IKBKB	F:AGGGTGGTTTTTTATTTTTATTTT R:AACCCCCACTAAAACTAACTTAA	55	117	1
IRF1	F:TTGGAGATTTAGGGAGTTAGGT R:CCCCTTACCTATCTTAAAAAACC	55	123	1
PTGS2	F:GTAATAGTAGGGAGGAAAAATTTTAA R:ATCCTAACAAACCCCAAA	55	111	1
TGFA	F:GTTTTTTTAGGTGGTTGGTTAAG R:CTTCAAACACCTCCCTACAATA	55	188	1

Rozkład normalny uzyskanych wyników został oceniony w teście Shapiro-Wilka. Wpływ procedury CMS na spożycie sacharozy analizowano za pomocą testu t-studenta dla danych o rozkładzie normalnym lub testu Manna-Whitney w odwrotnym przypadku. Uzyskane dane dotyczące poziomu ekspresji oraz stopnia metylacji analizowano za pomocą jednoczynnikowej analizy wariancji (one-way ANOVA) z testem post-hoc Tukeya. Jeśli dane nie miały rozkładu normalnego, interpretowane były przy użyciu modelu jednoczynnikowej analizy wariancji dla rang Kruskala-Walisa ANOVA, a następnie testu post-hoc Newmana-Keulsa.

Wyniki

Wyniki otrzymane w toku analiz z wykorzystaniem materiału klinicznego wykazały szereg zależności pomiędzy obecnością SNP w badanych genach a ryzykiem wystąpienia depresji (Tabela 4). Analiza rozkładu genotypów i alleli wykazała, że genotypy A/G (rs2166975) *TGFA* – G > A, A/C (rs2070729) *IRF1* – C > A oraz G/T (rs5029748) *IKBKB* – G > T były związane ze zwiększonym ryzykiem rozwoju depresji. Natomiast genotypy G/G (rs2166975) *TGFA* – G > A, T/T (rs5029748) *IKBKB* – G > T oraz T/T (rs4648308) *PTGS2* – C > T zmniejszały to ryzyko. Ponadto analiza statystyczna grupy podzielonej względem płci, wykazała że genotyp A/G (rs2166975) *TGFA* – G > A, G/T (rs5029748) *IKBKB* – G > T, jak również A/A (rs5275) *PTGS2* – A > G były pozytywnie skorelowane ze zwiększonym ryzykiem zachorowania na depresję u mężczyzn. W populacji żeńskiej możliwość rozwoju choroby była wyższa w przypadku posiadania genotypu A/C (rs2070729) *IRF1* – C > A, A/G (rs5275) *PTGS2* – A > G jak również C/T (rs4648308) *PTGS2* – C > T. Ponadto polimorfizmy genów *IKBKB* – G > T (rs5029748), *IRF1* - C > A (rs2070729) oraz *TNFA* – C > T (rs1799964) mogą wpływać na efektywność terapii lekami przeciwdepresyjnymi z grupy inhibitorów zwrotnego wychwytu serotoniny. Co więcej ciężkość nasilenia objawów epizodu depresyjnego również może być związana z obecnością polimorfizmów genów *IL1B* – C > G (rs1143623) oraz *TGFB* – A > G (rs1800469).

Tabela 4. Podsumowanie wpływu badanych polimorfizmów na efekt fenotypowy

Polimorfizm	Genotyp	Efekt fenotypowy
TGFA – G >A (rs2166975)	A/G	Wyższe ryzyko depresji w populacji ogólnej
		Wyższe ryzyko depresji w grupie mężczyzn
	G/G	Niższe ryzyko depresji w populacji ogólnej
TGFB – A > G (rs1800469)	A/A	Niższy wiek pierwszego zachorowania

	G/G	Cięższe nasilenie objawów epizodu depresyjnego
IRF1 - C > A (rs2070729)	A/C	Wyższe ryzyko depresji w populacji ogólnej
		Wyższe ryzyko depresji w grupie kobiet
		Gorsza odpowiedź na farmakoterapię
	A/A	Lepsza odpowiedź na farmakoterapię
IKBKB – G > T (rs5029748)	G/T	Wyższe ryzyko depresji w populacji ogólnej
		Wyższe ryzyko depresji w grupie mężczyzn
	T/T	Niższe ryzyko depresji w populacji ogólnej
	G/G	Lepsza odpowiedź na farmakoterapię
PTGS2 – C > T (rs4648308)	T/T	Niższe ryzyko depresji w populacji ogólnej
	C/T	Wyższe ryzyko depresji w grupie kobiet
PTGS2 – A > G (rs5275)	A/A	Wyższe ryzyko depresji w grupie mężczyzn
	A/G	Wyższe ryzyko depresji w grupie kobiet
TNFA – C > T (rs1799964)	T/T	Gorsza odpowiedź na farmakoterapię
	C/T	Lepsza odpowiedź na farmakoterapię
IL1B – C > G (rs1143623)	C/C	Cięższe nasilenie objawów epizodu depresyjnego

Wyniki otrzymane w toku badań z wykorzystaniem zwierzęcego modelu depresji wykazały, że zarówno procedura chronicznego łagodnego stresu jak i terapia wenlafaksyną wpływają na poziom ekspresji oraz stopień metylacji regionu promotorowego. Spożycie sacharozy u zwierząt poddanych procedurze stresowania spadło do około 60% wartości początkowych. Zastosowanie terapii wenlafaksyną wywołało efekt normalizujący u stresowanych szczurów.

Procedura CMS trwająca 7 tygodni spowodowała znaczny wzrost ekspresji genów *TGFA, TGFB, PTGS2, IRF1* oraz *IKBKB* w PBMCs u zwierząt, którym podawano sól fizjologiczną w porównaniu z grupą kontrolną. Trwające 5 tygodni leczenie wenlafaksyną spowodowało znaczny spadek ekspresji wszystkich badanych genów w grupie szczurów poddanych procedurze stresowania. Co ciekawe, efekt wywołany przez CMS oraz terapię wenlafaksyną znacznie różnił się w zależności od badanej struktury mózgu. Procedura CMS spowodowała istotny spadek ekspresji genów *TGFA* i *IKBKB* w hipokampie. Ponadto stres indukował niższą ekspresję *TGFA, TGFB* i *IKBKB* w ciele migdałowatym oraz w śródmózgowiu w przypadku *IKBKB*. Po podawaniu wenlafaksyny u stresowanych zwierząt wykazano spadek poziomu ekspresji genów *TGFA, TGFB* oraz *IRF1* w podwzgórzu, genu *IKBKB* w korze przedczołowej i w ciele migdałowatym. Z drugiej strony leczenie wenlafaksyną wpłynęło na wzrost ekspresji genu *TGFA* w hipokampie i jądrach zwojach podstawy jądra, jak również *PTGS2* w podwzgórzu.

Analiza stopnia metylacji regionów promotorowych badanych genów w PBMCs wykazała istotną zmianę statusu metylacji jedynie w przypadku promotora genu *IKBKB*, gdzie dwutygodniowa ekspozycja na CMS spowodowała wzrost metylacji w porównaniu z grupą kontrolną. Nie zaobserwowano istotnych różnic po zastosowaniu terapii wenlafaksyną. W przypadku analizy zmian dotyczących struktur mózgu, procedura stresowania spowodowała znaczny wzrost stopnia metylacji promotora genu *TGFA* w ciele migdałowatym, jak również w przypadku promotora *IRF1* w ciele migdałowatym i korze przedczołowej, oraz w przypadku promotora *PTGS2* w hipokampie i ciele migdałowatym. Jednakże procedura CMS spowodowała również spadek metylacji promotora genu *PTGS2* oraz *TGFA* w korze przedczołowej. Co ciekawe, przewlekła pięciotygodniowa administracja wenlafaksyny spowodowała znaczny wzrost stopnia metylacji promotora *IKBKB* w ciele migdałowatym i jądrach zwojów podstawy, oraz promotora *IRF1* w ciele migdałowatym. Podobny efekt zaobserwowano w przypadku promotora genu *TGFA*, gdzie stan metylacji był wyższy w hipokampie i ciele migdałowatym po terapii wenlafaksyną.

Podsumowanie

Depresja jest coraz poważniejszym problemem zarówno zdrowotnym jak i ekonomicznym. Choroba i jej objawy ograniczają prawidłowe funkcjonowanie pacjentów wpływając na wszystkie aspekty ich życia. Niestety pomimo intensywnych badań złożona patogeneza zaburzeń depresyjnych nie jest w pełni poznana. Dodatkowo chorobę charakteryzuje wysoki wskaźnik nawrotów, a także fakt, że ponad jedna trzecia pacjentów nie reaguje na farmakoterapię. Ponadto diagnostyka depresji oparta jest jedynie na obserwacji zgodnej z międzynarodowymi klasyfikacjami symptomów, nie uwzględniając potencjalnego udziału mechanizmów na poziomie molekularnym. Prowadzone do tej pory badania jednoznacznie wskazują na wieloczynnikową i złożoną sieć powiązanych ze sobą mechanizmów leżących u podstaw rozwoju choroby. Coraz więcej doniesień akcentuje istotny udział stanu zapalnego i aktywacji układu immunologicznego w depresji. Wyniki uzyskane w toku niniejszej dysertacji, potwierdzają to stanowisko. Polimorfizmy genów zaangażowanych w stan zapalny modulują ryzyko wystąpienia depresji, jak również wpływają na ciężkość nasilenia jej objawów oraz co ważne odpowiedź na farmakoterapię. Co więcej, badania przeprowadzone w trakcie realizacji tej rozprawy wykazały, że zarówno chroniczny łagodny stres jak i terapia wenlafaksyną wpływają na zmiany ekspresji genów związanych z aktywacją układu immunologicznego. Otrzymane wyniki wskazują, że badane geny mogą być odpowiedzialne za aktywację szlaków zapalnych w obecności bodźców stresowych. Procedura CMS, zarówno w PMBC jak i obszarach mózgu wiąże się ze zmianami w ekspresji tych genów, które z kolei mogą wywołać kaskadę zapalną. Innym kluczowym odkryciem jest fakt, że przewlekłe podawanie wenlafaksyny może powodować działanie przeciwzapalne, wpływając na ekspresję badanych genów. W związku z ograniczeniem możliwość prowadzenia badań na ludzkim mózgu, zrozumienie złożonych relacji i procesów zachodzących w obrębie tej tkanki wymaga wykorzystania modelu zwierzęcego. Użyty model chronicznego łagodnego stresu odzwierciedla objawy depresji występujące u ludzi i opiera się na ocenie poziomu anhedonii – obniżonej zdolności do odczuwania przyjemności, będącej jedną z głównych symptomów choroby. Wszystkie zgromadzone wyniki przemawiają za faktem, że stan zapalny może odgrywać istotną rolę w molekularnym podłożu depresji. Dlatego też, ważne jest prowadzenie badań w tym kierunku, które przyczynią się do opracowania nowych metod diagnostycznych choroby jak również spersonalizowanej terapii przeciwdepresyjnej.

Wnioski

1. Stan zapalny związany jest z molekularnym podłożem rozwoju depresji.

2. Polimorfizmy pojedynczego nukleotydu zlokalizowane w genach zaangażowanych w stan zapalny *(IL1A, IL1B, TNFA, TGFA, TGFB, IKBKB, IRF1, PTGS2)* wpływają na ryzyko występowania depresji, wiek pierwszego epizodu oraz stopień nasilenia objawów.

3. Chroniczny łagodny stres oraz terapia wenlafaksyną wpływają na zmiany poziomu ekspresji genów zaangażowanych w stan zapalny *(TGFA, TGFB, IKBKB, IRF1, PTGS2)* oraz stopień metylacji regionów promotorowych tych genów we krwi oraz strukturach mózgu.

4. Chroniczny łagodny stres oraz terapia wenlafaksyną wpływają na zmiany stopnia metylacji regionów promotorowych genów zaangażowanych w stan zapalny *(TGFA, TGFB, IKBKB, IRF1, PTGS2)* we krwi oraz strukturach mózgu.

5. Zmiany poziomu ekspresji genów oraz stopnia metylacji regionów promotorowych mogą być specyficzne dla danej tkanki.

Literatura uzupełniająca

1.	WHO. Depression. https://www.who.int/news-room/fact-sheets/detail/d. 2020. p. https://www.who.int/news-room/fact-sheets/detail/d.

2.	Al-Harbi KS. Treatment-resistant depression: Therapeutic trends, challenges, and future directions. Patient Preference and Adherence. 2012.

3.	Ionescu DF, Rosenbaum JF, Alpert JE. Pharmacological approaches to the challenge of treatment-resistant depression. Dialogues Clin Neurosci. 2015;17(2):111–26.

4.	Lopizzo N, Chiavetto LB, Cattane N, Plazzotta G, Tarazi FI, Pariante CM, et al. Gene-environment interaction in major depression: Focus on experience-dependent biological systems. Frontiers in Psychiatry. 2015.

5.	Capuron L, Miller AH. Immune system to brain signaling: Neuropsychopharmacological implications. Pharmacology and Therapeutics. 2011.

6.	Schiepers OJG, Wichers MC, Maes M. Cytokines and major depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2005.

7.	Evans DL, Charney DS, Lewis L, Golden RN, Gorman JM, Krishnan KRR, et al. Mood disorders in the medically ill: Scientific review and recommendations. Biological Psychiatry. 2005.

8.	Zorrilla EP, Luborsky L, McKay JR, Rosenthal R, Houldin A, Tax A, et al. The relationship of depression and stressors to immunological assays: A meta-analytic review. Brain, Behavior, and Immunity. 2001.

9.	Howren MB, Lamkin DM, Suls J. Associations of depression with c-reactive protein, IL-1, and IL-6: A meta-analysis. Psychosom Med. 2009;

10.	Miller AH, Maletic V, Raison CL. Inflammation and Its Discontents: The Role of Cytokines in the Pathophysiology of Major Depression. Biological Psychiatry. 2009.

11.	Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A Meta-Analysis of Cytokines in Major Depression. Biol Psychiatry. 2010;

12.	Minghetti L. Role of COX-2 in inflammatory and degenerative brain diseases. Subcell Biochem. 2007;

13.   Minghetti L. Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases. Journal of Neuropathology and Experimental Neurology. 2004.

14.   Gałecki P, Gałecka E, Maes M, Chamielec M, Orzechowska A, Bobińska K, et al. The expression of genes encoding for COX-2, MPO, iNOS, and sPLA2-IIA in patients with recurrent depressive disorder. J Affect Disord. 2012;

15.   Cassano P, Hidalgo A, Burgos V, Adris S, Argibay P. Hippocampal upregulation of the cyclooxygenase-2 gene following neonatal clomipramine treatment (a model of depression). Pharmacogenomics J [Internet]. 2006;6(6):381–7. Available from: https://doi.org/10.1038/sj.tpj.6500385

16.   Myint AM, Steinbusch HWM, Goeghegan L, Luchtman D, Kim YK, Leonard BE. Effect of the COX-2 inhibitor celecoxib on behavioural and immune changes in an olfactory bulbectomised rat model of depression. Neuroimmunomodulation. 2007;

17.   Abbasi SH, Hosseini F, Modabbernia A, Ashrafi M, Akhondzadeh S. Effect of celecoxib add-on treatment on symptoms and serum IL-6 concentrations in patients with major depressive disorder: Randomized double-blind placebo-controlled study. J Affect Disord. 2012;

18.   Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: When the immune system subjugates the brain. Nature Reviews Neuroscience. 2008.

19.   De Kloet ER, Joëls M, Holsboer F. Stress and the brain: From adaptation to disease. Nature Reviews Neuroscience. 2005.

20.   Guillemin GJ, Smythe G, Takikawa O, Brew BJ. Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons. Glia. 2005;

21.   Fujigaki H, Saito K, Fujigaki S, Takemura M, Sudo K, Ishiguro H, et al. The signal transducer and activator of transcription 1α and interferon regulatory factor 1 are not essential for the induction of indoleamine 2,3-dioxygenase by lipopolysaccharide: Involvement of p38 mitogen-activated protein kinase and nuclear factor-κB pathways, and synergistic effect of several proinflammatory cytokines. J Biochem. 2006;

22.   Zhu C Bin, Lindler KM, Owens AW, Daws LC, Blakely RD, Hewlett WA. Interleukin-

1 receptor activation by systemic lipopolysaccharide induces behavioral despair linked to MAPK regulation of CNS serotonin transporters. Neuropsychopharmacology. 2010;

23. Felger JC, Lotrich FE. Inflammatory cytokines in depression: Neurobiological mechanisms and therapeutic implications. Neuroscience. 2013.

24. Steiner J, Bielau H, Brisch R, Danos P, Ullrich O, Mawrin C, et al. Immunological aspects in the neurobiology of suicide: Elevated microglial density in schizophrenia and depression is associated with suicide. J Psychiatr Res. 2008;42(2):151–7.

25. Michelucci A, Heurtaux T, Grandbarbe L, Morga E, Heuschling P. Characterization of the microglial phenotype under specific pro-inflammatory and anti-inflammatory conditions: Effects of oligomeric and fibrillar amyloid-β. J Neuroimmunol. 2009;210(1–2):3–12.

26. Park J, Min JS, Kim B, Chae U Bin, Yun JW, Choi MS, et al. Mitochondrial ROS govern the LPS-induced pro-inflammatory response in microglia cells by regulating MAPK and NF-κB pathways. Neurosci Lett. 2015;

27. Vivien D, Ali C. Transforming growth factor-β signalling in brain disorders. Cytokine Growth Factor Rev. 2006;

28. Musil R, Schwarz MJ, Riedel M, Dehning S, Cerovecki A, Spellmann I, et al. Elevated macrophage migration inhibitory factor and decreased transforming growth factor-beta levels in major depression - No influence of celecoxib treatment. J Affect Disord. 2011;

29. Sutcigil L, Oktenli C, Musabak U, Bozkurt A, Cansever A, Uzun O, et al. Pro- and anti-inflammatory cytokine balance in major depression: Effect of sertraline therapy. Clin Dev Immunol. 2007;

30. Wray NR, Pergadia ML, Blackwood DHR, Penninx BWJH, Gordon SD, Nyholt DR, et al. Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. Mol Psychiatry [Internet]. 2010/11/02. 2012 Jan;17(1):36–48. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21042317

31. Myint AM, Leonard BE, Steinbusch HWM, Kim YK. Th1, Th2, and Th3 cytokine alterations in major depression. J Affect Disord. 2005;

32. Hong M, Zheng J, Ding ZY, Chen JH, Yu L, Niu Y, et al. Imbalance between Th17 and treg cells may play an important role in the development of chronic unpredictable

mild stress-induced depression in mice. Neuroimmunomodulation. 2012;

33. Frank MG, Hershman SA, Weber MD, Watkins LR, Maier SF. Chronic exposure to exogenous glucocorticoids primes microglia to pro-inflammatory stimuli and induces NLRP3 mRNA in the hippocampus. Psychoneuroendocrinology. 2014;

34. Han A, Yeo H, Park MJ, Kim SH, Choi HJ, Hong CW, et al. IL-4/10 prevents stress vulnerability following imipramine discontinuation. J Neuroinflammation. 2015;

35. Vogelzangs N, Duivis HE, Beekman ATF, Kluft C, Neuteboom J, Hoogendijk W, et al. Association of depressive disorders, depression characteristics and antidepressant medication with inflammation. Transl Psychiatry. 2012;

36. The 100 most important chemical compounds: a reference guide. Choice Rev Online. 2008;

37. Westenberg HGM, Sandner C. Tolerability and safety of fluvoxamine and other antidepressants. Int J Clin Pract. 2006;

38. Papp M. Models of affective illness: Chronic mild stress in the rat. Curr Protoc Pharmacol. 2012;

39. You Z, Luo C, Zhang W, Chen Y, He J, Zhao Q, et al. Pro- and anti-inflammatory cytokines expression in rat's brain and spleen exposed to chronic mild stress: Involvement in depression. Behav Brain Res. 2011;

Summary

Introduction

Depression (MDD, major depressive disorders), being one of the most frequently diagnosed mental diseases, affects over 260 million people worldwide and is a major contributor to the global burden of disease. Due to the constantly growing number of cases, it is estimated that depression is the second most common cause of social disability. People suffering from depression are accompanied by persistent sadness, lack of interest, socio-behavioral withdrawal and anxiety that limit their life and proper functioning [1]. Broad spectrum of symptoms, including psychotic behavior often become recurrent or chronic, increasing the risk of suicide attempts. Furthermore, one of the major problems is the high rate of relapse, as well as the fact that more than a 30 percent of patients do not respond to conventional antidepressant treatment [2,3]. Therefore, it is important to identify the incompletely understood pathogenesis and molecular mechanisms underlying depression.

Despite many years of research, the molecular aspects underlying the development of MDD have not been fully elucidated. Certainly, the disease is characterized by a multifactorial etiology, and the risk of its development is often determined by genetic, environmental and individual biological factors [4]. Increasingly, among the many processes contributing to the development of the disease, inflammation is considered as a factor playing an important role in the etiology of depression. One of the explanatory mechanisms is the "cytokine hypothesis", confirmed by the fact that depressed patients present higher levels of pro-inflammatory cytokines in the blood, such as interleukin-1α (IL-1α), interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α). Moreover, patients with MDD are characterizes by increased levels of other inflammatory markers, including acute phase proteins, prostaglandins, adhesion molecules and chemokines. For example, both prostaglandins and the enzyme responsible for their synthesis PTGS2 (Prostaglandin-endoperoxide synthase 2, COX-2) plays a significant role in triggering the inflammatory cascade in depression [12,13]. People suffering from depression are characterized by an increase in the expression of the *PTGS2* gene [14]. These reports are also confirmed by studies using an animal model of depression, suggesting a higher level of *PTGS2* expression in the brain [15]. Moreover, the administration of a selective COX-2 inhibitor reduced depressive behavior in rats as well as lowered the level of cytokines in the animals' hypothalamus [16]. Similarly, in patients with severe depression, selective inhibitor add-on therapy may not only alleviate the depressive behavior, but also reduce the level of pro-inflammatory cytokines in the serum [17]. Prolonged activation of the immune system and

chronic inflammation are strongly associated with the development and progression of the disease. Factors released during the activation of the immune system could act as neuromodulators, and thus can trigger a cascade of neurochemical, neuroendocrine and behavioral changes affecting many biological functions, such as activation of the HPA (hypothalamic-pituitary-adrenal) axis, metabolism of neurotransmitters or neuroplasticity, which are dysregulated in the development and progression of depression [6,18,19]. Stimulation of the immune system also affects the metabolism of tryptophan, through the ability of proinflammatory cytokines to regulate the enzyme indoleamine 2,3-dioxygenase (IDO). The increased activity of the IDO enzyme, stimulated by cytokines, and hence the increased conversion of tryptophan to kynurenine, reduces the amount of this amino acid as a precursor to serotonin [20,21]. Cytokines can also interfere with serotonergic neurotransmission by influencing the activity of the SERT (sodium-dependent serotonin transporter) enzyme [22]. In addition, peripheral inflammation, becoming chronic is reflected in the central nervous system (CNS) characterized by the mobilization of immune cells, called microglia [23,24]. Stimulated microglia is responsible for the production of cytokines in the CNS, which may contribute to the development of neuroinflammation and nervous system disruptions [25]. This process is closely related to the activation of the nuclear transcription factor NF-kappa β (NF-$\kappa\beta$), responsible for the production of pro-inflammatory cytokines [26]. Nevertheless, microglia also exerts a neuroprotective effect, stimulating anti-inflammatory factors to antagonize inflammation-induced damage. One of these molecules is the transforming growth factor β (TGF-β), which levels are thought to be unbalanced in patients with depression [25,27]. Lower TGF-β expression have been reported in depressed patients compared to healthy controls [28-31]. However, in studies using an animal model of depression, it was found that the level of TGF-β is elevated in individuals with symptoms of the disease [32]. Moreover, stress has been confirmed to cause inflammation in the brain areas such as the frontal cortex, hypothalamus, and the hippocampus [33,34]. What is more, it is believed that the administration of antidepressants may be effective in reducing pro-inflammatory cytokines in people with depression [35]. Currently, the first-line treatment of MDD includes selective serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors (SNRIs) and tricyclic antidepressants (TCAs) [36,37]. Nevertheless, the effect of antidepressants on the levels of inflammatory molecules in the peripheral and central nervous systems is not fully investigated. Due to the possibility of conducting research on human brain tissue only *post-mortem*, to understand the interplay between processes occurring in the nervous system during the disease,

it is necessary to use animal models of depression, such as the chronic mild stress (CMS) procedure [38]. Interestingly, research indicates an imbalance between pro- and anti-inflammatory cytokines in CMS-induced depressive behavior [39].

Taking together, activation of the immune system in depression is based on a complex network of closely interconnected pathways. Therefore the occurrence of any variation or disturbance in one of them may cause dysregulation and disruption of other related factors and thus trigger an inflammatory cascade. Furthermore, despite the confirmed involvement of the immune system in depression, the knowledge of inflammatory molecules other than cytokines, in particular those involved in neuroinflammation and brain function, in the pathogenesis of the disease is incomplete.

In this work, polymorphisms of genes involved in inflammatory processes that may affect the activity of the encoded proteins have been investigated. The effect of the chronic mild stress procedure, an animal model of depression, and venlafaxine therapy on the expression level of genes related to inflammation was assessed. Moreover, it was investigated whether the above-mentioned factors cause epigenetic changes in the studied genes, such as the methylation status of their promoter regions.

Aim of the study

The work aimed to determine the role of inflammation in the development of depression and the mechanism of action of antidepressant drugs. This aim was accomplished b:

- Examination of the prospective relationship between the appearance of single nucleotide polymorphism (SNP) located in inflammatory-related genes i.e. *IL1A, IL1B, TNFA, TGFA, TGFB, PTGS2, IRF1, IKBKB*, and the occurrence of MDD, age of onset, severity of episodes or antidepressant treatment efficacy
- Impact of the chronic mild stress (CMS) procedure in rats, which closely mirrors depression in humans, and chronic administration of serotonin-norepinephrine reuptake inhibitor, venlafaxine, on changes in *TGFA, TGFB, IRF1, PTGS2* and *IKBKB* expression at the mRNA level in peripheral blood mononuclear cells (PBMCs) and in selected brain structures (hippocampus, amygdala, midbrain, hypothalamus, prefrontal cortex and basal ganglia)
- Impact of the chronic mild stress (CMS) procedure in rats, which closely mirrors depression in humans, and chronic administration of serotonin-norepinephrine reuptake inhibitor, venlafaxine, on promoter methylation status changes in *TGFA, TGFB, IRF1, PTGS2* and *IKBKB* genes

Materials and methods

In vitro study

The blood samples were obtained from patients diagnosed with depression hospitalized at the Department of Adult Psychiatry, Medical University of Lodz and healthy controls selected randomly. Inclusion criteria and diagnosis were based on those outlined in ICD-10 (F32.0-F.32.2, F33.0-F33.8). The exclusion criteria included: axis I and axis II disorders, severe and chronic somatic diseases, inflammatory or autoimmune disorders, cancer and and injuries of the central nervous system. Participation in the experiment was voluntary and the purpose of the study was clearly presented. All of the subjects agreed by giving their written consent to participate in the experiment according to the protocol approved by the Bioethics Committee of the Medical University of Lodz (No. RNN/70/14/KE).

Genomic DNA was isolated from venous blood using Blood Mini Kit (A&A Biotechnology, Gdynia, Poland) in accordance to the manufacturer instruction. The purity and concentration of the DNA was measured spectrophotometrically by calculating the ratio between absorbance at 260 nm and 280 nm. The investigated SNPs were genotyped with TaqMan SNP Genotyping Assay using the real-time polymerase chain reaction (Real-Time PCR) (Bio-Rad CFX96 Real-Time PCR). A total of 11 polymorphisms were analyzed (Table 1).

Table 1. Characteristic of studied single nucleotide polymorphisms.

Gene	Rs number	Polymorphism	Localization
TGFA	rs2166975	g.70677994G>A	Exon
TGFB1	rs1800469	g.41354391A>G	5' UTR
IRF1	rs2070729	g.132484229C>A	Intron
IKBKB	rs5029748	g.42140549G>T	Intron
PTGS2	rs5275	g.186643058A>G	3' UTR
	rs4648308	g.186640617C>T	3' UTR
IL1A	rs17561	g.10749G>T	Ekson
IL1B	rs1143623	g.113595829C>G	5' UTR
	rs1143627	g.113594387G>A	5' UTR
TNFA	rs1799964	g.4970C>T	5' UTR
	rs1800629	g.31543031G>A	5' UTR

Statistical analysis of the distribution of genotypes and alleles of the studied polymorphisms was carried out using a multiple logistic regression model. The results was presented as odds ratio (OR) with 95% confidence interval (95% CI). In addition, the significant outcomes were further validated with the use of two approaches: the bootstrap-boosted multiple logistic regression (resampling with replacement, 10,000 iterations) and the cross-validated logistic regression (corresponding to the d-jackknife technique). The goodness of fit of logistic regression models was estimated with Hosmer-Lemeshow test. Normality of the studied group was verified with the Shapiro–Wilk test.

In vivo study

Male Wistar Han rats (Charles River, Germany) were used to induce chronic mild stress procedure. Animals were brought into the laboratory to adapt to the housing conditions. After acclimatization, the animals were trained to consume a 1% sucrose solution. Sucrose solution consumption is the most common, adequate way to quantify the behavioral effect of CMS procedure by measuring the ability to respond to reward stimuli. Consumption of the sucrose was verified once a week, under controlled conditions, until the experiment was ended. The animals were subjected to the standard CMS procedure (Table 2). Subsequently, both control and stressed rats were divided into two matched groups to receive daily administration of vehicle (1 mL/kg, IP) or venlafaxine (10 mg/kg, IP) for the next five weeks. At the end of the procedure, the rats were decapitated. Blood and brain samples (hippocampus, amygdala, hypothalamus, midbrain, prefrontal cortex and nucleus basal ganglia) were collected.

Table 2. Schedule of CMS procedure and detailed description of all applied stressors.

Experiment start		
5 weeks adaptation to 1% sucrose consumption test		
2 weeks without stress	2 weeks of initial stress	
5 weeks without stress and with	5 weeks of stress with saline administration	5 weeks of stress with venlafaxine administration

Stress procedure

Stressor	Duration	Number of periods
Food and water depriviation	10 – 14 hours	2 periods
45-degree cage tilt	10 – 14 hours	2 periods
soiled cage (250 ml water in sawdust bedding)	10 – 14 hours	2 periods
paired housing	10 – 14 hours	1 period
low intensity stroboscopic illumination (150 flashes/min)	10 – 14 hours	2 periods
Intermittent illumination (light on and off every two hours)	10 – 14 hours	2 periods
No stress	10 – 14 hours	3 periods

Final sucrose consumption test and decapitation

RNA and DNA isolation was performed using the commercial spin column methods: GenElute mammalian total RNA miniprep kit (Sigma-Aldrich, St. Louis, MO, USA) and QIAamp DNA mini kit (Qiagen, Hilden, Germany) respectively, following the manufacturer's instructions. Regarding brain regions commercial kit ISOLATE II RNA/DNA/protein kit (Bioline) was used. Samples were first homogenized and then sonicated before isolation procedure. The purity and concentration of the DNA and RNA was measured spectrophotometrically by calculating the ratio between absorbance at 260 nm and 280 nm.

Real-Time PCR and TaqMan Gene Expression Assay was used to examine the expression of the selected genes. To obtain complementary DNA (cDNA) as a template, the

reverse transcription reaction was performed with using a high-capacity cDNA reverse transcription kit (Applied Biosystems). The housekeeping gene 18S ribosomal RNA gene (18S) was applied as an internal control (reference gene) for all reverse transcription–quantitative polymerase chain reactions. For each sample, the gene expression of the target mRNA was calculated relative to a reference gene.

The methylation status of investigated gene promoters was obtained by methylation-sensitive high-resolution melting (MS-HRM). The DNA bisulfite conversion reaction was performed with a CiTi converter DNA methylation kit (A&A Biotechnology, Gdynia, Poland), according to the manufacturer's instruction. Methylated DNA (CpGenome™ rat methylated genomic DNA standard; Merck Millipore) and unmethylated DNA (CpGenome™ rat unmethylated genomic DNA standard; Merck Millipore) were used as controls for the MS-HRM experiments. Primers were designed for promoters containing CpG islands using Methyl Primer Express™ Software v 1.0 (Table 3).

Table 3. The specification of primers used for the analysis of methylation levels in the promoter regions of the studied genes.

Gene	Primer sequence (5'->3')	Tm [C°]	Product size [bp]	Number of CpG
IKBKB	F:AGGGTGGTTTTTTATTTTTATTTT R:AACCCCCACTAAAACTAACTTAA	55	117	1
IRF1	F:TTGGAGATTTAGGGAGTTAGGT R:CCCCTTACCTATCTTAAAAAACC	55	123	1
PTGS2	F:GTAATAGTAGGGAGGAAAAATTTTAA R:ATCCTAACAAACCCCAAA	55	111	1
TGFA	F:GTTTTTTTAGGTGGTTGGTTAAG R:CTTCAAACACCTCCCTACAATA	55	188	1

Normality of the collected data was verified with the Shapiro–Wilk test. The effect of stress procedure on sucrose consumption was analyzed by t-test for normally distributed data or the Mann–Whitney rank-sum test for non-normally distributed data. In addition, when the data were normally distributed, gene expression and methylation data were analyzed using one-way analysis of variance (one-way ANOVA), with Tukey's test as a post hoc test. If the data were not normally distributed, these relationships were tested using the Kruskal–Wallis one-way ANOVA on ranks, followed by post hoc Student–Newman–Keuls test.

Results

The obtained results showed a number of associations between the presence of SNP in the studied genes and the risk of depression (Table 3). Analysis of the distribution of genotypes

and alleles showed that, A/G genotype of *TGFA* – G > A (rs2166975), A/C of *IRF1* – C > A (rs2070729) as well as G/T of *IKBKB* – G > T (rs5029748) were associated with increased risk of depression development. In contrast, the G/G of *TGFA* – G > A (rs2166975), T/T of *IKBKB* – G > T (rs5029748) and T/T of *PTGS2* – C > T (rs4648308) genotypes reduced this risk. In addition, group was stratified to investigate prevalence of the disease in stratified male/female population. Such analysis revealed that, A/G genotype of *TGFA* – G > A (rs2166975) and A/A genotype of *PTGS2* – A > G (rs5275) were positively correlated with higher risk of depression occurrence in male population. In female group possibility of developing the disease was higher in the case of carrying A/C genotype of *IRF1* – C > A (rs2070729), A/G of *PTGS2* – A > G (rs5275) as well as C/T genotype of *PTGS2* – C > T (rs4648308). Moreover, polymorphisms of the *IKBKB* – G > T (rs5029748)*, IRF1* – C > A (rs2070729) and *TNFA– C > T* (rs1799964) genes may affect the effectiveness of antidepressant therapy with serotonin reuptake inhibitors. What is more, severity of the episodes could be connected with occurrence of the *IL1B* – C > G (rs1143623) and *TGFB* – A > G (rs1800469) polymorphisms.

Table 4. Summary of the impact of studied SNPs on phenotype

Polymorphism	Genotype	Phenotype effect
TGFA – G >A (rs2166975)	A/G	Higher risk of depression in the general population
		Higher risk of depression in the male group
	G/G	Lower risk of depression in the general population
TGFB – A > G (rs1800469)	A/A	Lower age of onset
	G/G	More severe symptoms of a depressive episode
IRF1 - C > A (rs2070729)	A/C	Higher risk of depression in the general population
		Higher risk of depression in the female group
		Worse treatment response
	A/A	Better treatment response
IKBKB – G > T (rs5029748)	G/T	Higher risk of depression in the general population
		Higher risk of depression in the male group
	T/T	Lower risk of depression in the general population
	G/G	Better treatment response
PTGS2 – C > T (rs4648308)	T/T	Lower risk of depression in the general population
	C/T	Higher risk of depression in the female group
PTGS2 – A > G (rs5275)	A/A	Higher risk of depression in the male group
	A/G	Higher risk of depression in the female group
TNFA – C > T (rs1799964)	T/T	Worse treatment response
	C/T	Better treatment response
IL1B – C > G (rs1143623)	C/C	More severe symptoms of a depressive episode

The results obtained using an animal model of depression showed that both the chronic mild stress procedure and venlafaxine administration affect the gene expression level and the methylation status in their promoter regions. After stress procedure, the consumption of sucrose solution decreased to approximately 60% of initial values. Chronic venlafaxine treatment normalized sucrose consumption in stressed rats.

Animals stressed for seven weeks and administered saline demonstrated significantly greater expression of *TGFA, TGFB, PTGS2, IRF1* and *IKBKB* genes in PBMCs compared to the control group. Chronic administration of venlafaxine for five weeks caused a significant decrease in the expression of all studied genes in stressed rats. Interestingly, the effect of the CMS and antidepressant administration on the mRNA expression of the studied genes clearly differed between brain structures. The CMS caused a significant decrease of *TGFA* and *IKBKB* expression in the hippocampus. Furthermore, stress induced lower expression of *TGFA, TGFB* and *IKBKB* in the amygdala, and in the midbrain in the case of *IKBKB*. After venlafaxine administration, the stressed animals demonstrated downregulation of *TGFA, TGFB* and *IRF1* in the hypothalamus as well as *IKBKB* gene in the amygdala and prefrontal cortex. On the other hand, venlafaxine treatment also increased the expression of *TGFA* in the hippocampus and nucleus basal ganglia, as well as *PTGS2* gene in the hypothalamus.

The only significant change in methylation status in PBMCs was found in the case of the *IKBKB* promoter, where two-week exposure to CMS caused increased methylation compared with non-stressed controls. No significant differences after venlafaxine treatment were observed. Regarding brain structures, CMS procedure significantly increased the methylation level of the *TGFA* promoter in the amygdala. Stressed animals also demonstrated a higher methylation status in the case of the *IRF1* promoter in the amygdala and prefrontal cortex, as well as in the case of *PTGS2* promoter in the hippocampus and amygdala. However, CMS also caused a decrease in *PTGS2* and *TGFA* promoter methylation in the prefrontal cortex. Interestingly, chronic five-week administration of venlafaxine resulted in significant increased *IKBKB* promoter methylation in the amygdala and nucleus basal ganglia, and *IRF1* promoter in the amygdala. A similar effect was observed in the case of the *TGFA* promoter, where the methylation status was higher in the hippocampus and amygdala after venlafaxine treatment.

35

Resume and conclusion

Depression is a serious health and economic problem. The disease and its symptoms affect all aspects of patients life, and thus limit the proper functioning. Unfortunately, despite intensive research, the complex pathogenesis of depressive disorders is still not fully understood. In addition, the disease is characterized by a high relapse rate and the fact that more than 30 percent of patients do not respond to conventional antidepressant drug therapy. Moreover, the diagnosis of depression is based only on observation according to the international classifications of symptoms, without taking into account the potential contribution of mechanisms at the molecular level. The studies conducted so far clearly indicate a multifactorial and complex network of interrelated mechanisms underlying the development of the disease. The growing body of evidence emphasizes the significant role of inflammation and activation of the immune system in depression. The results obtained in this dissertation confirm this statement. Polymorphisms of genes involved in inflammation modulate the risk of depression, as well as affect the severity of its symptoms and, importantly, the response to pharmacotherapy. Moreover, the this research showed that both chronic mild stress and venlafaxine therapy affect gene expression changes related to the activation of the immune system. The obtained results indicate that the studied genes may be responsible for the activation of inflammatory pathways in the presence of stress stimuli. The CMS procedure, in both PMBC and brain regions, induce changes in the expression of investigated genes, which in turn can trigger an inflammatory cascade. Another key finding is that chronic venlafaxine administration may cause anti-inflammatory effects by affecting the expression of the inflammation-related genes. As possibility of conducting research on the human brain is limited, understanding the complex relationships and processes occurring within this tissue requires the use of an animal models which reflect the symptoms of depression in humans. The validated chronic mild stress model, is based on the assessment of the level of anhedonia - the most common, adequate way to quantify the behavioral effect of CMS procedure by measuring the ability to respond to reward stimuli. All the results support the fact that inflammation may play an important role in the molecular aspect of depression etiology. Therefore, it is important to conduct research in such area, which might contribute to the development of new diagnostic methods of the disease as well as personalized antidepressant therapy.

Conclusion

1. Inflammation plays a significant role in the molecular basis of depression

2. Single nucleotide polymorphisms located in genes involved in inflammation (*IL1A, IL1B, TNFA, TGFA, TGFB, IKBKB, IRF1, PTGS2*) affect the risk of depression, age of first episode, severity of symptoms and response to the treatment

3. Chronic mild stress and venlafaxine treatment induce changes in the mRNA expression level of genes involved in inflammation (*TGFA, TGFB, IKBKB, IRF1, PTGS2*) in the blood and brain structures

4. Chronic mild stress and venlafaxine treatment in affect methylation status of the promoter regions of genes involved in inflammation (*TGFA, TGFB, IKBKB, IRF1, PTGS2*) in the blood and brain structures

5. Changes in the level of gene expression and the methylation of promoter regions may be tissue specific

References

1.   WHO. Depression. https://www.who.int/news-room/fact-sheets/detail/d. 2020. p. https://www.who.int/news-room/fact-sheets/detail/d.

2.   Al-Harbi KS. Treatment-resistant depression: Therapeutic trends, challenges, and future directions. Patient Preference and Adherence. 2012.

3.   Ionescu DF, Rosenbaum JF, Alpert JE. Pharmacological approaches to the challenge of treatment-resistant depression. Dialogues Clin Neurosci. 2015;17(2):111–26.

4.   Lopizzo N, Chiavetto LB, Cattane N, Plazzotta G, Tarazi FI, Pariante CM, et al. Gene-environment interaction in major depression: Focus on experience-dependent biological systems. Frontiers in Psychiatry. 2015.

5.   Capuron L, Miller AH. Immune system to brain signaling: Neuropsychopharmacological implications. Pharmacology and Therapeutics. 2011.

6.   Schiepers OJG, Wichers MC, Maes M. Cytokines and major depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2005.

7.   Evans DL, Charney DS, Lewis L, Golden RN, Gorman JM, Krishnan KRR, et al. Mood disorders in the medically ill: Scientific review and recommendations. Biological Psychiatry. 2005.

8.   Zorrilla EP, Luborsky L, McKay JR, Rosenthal R, Houldin A, Tax A, et al. The relationship of depression and stressors to immunological assays: A meta-analytic review. Brain, Behavior, and Immunity. 2001.

9.   Howren MB, Lamkin DM, Suls J. Associations of depression with c-reactive protein, IL-1, and IL-6: A meta-analysis. Psychosom Med. 2009;

10.   Miller AH, Maletic V, Raison CL. Inflammation and Its Discontents: The Role of Cytokines in the Pathophysiology of Major Depression. Biological Psychiatry. 2009.

11.   Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A Meta-Analysis of Cytokines in Major Depression. Biol Psychiatry. 2010;

12.   Minghetti L. Role of COX-2 in inflammatory and degenerative brain diseases. Subcell Biochem. 2007;

13.   Minghetti L. Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases. Journal of Neuropathology and Experimental Neurology. 2004.

14.   Gałecki P, Gałecka E, Maes M, Chamielec M, Orzechowska A, Bobińska K, et al. The expression of genes encoding for COX-2, MPO, iNOS, and sPLA2-IIA in patients with recurrent depressive disorder. J Affect Disord. 2012;

15.   Cassano P, Hidalgo A, Burgos V, Adris S, Argibay P. Hippocampal upregulation of the cyclooxygenase-2 gene following neonatal clomipramine treatment (a model of depression). Pharmacogenomics J [Internet]. 2006;6(6):381–7. Available from: https://doi.org/10.1038/sj.tpj.6500385

16.   Myint AM, Steinbusch HWM, Goeghegan L, Luchtman D, Kim YK, Leonard BE. Effect of the COX-2 inhibitor celecoxib on behavioural and immune changes in an olfactory bulbectomised rat model of depression. Neuroimmunomodulation. 2007;

17.   Abbasi SH, Hosseini F, Modabbernia A, Ashrafi M, Akhondzadeh S. Effect of celecoxib add-on treatment on symptoms and serum IL-6 concentrations in patients with major depressive disorder: Randomized double-blind placebo-controlled study. J Affect Disord. 2012;

18.   Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: When the immune system subjugates the brain. Nature Reviews Neuroscience. 2008.

19.   De Kloet ER, Joëls M, Holsboer F. Stress and the brain: From adaptation to disease. Nature Reviews Neuroscience. 2005.

20.   Guillemin GJ, Smythe G, Takikawa O, Brew BJ. Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons. Glia. 2005;

21.   Fujigaki H, Saito K, Fujigaki S, Takemura M, Sudo K, Ishiguro H, et al. The signal transducer and activator of transcription 1α and interferon regulatory factor 1 are not essential for the induction of indoleamine 2,3-dioxygenase by lipopolysaccharide: Involvement of p38 mitogen-activated protein kinase and nuclear factor-κB pathways, and synergistic effect of several proinflammatory cytokines. J Biochem. 2006;

22.   Zhu C Bin, Lindler KM, Owens AW, Daws LC, Blakely RD, Hewlett WA. Interleukin-

1 receptor activation by systemic lipopolysaccharide induces behavioral despair linked to MAPK regulation of CNS serotonin transporters. Neuropsychopharmacology. 2010;

23. Felger JC, Lotrich FE. Inflammatory cytokines in depression: Neurobiological mechanisms and therapeutic implications. Neuroscience. 2013.

24. Steiner J, Bielau H, Brisch R, Danos P, Ullrich O, Mawrin C, et al. Immunological aspects in the neurobiology of suicide: Elevated microglial density in schizophrenia and depression is associated with suicide. J Psychiatr Res. 2008;42(2):151–7.

25. Michelucci A, Heurtaux T, Grandbarbe L, Morga E, Heuschling P. Characterization of the microglial phenotype under specific pro-inflammatory and anti-inflammatory conditions: Effects of oligomeric and fibrillar amyloid-β. J Neuroimmunol. 2009;210(1–2):3–12.

26. Park J, Min JS, Kim B, Chae U Bin, Yun JW, Choi MS, et al. Mitochondrial ROS govern the LPS-induced pro-inflammatory response in microglia cells by regulating MAPK and NF-κB pathways. Neurosci Lett. 2015;

27. Vivien D, Ali C. Transforming growth factor-β signalling in brain disorders. Cytokine Growth Factor Rev. 2006;

28. Musil R, Schwarz MJ, Riedel M, Dehning S, Cerovecki A, Spellmann I, et al. Elevated macrophage migration inhibitory factor and decreased transforming growth factor-beta levels in major depression - No influence of celecoxib treatment. J Affect Disord. 2011;

29. Sutcigil L, Oktenli C, Musabak U, Bozkurt A, Cansever A, Uzun O, et al. Pro- and anti-inflammatory cytokine balance in major depression: Effect of sertraline therapy. Clin Dev Immunol. 2007;

30. Wray NR, Pergadia ML, Blackwood DHR, Penninx BWJH, Gordon SD, Nyholt DR, et al. Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. Mol Psychiatry [Internet]. 2010/11/02. 2012 Jan;17(1):36–48. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21042317

31. Myint AM, Leonard BE, Steinbusch HWM, Kim YK. Th1, Th2, and Th3 cytokine alterations in major depression. J Affect Disord. 2005;

32. Hong M, Zheng J, Ding ZY, Chen JH, Yu L, Niu Y, et al. Imbalance between Th17 and treg cells may play an important role in the development of chronic unpredictable

mild stress-induced depression in mice. Neuroimmunomodulation. 2012;

33. Frank MG, Hershman SA, Weber MD, Watkins LR, Maier SF. Chronic exposure to exogenous glucocorticoids primes microglia to pro-inflammatory stimuli and induces NLRP3 mRNA in the hippocampus. Psychoneuroendocrinology. 2014;

34. Han A, Yeo H, Park MJ, Kim SH, Choi HJ, Hong CW, et al. IL-4/10 prevents stress vulnerability following imipramine discontinuation. J Neuroinflammation. 2015;

35. Vogelzangs N, Duivis HE, Beekman ATF, Kluft C, Neuteboom J, Hoogendijk W, et al. Association of depressive disorders, depression characteristics and antidepressant medication with inflammation. Transl Psychiatry. 2012;

36. The 100 most important chemical compounds: a reference guide. Choice Rev Online. 2008;

37. Westenberg HGM, Sandner C. Tolerability and safety of fluvoxamine and other antidepressants. Int J Clin Pract. 2006;

38. Papp M. Models of affective illness: Chronic mild stress in the rat. Curr Protoc Pharmacol. 2012;

39. You Z, Luo C, Zhang W, Chen Y, He J, Zhao Q, et al. Pro- and anti-inflammatory cytokines expression in rat's brain and spleen exposed to chronic mild stress: Involvement in depression. Behav Brain Res. 2011;

Publikacje będące podstawą rozprawy doktorskiej
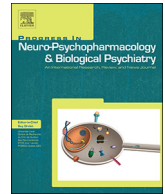
Major depressive disorders accompanying autoimmune diseases – Response to treatment

Katarzyna Bialek[a], Piotr Czarny[b], Justyna Strycharz[b], Tomasz Sliwinski[a,*]

[a] University of Lodz, Faculty of Environmental Protection, Laboratory of Medical Genetics, Lodz, Poland
[b] Department of Medical Biochemistry, Medical University of Lodz, Lodz, Poland

ABSTRACT

MDDs (major depressive disorders) belong to the most frequently diagnosed mental diseases and affect approximately 350 million people all over the world. A growing body of evidence suggests that inflammatory processes may play a significant role in the pathophysiology and progression of the disease. The comorbidity of MDDs with many other medical conditions, for example autoimmune diseases (ADs) caused by inflammation, has been observed on numerous occasions. In both cases, increased levels of pro-inflammatory cytokines, chemokines and other inflammatory agents are observed. Furthermore, higher rates of inflammatory markers are associated with a poorer response to antidepressant treatment. Additionally, the presence of any AD is associated with higher prevalence of depression and may reduce the chance of effective therapy. Interestingly, the administration of several anti-inflammatory agents used in AD treatment is positively correlated with a reduction of depressive symptoms. In conclusion, the factors contributing to the coexistence of depression as well as affecting antidepressant treatment effectiveness may lead to an alteration of the cytokine profiles in many autoimmune diseases.

1. Introduction

The leading aspect of research into the human brain is to understand its functioning at the molecular, cellular and systemic levels, which may contribute to the comprehension of the pathogenesis of brain diseases. These disorders constitute a cluster of leading health problems around the world. Among them, a depressive disorder (MDD, depression) represents one of the most frequently diagnosed diseases and a major contributing factor to the overall global burden of all diseases (WHO 2018). The number of depressed patients is increasing every year, making depression one of the most common diseases afflicting the human population. According to the World Health Organization, depression affects approximately 350 million people, i.e. approximately 5% of the global population. Moreover, up to 10% of the society in developed countries may suffer from depression (DiLuca and Olesen 2014). It is estimated that by 2020 MDD will have been the second most common cause of disability, directly behind cardiovascular diseases (Poniatowska-Leszczyńska and Małyszczak 2013, WHO 2018). Depression can occur in both sexes; however, women are about twice more likely to suffer from this disease than men (Seedat et al. 2009). Additionally, MDD is one of the most economically burdening diseases in the modern world with over 60% of all costs generated annually by

brain diseases (DiLuca and Olesen 2014). Furthermore, mood disorders, including MDD, increase the risk of suicide in relation to the general population (Bachmann 2018, Handley et al. 2018). Almost one million people commit suicide every year, which is the second leading cause of death among people aged 15–29 (Marcus et al. 2012, WHO 2018).

Despite the importance of the problem, knowledge of the pathogenesis of the disease is not complete, which is partly due to its multifactorial nature. However, the role of complex interrelations between genetic, environmental, biological as well as psychosocial factors is emphasized among many processes that may have an impact on the development and progression of the disease (Sullivan 2000, Dowlati et al. 2010). In spite of the availability of many antidepressants, about 30% of all patients do not respond to conventional methods of therapy (Al-Harbi 2012). Therefore, there is a strong need to understand the mechanisms underlying depressive disorders, which may contribute to the development of novel and personalized treatment options. It has been found that MDDs frequently accompany many other medical conditions, for example autoimmune diseases (ADs) caused by inflammation (Dantzer et al. 2008, Dowlati et al. 2010, Lotrich 2012, Alcocer-Gómez et al. 2014). Thus, the role of immune impairment in the course of depression has been studied for the past two decades (Miller and Raison 2015). Nevertheless, the mechanisms underlying
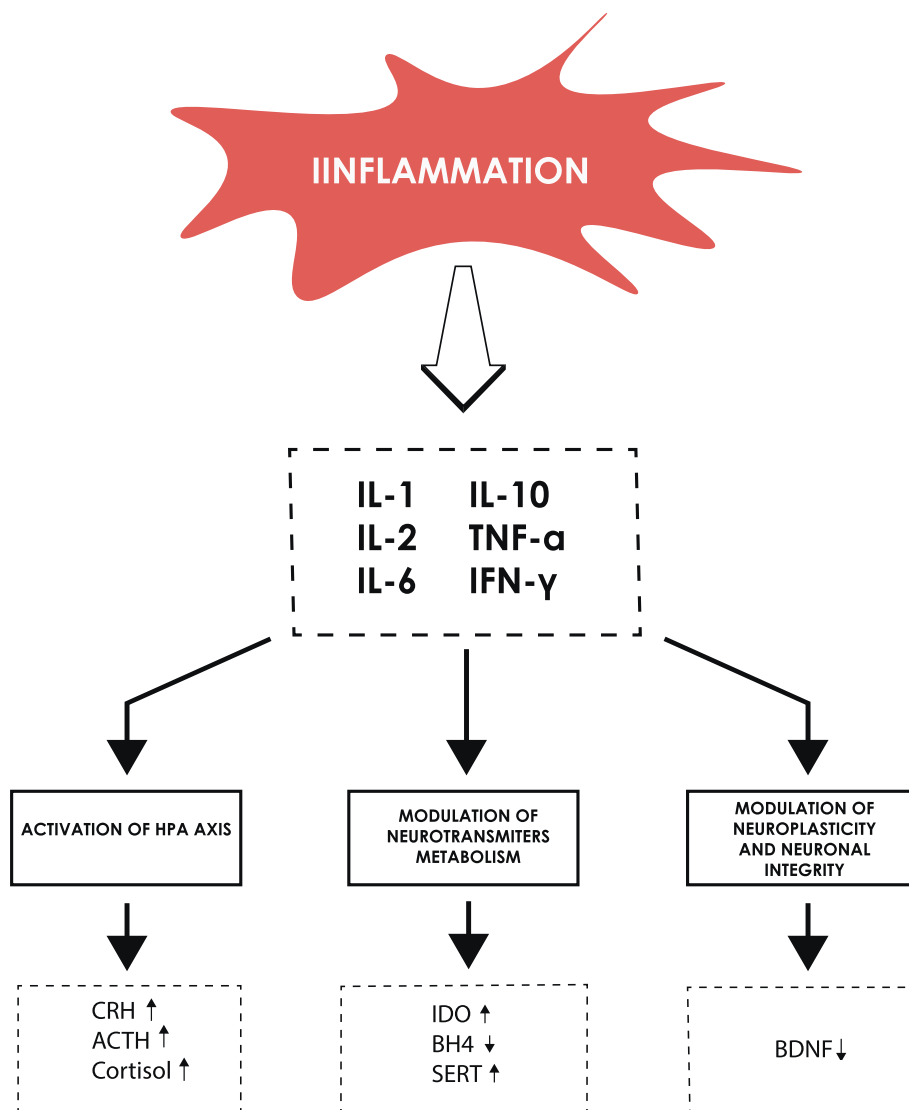
Fig. 1. The impact of inflammation on biological processes connected with neurotransmission.
↑ increased level/activity; decreased level/activity.
CRH – corticotropin-releasing hormone; ACTH – adrenocorticotropic hormone; IDO- indoleamine 2,3-dioxygenase enzyme; BH4 – tetrahydrobiopterin; SERT – sodium-dependent serotonin transporter; BDNF – brain-derived neurotrophic factor.

this phenomenon remain unclear (Kessler and Bromet 2013). ADs represent a cluster of disorders accompanied by chronic inflammation, which develop as a result of a reduction or loss of immunological tolerance to self-antigens (Anaya 2012). There is a growing body of evidence indicating that several autoimmune diseases are characterized by an increased immune response (Khaibullin et al. 2017). The aim of this paper is to elucidate, based on the available research results and literature, the role of the relationship between the comorbidity of MDD and ADs as well as the reaction to antidepressant treatment under conditions with an altered immune response. We have put forward a hypothesis that inflammation in the course of an autoimmune disease may significantly reduce the chance of a positive response to MDD treatment. In this article, instead of a systematic comprehensive literature review, we provided a meaningful synthesis of available literature data.

2. Depression and inflammation

Inflammation is characterized by a number of behavioral, autonomic and endocrine changes that affect the balance of physiological processes (Dantzer et al. 2008). Inflammatory processes cause a broad spectrum of symptoms and behavioral changes observed in both illness and depressive disorders. During inflammation, the most important cells are macrophages (Jiang et al. 2014), since they stimulate the immune system to produce prostaglandins and a variety of cytokines (Sokol and Luster 2015). The secretion of pro-inflammatory cytokines results in the release of C-reactive proteins (CRP) by the liver, thus enabling stimulation of and communication with other cells of the immune system (Sokol and Luster 2015). In patients suffering from MDD, the activation of inflammatory pathways is manifested by significantly elevated levels of acute phase proteins such as CRP, α1-antitrypsin or haptoglobin (Raison et al. 2006). Moreover, the expression of other inflammatory mediators, e.g. prostaglandins, adhesion molecules and chemokines such as monocyte chemoattractant protein-1 (MCP-1) and *E*-selectin, is also higher. (Raison et al. 2006, Raison et al. 2009). In fact, prolonged activation of the immune system and chronically persistent inflammation are strongly associated with the development and progression of the disease. Patients with MDD have higher levels of pro-inflammatory cytokines in blood, including interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin 6 (IL-6), and

tumor necrosis factor alpha (TNF-α) (Haapakoski et al. 2015). The cytokines released during immune system stimulation modulate many biological functions such as activation of the HPA axis (Hypothalamic–Pituitary–Adrenal axis), neuroplasticity and modification of neurotransmitters metabolism. HPA axis activation by cytokines causes a release of the corticotrophin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and cortisol. Activated hypothalamus synthetizes CRH, which binds to specific receptors in the anterior pituitary. Subsequently, secreted ACTH causes release of glucocorticoids (GC) in the adrenal glands (de Kloet et al. 2005).To reinstate homeostasis, this process is regulated by negative feedback mechanisms, precisely GCs bind to glucocorticoid receptors (GR) and further inhibit the release of CRH. In MDD, the negative feedback is reduced by impaired sensitivity of GRs and thus hypersecretion of CRH and increased synthesis of GCs (Holsboer 2000, Pariante and Miller 2001). Moreover, cytokines are found to be able to cause glucocorticoid resistance by direct interaction with GR (Miller et al.1999; Pariante et al. 1999). The end products of this system have. Been known to be able to cross blood-brain barrier and influence the brain functioning, therefore leading to psychopathology in nervous system (McKay and Cidlowski 2003).The levels of all of these neurotransmitters have been found to be increased in MDD patients (Pariante and Miller 2001, Pace et al. 2007). Apart from this, the activity of the immune system also affects tryptophan metabolism. Pro-inflammatory cytokines are involved in the regulation of the indoleamine 2,3-dioxygenase enzyme (IDO), which is responsible for the conversion of tryptophan to kynurenine. Upregulation of IDO results in a reduced amount of tryptophan as a serotonin precursor (Guillemin et al. 2005, Fujigaki et al. 2006). Moreover, cytokines may interfere with serotonergic neurotransmission by affecting SERT (sodium-dependent serotonin transporter) enzyme activity (Zhu et al. 2006). Furthermore, inflammation and cytokines have been shown to influence dopamine (DA) synthesis through decreasing tetrahydrobiopterin (BH4) availability. BH4 is an important enzyme cofactor for tyrosine hydroxylase (TH) responsible for tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) conversion during dopamine (DA) synthesis (Neurauter et al. 2008). Simultaneously, BH4 is required for arginine to nitric oxide (NO) conversion by nitric oxide synthase (NOS) (Haroon et al. 2012). Cytokines can stimulate NOS activity, and thus increase the utilization of BH4, which results in its deficiency in DA synthesis (Cunnington and Channon 2010).

In addition, inflammatory mediators can negatively affect neurotransmission and neuronal integrity (Felger and Lotrich 2013). As outlined above, inflammation affects many biological processes, such as neurotransmitters metabolism, neuroplasticity, neuroendocrine function and brain activity, all of which are involved in the development and progression of MDD (Fig. 1). Taken together, immune processes can cause depression through at least several mechanisms of action.

3. The relationship between depression and autoimmune disorders

The association between autoimmunity and mood disorders is probably the result of increased inflammatory activity and has been observed for various autoimmune diseases (Siegmann et al. 2018). Such an association has also been found for depression (Chosidow et al. 2010, Kheirandish et al. 2015, Khaibullin et al. 2017, Muscatello et al. 2017, Marrie et al. 2018). Previously, studies focused on the prevalence of mood disorders, especially depression, in patients suffering from ADs; now, there is a growing body of evidence indicating that the association may be bidirectional, and autoimmunity could be considered a potential cause of certain mental disorders (Dowlatshahi et al. 2014). Accordingly, patients suffering from ADs are exposed to a higher risk of depression development (Euesden et al. 2017) induced by brain-reactive antibodies or other inflammatory agents (Katzav et al. 2007, Chen et al. 2009, Diamond et al. 2009). In addition, it was confirmed that depressed patients with severe symptoms had increased levels and

reactivity of autoantibodies (Diamond et al. 2009, Laske et al. 2008). Andersson et al. (2015) found that rheumatoid arthritis, psoriasis vulgaris, systemic lupus erythematosus, ulcerative colitis, Graves' disease, multiple sclerosis, Crohn's disease and type 1 diabetes are the ADs most frequently co-occurring with MDDs. Furthermore, an analysis performed by Benros et al. (2013) established that a greater risk of depression development is associated with autoimmune diseases suggesting that autoimmunity is a significant factor in the pathophysiology of mood disorders. The bidirectional interaction of the immune system and the central nervous system may at least partially explain the relationship between ADs and depression. Although inflammation may be systemic, pro-inflammatory agents such as cytokines can affect the brain causing a cascade of actions in the central nervous system leading to pathologies (Hodes et al. 2014). Peripheral inflammation occurring in autoimmune diseases is able to induce "sickness behavior", i.e. a group of behavioral and physiological changes present during infection development such as fatigue, malaise and anhedonia (Dantzer et al. 2008). It has been revealed that even though inflammation is systemic peripheral cytokines are able to affect and send a signal to the brain through multiple pathways, including neural, cellular and humoral. These mechanisms include: (i) passage of cytokines through leaky regions in the blood brain barrier (BBB); (ii) active transport through BBB via cytokine-specific transport molecules; (iii) activation of the cells (e.g. endothelial cells) responsible for the release of inflammatory mediators (e.g. prostaglandins); (iv) transmission of cytokine signals through binding to specific receptors associated with afferent nerve fibers including the vagus nerve (D'Mello et al. 2009, Dantzer et al. 2008, Miller et al. 2009).

MDD often accompanies many other medical conditions, being related to immune system disruption. In this section, we emphasize the link between depression and autoimmune diseases in the context of anomalous inflammatory activity.

3.1. Multiple sclerosis and inflammation

Multiple sclerosis (MS) is a demyelinating disease with a wide range of clinical manifestations, the etiology of which has not been thoroughly defined so far. Patients with MS are found to have higher prevalence of major depression (Feinstein 2004). Moreover, MS is associated with a predominantly Th1 (T helper cells type I) cytokine pattern, which is characterized by elevated levels of pro-inflammatory cytokines, i.e. TNF tumor necrosis factor α (TNF-α), interleukin (IL-) IL-6, IL-12, Il-23, and decreased levels of anti-inflammatory interleukins – IL-10 (Kato and Suzumara 2003, Kang and Kim 2006, Khaibullin et al. 2017, Palle et al. 2017). Moreover, increased levels of CC and CXC chemokines, which stimulate leukocyte recruitment, i.e. CCL4 (macrophage inflammatory protein 1β), CCL22 (macrophage-derived chemokine) and CXCL10 (interferon-gamma-induced protein 10), have been found, suggesting T cell activation (Khaibullin et al. 2017). Interestingly, altered cytokine profiles in MS patients are similar to those found in depressed patients (Ratchford 2008). Furthermore, mRNA levels of IFN-γ and TNF-α are increased in such patients and both cytokines are correlated significantly with scores on the Beck Depression Inventory (Kahl and Kim 2002).

3.2. Hashimoto's thyroiditis, Grave's disease, Type 1 diabetes and inflammation

Changes in the level of proinflammatory cytokines are also found in other autoimmune diseases such as Hashimoto's thyroiditis, Grave's disease and Type 1 diabetes (T1D). Several studies have confirmed the connection between symptoms of depressive episodes in individuals suffering from T1D (Powers et al. 2016, Muscatello et al. 2017, Trief et al. 2017). Moreover, it has been shown that the autoimmune response in the disease is associated with the upregulation of IL-1, IL-2, IL-12, IL-18 and IL-23/IL-17 (Kikodze et al. 2013, Costa et al. 2010). In

case of Hashimoto's thyroiditis, a dominant immune response is characterized by the increased expression of IL-2, IL-12, IL-18, IL-1β, IL-8, IFN-γ and TNF-α (Phenekos et al. 2004, Lichiardopol and Mota 2009, Konca-Degertekin 2016), while Grave's disease is predominated by the presence of Th2 (T helper cells type II) cytokines, i.e. IL-4, IL-5 and IL-6 (Phenekos et al. 2004).

3.3. Rheumatoid arthritis and inflammation

Rheumatoid arthritis (RA) is an autoimmune inflammatory disorder with common fluctuations in the level of cytokines. RA is a syndrome characterized by stiffness, inflammation and pain leading to articular destruction and functional decline. Furthermore, patients with RA are exposed to a higher risk of depressive disorders than the general population (Mateen et al. 2017, Marrie et al. 2018). Cytokines such as TNF-α, IFN-γ, IL-1, IL-12 play an important role in the pathophysiology of RA. Moreover, studies have confirmed elevated levels of IL-1β, IL-6, IL-17 as well as TNF-α in RA patients (Mateen et al. 2017). Interestingly, several of those cytokines, e.g. TNF-α as well as IL-6, have been linked to depressive disorders (Hodes et al. 2016). Based on this, inflammation may be a potential factor increasing the risk of depression in people with RA.

3.4. Psoriasis vulgaris and inflammation

Psoriasis vulgaris is a chronic, multisystemic, immune-mediated disorder with unknown etiology (Menter et al. 2010). It has been confirmed that pro-inflammatory cytokines are also involved in its pathogenesis and progression. Patients with psoriasis are found to have elevated levels of TNF-α, IL-12, IL-17, IL-23 and IFN-γ (Baliwang et al. 2015). Based on screening studies, depression may affect up to 55% of patients with psoriasis, while the incidence of mood disorders is higher in the case of severe psoriasis compared to its mild form (Chosidow et al. 2010).

3.5. Systemic lupus erythematosus and inflammation

A similar trend can be found in systemic lupus erythematosus (SLE), i.e. a chronic and progressive disorder affecting multiple organs with a wide range of clinical manifestations. In the course of the disease both innate and adaptive immune systems become dysfunctional (Guervitz et al. 2013). Interestingly, the prevalence of depression in the people suffering from SLE is two times higher compared to the general population (Bachen et al., 2009, Kheirandish et al. 2015). Furthermore, it seems that SLE is an autoimmune disease with a prevailing Th2 response. On the other hand, during advanced stages of the disease, an ongoing Th1 response is observed, characterized by elevated levels of IL-12, IL-17 IL-23, IL-18, TNF-α, as well as IFN-γ (Nakashima et al. 2006, Mangini et al. 2007, Mok et al. 2010).

4. Inflammation and response to treatment

4.1. Inflammation and pharmacotherapy

Psychopharmacological approaches to MDDs include many types of therapies. Several antidepressant drugs are available, which provide different mechanisms of action (Table 1). Most of them usually affect the metabolism of monoamines. It is known that some antidepressants have anti-inflammatory and neuroprotective activity, which may partly be attributed to their ability of downregulation of pro-inflammatory cytokine production and upregulation of the production of anti-inflammatory cytokines (Obuchowicz et al. 2014). Interestingly, evidence suggests that insufficient therapeutic benefits of antidepressant treatment may be related to immune system impairment (Carvalho et al. 2013).

The therapeutic effects of antidepressant drugs on the levels of pro-

inflammatory cytokines have been investigated in many studies (Xia et al., 1996, Maes et al. 2005, Obuchowicz et al. 2014). Drugs from the group of tricyclic antidepressants (TCA) as well selective serotonin reuptake inhibitors (SSRI) have anti-inflammatory effects on cytokine secretion. Drugs from both groups, including imipramine and fluoxetine, are able to decrease the levels of IL-6, IL-1β and TNF-α, while imipramine has the additional ability to prevent morphological changes and activate microglia (Obuchowicz et al. 2014). Furthermore, these antidepressants are capable of reducing the levels of IFN-γ. Moreover, the investigated antidepressants increase the secretion of anti-inflammatory cytokines, e.g. IL-4 (Alboni et al. 2013). In addition, a pharmacotherapy based on the application of antidepressant drugs diminishes CRP concentrations (Hiles et al. 2012). On the other hand, mirtazapine, which belongs to the group of third-generation drugs, increases the production of IL-22 (Munzer 2013). There is also evidence that the success rate of the therapy increases when the antidepressants are combined with anti-inflammatory drugs (Akhondzadeh et al., 2009, Hashemian et al., 2011, Abbasi 2012). The prominent example includes nonsteroidal anti-inflammatory drugs (NSAIDs), such as selective cyclooxygenase-2 (COX-2) inhibitors. As mentioned previously, pro-inflammatory molecules can trigger a cascade of inflammatory processes, including induction of cyclooxygenases (COXs) – main enzymes involved in prostaglandin production (Harden et al., 2015). Treatment targeting COX enzymes may have an advantageous result in MDD patients with increased levels of pro-inflammatory cytokines. The patients who received add-on medication of celecoxib (COX-2 inhibitor) showed a significant improvement in scores on the Hamilton Depression Rating Scale (Müller et al., 2006). Moreover, it has been shown that celecoxib reduces serum IL-6 concentration and increases treatment response in MDD patients (Abbasi et al., 2012). Those studies suggest an adjuvant role of NSAIDs in MDD treatment. In addition, there are several other drugs providing an anti-inflammatory mechanism of action which may enhance the effects of antidepressant drugs. Particularly, statins, i.e. a group of lipid-lowering medications, display properties allowing for the reduction of depressive symptoms. One study indicates that the antidepressant effect of fluoxetine increases when this drug is administrated with lovastatin (Ghanizadeh 2013). Similar properties are demonstrated by simvastatin. Simvastatin-treated patients experienced a greater response to antidepressant treatment as well as significantly more pronounced reduction in Hamilton Depression Rating Scale (HDRS) scores (Gougol 2015).

4.2. Impact of inflammatory processes on response to treatment

It has been reported that patients who do not respond to antidepressant treatment are more likely to have higher rates of inflammatory markers compared to the responding ones. Moreover, patients who do not respond to antidepressants exhibit increased inflammatory markers compared to those after a successful therapy (Strawbridge et al. 2015). This was further confirmed when Haroon et al. (2018) reported that a number of failed antidepressant treatment attempts was associated with increased plasma levels of inflammation markers, i.e. TNF-α, IL-6, sTNFR2 and CRP. Furthermore, there are a few studies indicating an association between increased TNF levels in patients and unsuccessful therapy (Eller et al. 2008, Gupta et al. 2016, Strawbridge et al. 2015). CRP is considered to be another candidate for being a marker or risk factor for treatment-resistant depression (Chamberlain et al. 2018, Raison et al. 2013, Haroon 2018). It has been found that a subgroup of patients with such type of depression have increased concentrations of CRP compared to other patients (Chamberlain et al. 2018). Another study showed higher levels of CRP in patients with multiple antidepressant treatment trials (Haroon 2018). Additionally, depressed patients with high rates of inflammatory agents respond better to novel or adjuvant therapies than conventional treatment (Jha et al. 2017, Yang et al. 2015, Shelton et al. 2015). The mentioned evidence proves that non-responsiveness to traditional

Table 1
Classification of antidepressant drugs (National Collaboration Centre for Mental Health 2010).

Antidepressant drugs group	Mechanism	Example
Tricyclic antidepressants (TCAs)	Increasing noradrenergic and serotonergic neurotransmission by inhibiting the reuptake of monoamine neurotransmitters into the pre-synaptic neuron	Amitriptyline, Imipramine, Doxepin, Nortriptyline
Selective serotonin reuptake inhibitors (SSRIs)	Inhibiting the reuptake of serotonin into the presynaptic neuron	Citalopram, Fluoxetine, Fluvoxamine, Paroxetine, Sertaline
Monoamine oxidase inhibitors	Increasing monoamine neurotransmission by binding irreversibly to monoamine oxidase (enzyme that degrades monoamine neurotransmitters e.g. serotonin and NA)	Maclobemide, Phenelzine
'Third-generation' drugs	Variable types of action, including inhibition of the reuptake of monoamines as well as blocking receptors that modulate release of neurotransmitters	Duloxetine, Mirtazapine, Venlafaxine

treatment may be related to inflammation.

4.3. Inflammatory mechanisms affecting response to treatment

Inflammation in the course of depression may influence the effectiveness of treatment with antidepressants via several mechanisms of action (Miller and Raison, 2015). The majority of traditional antidepressant drugs used in the pharmacotherapy of MDD exert a clinical effect by blocking the reuptake of monoamines, e.g. SSRIs inhibit the reuptake of serotonin (National Collaborating Centre for Mental Health 2000). In fact, pro-inflammatory cytokines disrupt this mechanism of action by increasing the expression and, hence, the function of monoamine transporters, mainly those for serotonin. More precisely, TNF and IL-1β activate p38 MAPKs (mitogen-activated protein kinases), which results in an increased activity of monoamine transporters, thus decreasing availability of serotonin in the synapses (Zhu et al. 2010). Inflammatory cytokines have also been shown to reduce neurotransmitter synthesis by modulating the activity of the enzymes involved in monoamine synthesis, including indoleamine 2,3 dioxygenase (IDO) (Capuron et al. 2013). Additionally, inflammatory processes may be associated with disruptions in glutamate metabolism (Haroon et al. 2016). It has been shown that pro-inflammatory proteins, including cytokines, can simultaneously increase the release of glutamate by astrocytes and decrease glutamate reuptake through downregulation of its transporters. In consequence, elevated concentrations of this neurotransmitter may result in a reduction in the brain-derived neurotrophic factor (BDNF), which can lead to neurotoxicity (Haroon et al. 2017).

5. Impact of anti-inflammatory agents on depressive symptoms

As mentioned above, pro-inflammatory cytokines and other immune agents are upregulated in various autoimmune diseases, while their elevated levels may affect response to antidepressant treatment. These facts have allowed putting forward a hypothesis that co-occurrence of autoimmune diseases in the course of depressive disorders may reduce the chance of a positive response to treatment. Moreover, some piece of evidence shows that this relation is bidirectional, and depression may affect the efficacy of treatment in autoimmune diseases. Nonetheless, the administration of several anti-inflammatory and biological agents in AD therapy is positively correlated with decreased depressive symptoms (Uguz et al. 2009, Figueiredo-Braga et al. 2018). The antidepressant properties of anti-cytokine agents have been investigated in recent studies (Table 2).

In case of rheumatoid arthritis, the co-existence of MDD weakens the response to disease-modifying antirheumatic drugs (DMARDs) and glucocorticoid treatment (Matcham et al. 2016). Hider et al. (2009) evaluated the impact of anti-TNF therapy in depressed RA patients on their mood alterations. This study showed that patients with severe depression were more likely to respond worse to a therapy based on cytokine inhibitors. Nevertheless, Braga et al. (2018) investigated the influence of the use of biological therapeutics on depressive symptoms, i.e. Abatacept, Adalimumab, Etanercept, Golimumab, Infliximab,

Rituximab and Tocilizumab; however, the results were inconclusive. Researchers found that several biological drugs used in RA therapy were able to lessen depressive symptoms. It was indicated that using Tocilizumab – an IL-6 inhibitor – in the patients with RA led to an improvement in the symptoms of depression. In addition, Sulfasalazine – a non-biological agent – has a therapeutic impact on symptoms of depression (Figueiredo-Braga et al. 2018). However, other biological medications, i.e. Abatacept, Adalimumab and Golimumab, show neither positive nor negative correlation with depression (Figueiredo-Braga et al. 2018). Another research investigated whether TNF-α antagonists, i.e. Etanercept or Infliximab, could have an impact on the severity of symptoms of depression in patients suffering RA. This further confirms that the use of anti-TNF drugs may lower the prevalence of depression symptoms (Uguz et al. 2009).

According to a growing number of research studies on the interactions between psoriasis and depression, biological therapeutics commonly used in the treatment of this type of AD, i.e. TNF-α inhibitors, IL12/23 blockers and IL-17 blockers, may be potentially used in the therapy of depression (Patel et al. 2017). Data confirmed an almost 50% reduction in depressive disorders in the patients with psoriasis and psoriasis arthritis who were treated with anti-TNF-α biological medications (Wu et al. 2016). According to other research, Etanercept reduced depression symptoms (Tyring et al. 2006). Moreover, as compared to the placebo group, Adalimumab displayed properties in reducing symptoms of depression in the patients suffering from moderate to severe psoriasis (Menter et al. 2010). Another research, conducted by Wu et al. (2016), confirmed the antidepressant effect of anti-TNF-α therapy using Etanercept, Adalimumab or Golimumab in the treatment of psoriasis. The drop of depression prevalence was observed within the first 3 months and continued for the next 24 months (Wu et al. 2016). Furthermore, other cytokine inhibitors, particularly Brodalumab, Secukinumab and Ixekizumab belonging to the group of IL-17 inhibitors, as well as Kriakinumab and Ustekinumab from the group of IL-12/23 inhibitors, also play an important role in depression treatment. Moreover, Ustekinumab has been found to reduce depression symptoms and decrease the risk of depression development by more than 50%, as measured by the Hospital Anxiety and Depression Scale (HADS) (Langley et al. 2010).

Infliximab is also associated with decreased symptoms of depression in patients with Crohn's disease (CD) and ulcerative colitis (UC), which are the principal types of the inflammatory bowel disease (IBD) (Guloksuz et al. 2013, Horst et al. 2015). Guloksuz et al. (2013) reported that Infliximab distribution was positively correlated with a significant reduction in Hamilton Depression Rating Scale (HDRS) and Beck Depression Inventory (BDI) scores. This discovery is in line with a retrospective study of IBD patients. A reduction in the severity of depression symptoms occurred in investigated patients suffering from CD as well as UC treated with Infliximab. Moreover, the probability of development of depressive disorders, measured as a percentage of patients with IBD exposed to the risk of depression, was reduced as a result of treatment based on the administration of Infliximab. In case of CD patients, the risk decreased by about 30% and for UC the risk was reduced by 18% (Horst et al. 2015).

Table 2

Summary of the studies assessing pro-inflammatory agents in the treatment of depressive symptoms.

Drugs	Medical condition	Result	Authors
Tocilizumab	Patients with RA and depressive symptoms	Tocilizumab was associated with decreased depressive symptoms	Figueiredo-Braga et al. 2018
TNF-α antagonists	Patients with RA diagnosed with psychiatric disorders	TNF-α antagonists may have an impact on the severity of depressive symptoms	Uguz et al., 2009
	Patients with psoriasis vulgaris or psoriatic arthritis	Anti-TNF-α reduced symptoms of depressive disorders in patients	Wu et al. 2016
	Patients with inflammatory bowel disease	Anti-TNF-α reduced the risk from moderate to severe	Horst et al. 2015
Adalimumab	Patients with moderate to severe psoriasis vulgaris	Adalimumab displayed properties in reducing depressive symptoms	Menter et al. 2010
Entanercept	Patients with moderate to severe psoriasis and symptoms of depression	Etanercept was found to reduce depression symptoms	Tyring et al. 2006
Ustekinumab	Patients with psoriasis vulgaris	Ustekinumab reduced depression symptoms and decreased the risk of depression development	Langley et al. 2010
Infliximab	Patients with Crohn's disease	Infliximab reduced the severity of depressive symptoms	Guloksuz et al., 2013
	Patients with ankylosing spondylitis	Infliximab may be effective in depression treatment	Ertenli et al., 2012
	Patients with ankylosing spondylitis	Infliximab reduced depressive symptoms	Ersözlü-Bozkırlı et al. 2015
	Patients with treatment-resistant depression	Infliximab reduced levels of inflammatory biomarkers	Raison et al., 2013

A potential role of Infliximab in the treatment of depressive symptoms was suggested in a longitudinal study involving patients with ankylosing spondylitis (Ertenli et al. 2012). TNF-α inhibitor administration was associated with lower BDI scores. Bozkirli et al. (2015) observed similar results in a prospective observational study of patients with AS. A diagnosis of depression, carried out using BDI before and after Infliximab administration, demonstrated an improvement in depressive symptoms after a 12-week period (Ersözlü-Bozkırlı et al. 2015).

There are also studies evaluating inhibitors of cytokines as potential therapeutics in patients with depressive disorders without any accompanying autoimmune disease. Recently published meta-analysis subjected randomized clinical trials (RCTs) studying the effect of anti-inflammatory drugs on patients with MDD and depressive symptoms. It is particularly significant paper, since it is the first study collecting evidence from all RCTs investigating antidepressant treatment effects of anti-inflammatory agents. Authors analyzed 36 RCTs, examined NSAIDs, cytokine-inhibitors, statins, minocycline, pioglitazone and glucocorticoids. It revealed that among investigated RCTs, administration of anti-inflammatory drugs improved antidepressant treatment compared to placebo. Moreover, both NSAID and cytokine-inhibitors monotherapy exerted a better improvement in depressive symptoms than placebo. (Köhler-Forsberg et al. 2019). Similarly, Maes et al. conducted a randomized controlled trial using Infliximab and involving patients with a major depressive disorder (MDD) as well as antidepressant-resistant depression. Despite the fact that the trial was interrupted predominantly, Infliximab exerted some therapeutic effects (Maas et al. 2010). Another research confirmed that TNF-α inhibition did result in a significant change in Hamilton Depression Rating Scale scores in the patients suffering from treatment-resistant depression and receiving Infliximab as compared to the group taking placebo (Raison et al. 2013). Nonetheless, a significant correlation was found between anti-inflammatory treatment and the baseline level of inflammatory biomarkers, namely the patients with elevated CRP concentrations exhibited a superior treatment response (Raisson et al. 2013). Although IL-6 inhibition is associated with a reduction in the severity of depressive symptoms in comorbidities such as RA, there have been no studies to date on the efficacy of IL-6 inhibitors in MDD patients without autoimmune diseases. Several trials of treatment with biological agents in mental disorders have been conducted. For instance, clinical studies investigating the effect of Sirukumab (NCT02473289) and Tocilizumab (NCT02660528) have been completed recently; however, the results have not been published yet.

There is also an extensive number of RCTs investigating the role of Omega-3 polyunsaturated fatty acids (PUFAs) in depression, which were the subject of several precise meta-analyses. These meta-analyses confirmed that supplementation with both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) showed beneficial results in MDD.

(Grosso et al., 2014, Hallahanet al., 2016, Lin et al., 2012, Lin and Su, 2007, Martins, 2009, Mocking et al., 2016, Sublette et al., 2011). Moreover, both DHA and EPA were well-tolerated by patients (Su et al., 2014) and significantly associated with decreased severity of MDD (Su et al., 2018). Interestingly, it is suggested that EPA, which is considered as one of the major anti-inflammatory nutraceutical agents, exerts better effect than DHA (Su, 2009, 2012, Su, 2015, Su et al., 2014, Su et al., 2018).

5.1. Depressive disorders and multiple sclerosis

Although depression and multiple sclerosis often coexist, there are very few studies examining MDD treatment in patients suffering from MS, being mainly based on the administration of antidepressant drugs. Although a high percentage of individuals were treated successfully (Patten 2009), depressive symptoms still persisted in some patients (Raissi et al. 2015). Interestingly, it has been found that sertraline treatment effectively reduces depression when measured with BDI, but not with HDRS (Mohr et al., 2001a, b). Similarly, paroxetine does not result in a significant improvement of the HDRS score as compared to placebo (Ehde et al. 2008). Another study conducted by Mohr et al. (2001a, b) provides data suggesting that decreased depressive symptoms in MS during treatment with Sertaline are associated with reduced levels of IFN-γ expression, measured with the use of the enzyme-linked immunosorbent assay (Mohr et al., 2001a, b). According to the American Academy of Neurology, the amount of evidence is insufficient to either refute or support the hypothesis that antidepressant therapy is effective in MS patients with depression (Minden et al. 2014). Even though there is no study indicating that a poorer response to treatment in patients with MS is associated with inflammation, inflammatory agents including cytokines may be a potential cause of this. As mentioned previously, inflammatory processes may contribute to decreased antidepressant efficacy. There is a large body of evidence indicating that the non-responsiveness is associated with higher rates of inflammatory markers (Strawbridge et al. 2015, Haroon et al. 2018), which allows us to suppose that elevated levels of cytokines, i.e. TNF-α, IL-6, IL-12 and Il-23, in MS patients may be factors influencing antidepressant therapy.

6. Future perspectives

The data collected in this review have shown some promising information. Although autoimmune diseases and depression retain their own individual sets of inflammatory cytokines in their pathogenesis and course of the disease, many alterations in cytokines can be found in both disorders. The research studies evaluating therapy with cytokine inhibitors in patients with depressive disorders without any

autoimmune comorbidities are limited. Nonetheless, those studies provide data regarding a potential antidepressant role of cytokine blockers. The antidepressant efficacy of these biological agents has been confirmed mainly in the studies that evaluated reduction in the severity of depressive symptoms assessed as a secondary outcome in auto-immune diseases. A disruption of the immune system in depressive disorders is clearly dissimilar to any other inflammatory condition. In fact, there is a limited number of available cytokine inhibitors that could be used as pro-inflammatory cytokine blockers in the course of depression. Furthermore, some patients respond to antidepressant therapy, whereas many individuals are more likely to be non-responders. Thus, the heterogeneity of MDD patients may be another limitation of using this type of treatment. It is known that prolonged activation of the immune system and chronically persistent inflammation are strongly associated with the development and progression of MDD (Haapakoski et al. 2015). Furthermore, an elevation of pro-inflammatory agents may affect response to antidepressant treatment. Thus, targeting inflammatory pathways, particularly in proinflammatory cytokines, may be promising for the treatment of the disease. Therefore, there is a strong need for future research in this field, which may contribute to the development of novel and personalized therapeutic methods.

7. Conclusion

The objective of this review was to evaluate the relationship between an altered immune response in both depressive disorders and autoimmune diseases, and a response to treatment in depression accompanied by autoimmune disorders. Another purpose of this study was to assess the efficacy of cytokine inhibitors in reducing the severity of depressive symptoms in AD patients suffering from MDDs. The factors contributing to the coexistence of depression as well as the ones affecting antidepressant treatment effectiveness may lead to an alteration of cytokine profiles in many autoimmune diseases. Furthermore, inhibition of pro-inflammatory cytokines may serve as a helpful therapeutic approach in depression in the case of lack of response to conventional and traditional treatment options.

Acknowledgements

References

Abbasi, S.H., Hosseini, F., Modabbernia, A., Ashrafi, M., Akhondzadeh, S., 2012. Effect of celecoxib add on treatment on symptoms and serum IL-6 concentrations in patients with major depressive disorder: randomized double-blind placebo-controlled study. J Affect Disord. 10;141 (2–3), 308–314.

Akhondzadeh, S., Jafari, S., Raisi, F., Nasehi, A.A., Ghoreishi, A., Salehi, B., Mohebbi-Rasa, S., Raznahan, M., Kamalipour, A., 2009. Clinical trial of adjunctive celecoxib treatment in patients with major depression: a double blind and placebo controlled trial. Depress Anxiety. 26 (7), 607–611.

Alboni, S., Benatti, C., Montanari, C., Tascedda, F., Brunello, N., 2013. Chronic anti-depressant treatments resulted in altered expression of genes involved in inflammation in the rat hypothalamus. Eur J Pharmacol. 5 (721(1–3)), 158–167.

Alcocer-Gómez, E., de Miguel, M., Casas-Barquero, N., Núñez-Vasco, J., Sánchez-Alcazar, J.A., Fernández-Rodríguez, A., Cordero, M.D., 2014. NLRP3 inflammasome is activated in mononuclear blood cells from patients with major depressive disorder. Brain Behav. Immun. 36, 111–117.

Al-Harbi, K.S., 2012. Treatment-resistant depression: therapeutic trends, challenges, and future directions. Patient Prefer Adherence. 6, 369–388.

Anaya, J.M., 2012. Common mechanisms of autoimmune diseases (the autoimmune tautology). Autoimmunity Reviews 11, 781–784.

Andersson, N.W., Gustafsson, L.N., Okkels, N., Taha, F., Cole, S.W., Munk-Jørgensen, P., Goodwin, R.D., 2015. Depression and the risk of autoimmune disease: a nationally representative, prospective longitudinal study. Psychol. Med. 45 (16), 3559–3569.

Bachen, E.A., Chesney, M.A., Criswell, L.A., 2009. Prevalence of mood and anxiety disorders in women with systemic lupus erythematosus. Arthritis Rheum. 61 (6), 822–829.

Bachmann, S., 2018. Epidemiology of suicide and the psychiatric perspective. Int. J. Environ. Res. Public Health 15 (7), 1425.

Baliwag, J., Barnes, D.H., Johnston, A., 2015. Cytokines in psoriasis. Cytokine. 73, 342–350.

Benros, M.E., Waltoft, B.L., Nordentoft, M., Ostergaard, S.D., Eaton, W.W., Krogh, J., Mortensen, P.B., 2013. Autoimmune diseases and severe infections as risk factors for mood disorders: a nationwide study. JAMA Psychiatry. 70 (8), 812–820.

Kessler, R.C., Bromet, E.J., 2013. The epidemiology of depression across cultures. Annu. Rev. Public Health 34, 119–138.

Capuron, L., Neurauter, G., Musselman, D.L., Lawson, D.H., Nemeroff, C.B., Fuchs, D., Miller, A.H., 2013. Interferon-alpha-induced changes in tryptophan metabolism. relationship to depression and paroxetine treatment. Biol. Psychiatry 54, 906–914.

Carvalho, L.A., Torre, J.P., Papadopoulos, A.S., Poon, L., Juruena, M.F., Markopoulou, K., Cleare, A.J., Pariante, C.M., 2013 May 15. Lack of clinical therapeutic benefit of antidepressants is associated overall activation of the inflammatory system. J. Affect. Disord. 148 (1), 136–140.

Chamberlain, S.R., Cavanagh, J., de Boer, P., Mondelli, V., Jones, D.N.C., Drevets, W.C., Cowen, P.J., Harrison, N.A., Pointon, L., Pariante, C.M., Bullmore, E.T., 2018. Treatment-resistant depression and peripheral C-reactive protein. Br. J. Psychiatry 16, 1–9.

Chen, P., Jiang, T., Ouyang, J., Chen, Y., 2009. Depression, another autoimmune disease from the view of autoantibodies. Med. Hypotheses 73 (4), 508–509.

Chosidow, O., Dellavalle, R.P., Do, D., et al., 2010. The risk of depression, anxiety, and suicidality in patients with psoriasis. Arch. Dermatol. 146, 891–895.

Costa, V.S., Mattana, T.C., da Silva, M.E., 2010. Unregulated IL-23/IL-17 immune response in autoimmune diseases. Diabetes Res. Clin. Pract. 88 (3), 222–226.

Cunnington, C., Channon, K.M., 2010. Tetrahydrobiopterin: pleiotropic roles in cardiovascular pathophysiology. Heart 96, 1872–1877.

Dantzer, R., O'Connor, J.C., Freund, G.G., et al., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat. Rev. Neurosci. 9 (1), 46–56.

de Kloet, E.R., Joels, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 6, 463–475.

Diamond, B., Huerta, P.T., Mina-Osorio, P., Kowal, C., Volpe, B.T., 2009. Losing your nerves? Maybe it's the antibodies. Nat Rev Immunol. 9 (6), 449–456.

DiLuca, M., Olesen, J., 2014. The cost of brain diseases: a burden or a challenge? Neuron 18;82 (6), 1205–1208.

D'Mello, C., Le, T., Swain, M.G., 2009. Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factoralpha signaling during peripheral organ inflammation. J. Neurosci. 29, 2089–2102.

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K., Lanctôt, K.L., 2010. A metaanalysis of cytokines in major depression. Biol. Psychiatry 67 (5), 446–457.

Dowlatshahi, E.A., Wakkee, M., Arends, L.R., Nijsten, T., 2014. The prevalence and odds of depressive symptoms and clinical depression in psoriasis patients: a systematic review and meta-analysis. J. Investig. Dermatol. 134, 1542–1551.

Ehde, D.M., Kraft, G.H., Chwastiak, L., Sullivan, M.D., Gibbons, L.E., Bombardier, C.H., Wadhwani, R., 2008. Efficacy of paroxetine in treating major depressive disorder in persons with multiple sclerosis. Gen. Hosp. Psychiatry 30 (1), 40–48.

Eller, T., Vasar, V., Shlik, J., Maron, E., 2008. Pro-inflammatory cytokines and treatment response to escitalopram in major depressive disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 32, 445–450.

Ersözlü-Bozkırlı, E.D., Keşkek, S.O., Bozkırlı, E., Yücel, A.E., 2015. The effect of infliximab on depressive symptoms in patients with ankylosing spondylitis. Acta Reumatol. Port. 40 (3), 262–267.

Ertenli, I., Ozer, S., Kiraz, S., Apras, S.B., Akdogan, A., Karadag, O., Calguneri, M., Kalyoncu, U., 2012. Infliximab, a TNF-α antagonist treatment in patients with ankylosing spondylitis: the impact on depression, anxiety and quality of life level. Rheumatol Int. 32 (2), 323–330.

Euesden, J., Danese, A., Lewis, C.M., Maughan, B., 2017. A bidirectional relationship between depression and the autoimmune disorders - New perspectives from the National Child Development Study. PLoS One 6;12 (3) e0173015.

Feinstein, A., 2004. The neuropsychiatry of multiple sclerosis. Can. J. Psychiatr. 49 (3), 157–163.

Felger, J.C., Lotrich, F.E., 2013. Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. Neuroscience. 29 (246), 199–229.

Figueiredo-Braga, M., Cornaby, C., Cortez, A., Bernardes, M., Terroso, G., Figueiredo, M., CDS, Mesquita, Costa, L., Poole, B.D., 2018. Influence of Biological Therapeutics, Cytokines, and Disease Activity on Depression in Rheumatoid Arthritis. J Immunol Res 2018, 1–9 5954897.

Fujigaki, H., Saito, K., Fujigaki, S., Takemura, M., Sudo, K., Ishiguro, H., Seishima, M., 2006. The signal transducer and activator of transcription 1alpha and interferon regulatory factor 1 are not essential for the induction of indoleamine 2,3-dioxygenase by lipopolysaccharide: involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB pathways, and synergistic effect of several proinflammatory cytokines. J. Biochem. 139 (4), 655–662.

Ghanizadeh, A., Hedayati, A., 2013. Augmentation of fluoxetine with lovastatin for treating major depressive disorder, a randomized double-blind placebo controlled-clinical trial. Depress Anxiety. 30 (11), 1084–1088.

Gougol, A., Zareh-Mohammadi, N., Raheb, S., Farokhnia, M., Salimi, S., Iranpour, N., Yekehtaz, H., Akhondzadeh, S., 2015. Simvastatin as an adjuvant therapy to fluoxetine in patients with moderate to severe major depression: a double-blind placebo-controlled trial. J. Psychopharmacol. 29 (5), 575–581.

Grosso, G., Pajak, A., Marventano, S., Castellano, S., Galvano, F., Bucolo, C., Drago, F., Caraci, F., 2014. Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials. PLoS One 9, e96905.

Guillemin, G.J., Smythe, G., Takikawa, O., Brew, B.J., 2005. Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes,

and neurons. Glia 1;49 (1), 15–23.

Guloksuz, S., Wichers, M., Kenis, G., Russel, M.G., Wauters, A., Verkerk, R., Arts, B., van Os, J., 2013 Mar 27. Depressive symptoms in crohn's disease: relationship with immune activation and tryptophan availability. PLoS ONE 8 (3) e60435.

Gupta, R., Gupta, K., Tripathi, A., Bhatia, M., Gupta, L., 2016. Effect of mirtazapine treatment on serum levels of brain-derived neurotrophic factor and tumor necrosis factor alpha in patients of major depressive disorder with severe depression. Pharmacology 97, 184–188.

Gurevitz, S.L., et al., 2013. Systemic lupus erythematosus: a review of the disease and treatment options. Consult. Pharm. 28 (2), 110–121.

Haapakoski, R., Mathieu, J., Ebmeier, K.P., Alenius, H., Kivimäki, M., 2015. Cumulative meta-analysis of interleukins 6 and 1β, tumour necrosis factor α and C-reactive protein in patients with major depressive disorder. Brain Behavior and Immunity. 49, 206–215.

Hallahan, B., Ryan, T., Hibbeln, J.R., Murray, I.T., Glynn, S., Ramsden, C.E., SanGiovanni, J.P., Davis, J.M., 2016. Efficacy of omega-3 highly unsaturated fatty acids in the treatment of depression. Br. J. Psychiatry 209 192–20.

Handley, T., Rich, J., Davies, K., Lewin, T., Kelly, B., 2018. The challenges of predicting suicidal thoughts and Behaviours in a sample of rural Australians with depression. Int. J. Environ. Res. Public Health 15 (5), 928.

Harden, L.M., Kent, S., Pittman, Q.J., Roth, J., 2015 Nov Nov. Fever and sickness behavior: Friend or foe? Brain Behav Immun 50, 322–333.

Haroon, E., Fleischer, C.C., Felger, J.C., Chen, X., Woolwine, B.J., Patel, T., Hu, X.P., Miller, A.H., 2016. Conceptual convergence: increased inflammation is associated with increase basal ganglia glutamate in patients with major depression. Mol. Psychiatry 21, 1351–1357.

Haroon, E., Raison, C.L., Miller, A.H., 2012. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuropsychopharmacology. 37 (1), 137–162.

Haroon, E., Miller, A.H., Sanacora, G., 2017. Inflammation, glutamate, and glia: a trio of trouble in mood disorders. Neuropsychopharmacology. 42, 193–215.

Haroon, E., Daguanno, A.W., Woolwine, B.J., Goldsmith, D.R., Baer, W.M., Wommack, E.C., Felger, J.C., Miller, A.H., 2018. Antidepressant treatment resistance is associated with increased inflammatory markers in patients with major depressive disorder. Psychoneuroendocrinology. 95, 43–49.

Hashemian, F., Majd, M., Hosseini, S.M., Sharifi, A., Panahi, M.V., Bigdeli, O., 2011. A randomized, double-blind, placebo-controlled trial of celecoxib augmentation of sertraline in the treatment of a drug-naive women with major depression. Klin Psikofarmakol Bul 21, 183–184.

Hider, S.L., Tanveer, W., Brownfield, A., Mattey, D.L., Packham, J.C., 2009. Depression in RA patients treated with anti-TNF is common and under-recognized in the rheumatology clinic. Rheumatology (Oxford) 48 (9), 1152–1154.

Hiles, S.A., Baker, A.L., de Malmanche, T., Attia, J., 2012. A meta-analysis of differences in IL-6 and IL-10 between people with and without depression: exploring the causes of heterogeneity. Brain Behav. Immun. 26, 1180–1188.

Hodes, G.E., Pfau, M.L., Leboeuf, M., Golden, S.A., Christoffel, D.J., Bregman, D., Rebusi, N., Heshmati, M., Aleyasin, H., Warren, B.L., Lebonté, B., Horn, S., Lapidus, K.A., Stelzhammer, V., Wong, E.H., Bahn, S., Krishnan, V., Bolaños-Guzman, C.A., Murrough, J.W., Merad, M., Russo, S.J., 2014. Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. Proc. Natl. Acad. Sci. U. S. A. 111, 16136–16141.

Hodes, G.E., Ménard, C., Russo, S., 2016. Integrating interleukin-6 into depression diagnosis and treatment. Neurobiology of Stress 4, 15–22.

Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology. 23, 477–501.

Horst, S., Chao, A., Rosen, M., Nohl, A., Duley, C., Wagnon, J.H., Beaulieu, D.B., Taylor, W., Gaines, L., Schwartz, D.A., 2015. Treatment with immunosuppressive therapy may improve depressive symptoms in patients with inflammatory bowel disease. Dig. Dis. Sci. 60 (2), 465–470.

http://www.who.int/news-room/fact-sheets/detail/depression.

Jha, M.K., Minhajuddin, A., Gadad, B.S., Trivedi, M.H., 2017. Platelet-derived growth factor as an antidepressant treatment selection biomarker: higher levels selectively predict better outcomes with bupropion-SSRI combination. Int J Neuropsychopharmacol. 1;20 (11), 919–927.

Jiang, Z., Jiang, J.X., Zhang, G.X., 2014. Macrophages: a double-edged sword in experimental autoimmune encephalomyelitis. Immunol. Lett. 160 (1), 17–22.

Kahl, K.G., Kruse, N., Faller, H., Weiss, H., Rieckmann, P., 2002. Expression of tumor necrosis factor-alpha and interferon-gamma mRNA in blood cells correlates with depression scores during an acute attack in patients with multiple sclerosis. Psychoneuroendocrinology. 27 (6), 671–681.

Kang, B.Y., Kim, T.S., 2006. Targeting cytokines of the interleukin-12 family in autoimmunity. Curr. Med. Chem. 13 (10), 1149–1156.

Kato, H., Suzumura, A., 2003. Cytokines in MS lesion. Nippon Rinsho 61 (8), 1428–1434.

Katzav, A., Solodeev, I., Brodsky, O., et al., 2007. Induction of autoimmune depression in mice by anti-ribosomal P antibodies via the limbic system. Arthritis Rheum. 56 (3), 938–948.

Khaibullin, T., Ivanova, V., Martynova, E., Cherepnev, G., Khabirov, F., Granatov, E., Rizvanov, A., Khaiboullina, S., 2017. Elevated Levels of Proinflammatory Cytokines in Cerebrospinal Fluid of Multiple Sclerosis Patients. Front Immunol 18 (8), 531.

Kheirandish, M., Faezi, S.T., Paragomi, P., Akhlaghi, M., Gharibdoost, F., Shahali, A., 2015. Prevalence and severity of depression and anxiety in patients with systemic lupus erythematosus: an epidemiologic study in Iranian patients. Mod. Rheumatol. 25 (3), 405–409.

Kikodze, N., Pantsulaia, I., Rekhviashvili, Kh., Iobadze, M., Dzhakhutashvili, N., Pantsulaia, N., Kukuladze, N., Bikashvili, N., Metreveli, D., Chikovani, T., 2013. Cytokines and T regulatory cells in the pathogenesis of type 1 diabetes. Georgian Med. News 222, 29–35.

Köhler-Forsberg, O., 2019. N Lydholm C, Hjorthøj C, Nordentoft M, Mors O, Benros ME. Efficacy of anti-inflammatory treatment on major depressive disorder or depressive symptoms: meta-analysis of clinical trials. Acta Psychiatr. Scand. 139 (5), 404–419.

Konca Degertekin, C., Aktas Yilmaz, B., Balos Toruner, F., Kalkanci, A., Turhan Iyidir, O., Fidan, I., Yesilyurt, E., Cakır, N., Kustimur, S., Arslan, M., 2016. Circulating Th17 cytokine levels are altered in Hashimoto's thyroiditis. Cytokine. 80, 13–17.

Langley, R.G., Feldman, S.R., Han, C., et al., 2010. Ustekinumab significantly improves symptoms of anxiety, depression, and skin-Psoriasis, Depression, and Inflammation Overlap related quality of life in patients with moderate-to-severe psoriasis:results from a randomized, double-blind, placebo-controlled phase III trial. J. Am. Acad. Dermatol. 63, 457–465.

Laske, C., Zank, M., Klein, R., et al., 2008. Autoantibody reactivity in serum of patients with major depression, schizophrenia and healthy controls. Psychiatry Res. 158 (1), 83–86.

Lichiardopol, C., Mota, M., 2009. The thyroid and autoimmunity. Rom. J. Intern. Med. 47 (3), 207–215.

Lin, P.Y., Chiu, C.C., Huang, S.Y., Su, K.P., 2012. a meta-analytic review of polyunsaturated fatty acid compositions in dementia. J Clin Psychiatry 73, 1245–1254.

Lin, P.Y., Su, K.P., 2007. A meta-analytic review of double-blind, placebo-controlled trials of antidepressant efficacy of omega-3 fatty acids. J Clin.Psychiatry 68, 1056–1061.

Lotrich, F., 2012. Inflammatory cytokines, growth factors, and depression. Curr. Pharm. Des. 18 (36), 5920–5935.

Maas DW, Westendorp RG, Willems JM, de Craen AJ, van der Mast RC. TNF-α antagonist infliximab in the treatment of depression in older adults: results of a prematurely ended,randomized, placebo-controlled trial. J. Clin. Psychopharmacol. 2010;30(3):343–5.

Maes, M., Kenis, G., Kubera, M., De Baets, M., Steinbusch, H., Bosmans, E., 2005. The negative immunoregulatory effects of fluoxetine in relation to the cAMP-dependent PKA pathway. Int. Immunopharmacol. 5 (3), 609–618.

Mangini, A.J., Lafyatis, R., Van Seventer, J.M., 2007. Type I interferons inhibition of inflammatory T helper cell responses in systemic lupus erythematosus. Ann. N. Y. Acad. Sci. 1108, 11–23.

Marcus, M.M., Yasamy, T., Ommeren, M., Chisholm, D., Saxena, S., 2012. Depression: A global public health concern. 1. pp. 6–8.

Marrie, R., Hitchon, C., Walld, R., et al., 2018. Increased burden of psychiatric disorders in rheumatoid arthritis. Arthritis Care & Research 7 (70), 970–978.

Martins, J.G., 2009. EPA but not DHA appears to be responsible for the efficacy of omega-3 long chain polyunsaturated fatty acid supplementation in depression: evidence from a meta-analysis of randomized controlled trials. J.Am.Coll.Nutr. 28, 525–542.

Matcham, F., Norton, S., Scott, D.L., Steer, S., Hotopf, M., 2016. Symptoms of depression and anxiety predict treatment response and long-term physical health outcomes in rheumatoid arthritis: secondary analysis of a randomized controlled trial. Rheumatology 55, 268–278.

Mateen, S., Moin, S., Shahzad, S., Khan, A.Q., 2017. Level of inflammatory cytokines in rheumatoid arthritis patients: correlation with 25-hydroxy vitamin D and reactive oxygen species. PLoS One 12 (6), e0178879.

McKay, L.I., Cidlowski, J.A., Physiologic and pharmacologic effects of corticosteroids. In: Kufe, D.W., Pollock, R.E., Weichselbaum, R.R., Bast, R.C., Gansler, T.S., Holland, J.F., Frei, E. (Eds.), Holland-Frei Cancer Medicine, 2003. 6th Edn. B.C. Decker, Hamilton, ON.

Menter, A., Augustin, M., Signorovitch, J., Yu, A.P., Wu, E.Q., Gupta, S.R., Bao, Y., Mulani, P., 2010. The effect of adalimumab on reducing depression symptoms in patients with moderate to severe psoriasis: a randomized clinical trial. J. Am. Acad. Dermatol. 62 (5), 812–818.

Miller, A.H., Raison, C.L., 2015. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol 16 22 ± 34.

Miller, A.H., Pariante, C.M., Pearce, B.D., 1999. Effects of cytokines on glucocorticoid receptor expression and function. Glucocorticoid resistance and relevance to depression.Adv. Exp. med. Biol. 461, 107–116.

Miller, A.H., Maletic, V., Raison, C.L., 2009. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol. Psychiatry 65, 732–741.

Minden, S.L., Feinstein, A., Kalb, R.C., Miller, D., Mohr, D.C., Patten, S.B., Bever Jr., C., Schiffer, R.B., Gronseth, G.S., Narayanaswami, P., 2014. Guideline Development Subcommittee of the American Academy of Neurology. Evidence-based guideline: assessment and management of psychiatric disorders in individuals with MS: report of the Guideline Development Subcommittee of the American Academy of Neurology. Neurology. 82 (2), 174–181.

Mocking, R.J., Harmsen, I., Assies, J., Koeter, M.W., Ruhe, H.G., Schene, A.H., 2016. Meta-analysis and meta-regression of omega-3 polyunsaturated fatty acid supplementation for major depressive disorder. Transl. Psychiatry 6, e756.

Mohr, D.C., Goodkin, D.E., Islar, J., Hauser, S.L., Genain, C.P., 2001a. Treatment of depression is associated with suppression of nonspecific and antigen-specific T(H)1 responses in multiple sclerosis. Arch. Neurol. 58 (7), 1081–1086.

Mohr, D.C., Boudewyn, A.C., Goodkin, D.E., Bostrom, A., Epstein, L., 2001b. Comparative outcomes for individual cognitive-behavior therapy, supportive-expressive group psychotherapy, and sertraline for the treatment of depression in multiple sclerosis. J.Consult. Clin. Psychol. 69 (6), 942–949.

Mok, M.Y., Wu, H.J., Lo, Y., Lau, C.S., 2010. The relation of interleukin 17 (IL-17) and IL-23 to Th1/Th2 cytokines and disease activity in systemic lupus erythematosus. J. Rheumatol. 37 (10), 2046–2052.

Müller, N., Schwarz, M.J., Dehning, S., Douhe, A., Cerovecki, A., Goldstein-Müller, B., Spellmann, I. Hetzel, Maino, G., Kleindienst, K., Möller, N., Arolt, H.J., Riedel, V.M., 2006. The cyclooxygenase-2 inhibitor celecoxib has therapeutic effects in major depression: results of a double-blind, randomized, placebo controlled, add-on pilot study to reboxetine. Mol. Psychiatry 11 (7), 680–684.

Muscatello, M.R., Troili, G.M., Pandolfo, G., Mento, C., Gallo, G., Lanza, G., Pintaudi, B., Di Vieste, G., Di Benedetto, A., Zoccali, R.A., Bruno, A., 2017. Depression, anxiety and anger in patients with type 1 diabetes mellitus. Recenti Prog. Med. 108 (2), 77–82.

Nakashima, H., Akahoshi, M., Masutani, K., 2006. Th1/Th2 balance of SLE patients with lupus nephritis. Rinsho Byori 54 (7), 706–713.

National Collaborating Centre for Mental Health (UK), 2000. Depression: The Treatment and Management of Depression in Adults (Updated Edition). British Psychological Society.

Neurauter, G., Schrocksnadel, K., Scholl-Burgi, S., Sperner-Unterweger, B., Schubert, C., Ledochowski, M., et al., 2008. Chronic immune stimulation correlates with reduced phenylalanine turnover. Curr. Drug Metab. 9, 622–627.

Obuchowicz, E., Bielecka, A.M., Paul-Samojedny, M., 2014. Imipramine and fluoxetine inhibit LPS-induced activation and affect morphology of microglial cells in the rat glial culture. Pharmacol. Rep. 66, 34–43.

Pace, T.W., Hu, F., Miller, A.H., 2007 Jan. Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. Brain Behav. Immun. 21 (1), 9–19.

Palle, P., Monaghan, K.L., Milne, S.M., Wan, E.C.K., 2017. Cytokine signaling in multiple sclerosis and its therapeutic applications. Med Sci. 13, 5(4).

Pariante, C.M., Miller, A.H., 2001. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biol Psychiatry 1;49 (5), 391–404.

Pariante, C.M., Pearce, B.D., Pisell, T.L., Sanchez, C.I., Po, C., Su, C., Miller, A.H., 1999. The proinflammatory cytokine, interleukin-1alpha, reduces glucocorticoid receptor translocation and function. Endocrinology. 140 (9), 4359–4366.

Patel, N., Nadkarni, A., Cardwell, L.A., Vera, N., Frey, C., Patel, N., Feldman, S.R., 2017 Oct. Psoriasis, depression, and inflammatory overlap: a review. Am. J. Clin. Dermatol. 18 (5), 613–620.

Patten, S.B., 2009. Antidepressant treatment for major depression in multiple sclerosis: the evolving efficacy literature. Int. J. MS Care. 11, 174–179.

Phenekos, C., Vryonidou, A., Gritzapis, A.D., Baxevanis, C.N., Goula, M., Papamichail, M., 2004. Th1 and Th2 serum cytokine profiles characterize patients with Hashimoto's thyroiditis (Th1) and Graves' disease (Th2). Neuroimmunomodulation 11 (4), 209–213.

Poniatowska-Leszczyńska, K., Małyszczak, K., 2013. Depresja a patologia osobowości w ujęciu psychodynamicznym. Postępy Psychiatrii i Neurologii. 22, 201–209.

Powers, M., Richter, S., Ackard, D., Craft, Ch., 2016. Diabetes distress among persons with type 1 diabetes. Americian Association of Diabetes Education. 43, 105–113.

Raison, C.L., Capuron, L., Miller, A.H., 2006. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol. 27 (1), 24–31.

Raison, C.L., Borisov, A.S., Majer, M., Drake, D.F., Pagnoni, G., Woolwine, B.J., Vogt, G.J., Massung, B., Miller, A.H., 2009. Activation of central nervous system inflammatory pathways by interferon-alpha: relationship to monoamines and depression. Biol Psychiatry 15;65 (4), 296–303.

Raison, C.L., Rutherford, R.E., Woolwine, B.J., Shuo, C., Schettler, P., Drake, D.F., Haroon, E., Miller, A.H., 2013. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory Biomarkers. JAMA Psychiatry 70 (1), 31–41.

Raissi, A., Bulloch, A.G., Fiest, K.M., McDonald, K., Jette, N., Patten, S.B., 2015. Exploration of undertreatment and patterns of treatment of depression in multiple sclerosis. Int. J. MS Care. 17 (6), 292–300.

Ratchford, J., 2008. The immunology of multiple sclerosis. The Neurology Report. 2, 5–11.

Seedat, S., Scott, K.M., Angermeyer, M.C., Berglund, P., Bromet, E.J., Brugha, T.S., Demyttenaere, K., de Girolamo, G., Haro, J.M., Jin, R., Karam, E.G., Kovess-Masfety, V., Levinson, D., Medina Mora, M.E., Ono, Y., Ormel, J., Pennell, B.E., Posada-Villa, J., Sampson, N.A., Williams, D., Kessler, R.C., 2009. Cross-national associations between gender and mental disorders in the World Health Organization World Mental Health Surveys. Arch. Gen. Psychiatry 66 (7), 785–795.

Shelton, R.C., Falola, M., Li, L., Zajecka, J., Fava, M., Papakostas, G.I., 2015. The pro-inflammatory profile of depressed patients is (partly) related to obesity. J. Psychiatr. Res. 70, 91–97.

Siegmann, E.M., Müller, H.H.O., Luecke, C., Philipsen, A., Kornhuber, J., Grömer, T.W., 2018 Jun 1. Association of Depression and Anxiety Disorders with Autoimmune Thyroiditis: a systematic review and Meta-analysis. JAMA Psychiatry. 75 (6), 577–584.

Sokol, C.L., Luster, A.D., 2015. The chemokine system in innate immunity. Cold Spring Harb. Perspect. Biol. 7 (5).

Strawbridge, R., Arnone, D., Danese, A., Papadopoulos, A., Herane Vives, A., Cleare, A.J., 2015. Inflammation and clinical response to treatment in depression: a meta-analysis. Eur. Neuropsychopharmacol. 25, 1532–1543.

Su, K.P., 2009. Biological mechanism of antidepressant effect of Omega-3 fatty acids: how does fish oil act as a 'Mind-body Interface'? Neurosignals 17, 144–152.

Su, K.P., 2012. Inflammation in psychopathology of depression: clinical, biological, and therapeutic implications. BioMedicine 2, 68–74.

Su, K., Nutrition, P., 2015. Psychoneuroimmunology and depression: the therapeutic implications of omega-3 fatty acids in interferon-alpha-induced depression. Biomedicine (Taipei) 5, 21.

Su, K.P., Lai, H.C., Yang, H.T., Su, W.P., Peng, C.Y., Chang, J.P., Chang, H.C., Pariante, C.M., 2014. Omega-3 fatty acids in the prevention of interferon-alpha-induced depression: results from a randomized, controlled trial. Biol. Psychiatry 76, 559–566.

Su, K.P., Yang, H.T., Chang, J.P., Shih, Y.H., Guu, T.W., Kumaran, S.S., Gałecki, P., Walczewska, A., Pariante, C.M., 2018. Eicosapentaenoic and docosahexaenoic acids have different effects on peripheral phospholipase A2 gene expressions in acute depressed patients. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 80, 227–233.

Sublette, M.E., Ellis, S.P., Geant, A.L., Mann, J.J., 2011. Meta-analysis of the effects of eicosapentaenoic acid (EPA) in clinical trials in depression. J Clin Psychiatry 72, 1577–1584.

Sullivan, P.F., Neale, M.C., Kendler, K.S., 2000. Genetic epidemiology of major depression: review and meta-analysis. Am. J. Psychiatry 157 (10), 1552–1562.

Trief, P.M., Jiang, Y., Beck, R., Huckfeldt, P.J., Knight, T., Miller, K.M., Weinstock, R.S., 2017. Adults with type 1 diabetes: partner relationships and outcomes. J. Health Psychol. 22 (4), 446–456.

Tyring, S., Gottlieb, A., Papp, K., et al., 2006. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. Lancet. 367 (9504), 29–35.

Uguz, F., Akman, C., Kucuksarac, S., Tufekci, O., 2009. Anti-tumor necrosis factor-alpha therapy is associated with less frequent mood and anxiety disorders in patients with rheumatoid arthritis. Psychiatry Clin. Neurosci. 63 (1), 50–55.

Wu, C.-Y., Chang, Y.-T., Juan, C.-K., et al., 2016. Depression and insomnia in patients with psoriasis and psoriatic arthritis taking tumor necrosis factor antagonists. Medicine 95, e3816.

Xia, Z., DePierre, J.W., Nässberger, L., 1996. Tricyclic antidepressants inhibit IL-6, IL-1beta and TNF-alfa release in human blood monocytes and IL-2 and interferon-gamma in T cells. Immunopharmacology. 34, 27–37.

Yang, L., Zhao, Y., Wang, Y., et al., 2015. The effects of psychological stress on depression. Curr. Neuropharmacol. 13 (4), 494–504.

Zhu, C.B., Blakely, R.D., Hewlett, W.A., 2006. The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. Neuropsychopharmacology. 31 (10), 2121–2131.

Zhu, C.B., Lindler, K.M., Owens, A.W., Daws, L.C., Blakely, R.D., Hewlett, W.A., 2010. Interleukin-1 receptor activation by systemic lipopolysaccharide induces behavioral despair linked to MAPK regulation of CNS serotonin transporters. Neuropsychopharmacology 35, 2510–2520.

BRIEF COMMUNICATION

Preliminary Study of the Impact of Single-Nucleotide Polymorphisms of *IL-1α*, *IL-1β* and *TNF-α* Genes on the Occurrence, Severity and Treatment Effectiveness of the Major Depressive Disorder

Katarzyna Bialek[1] · Piotr Czarny[2] · Cezary Watala[3] · Ewelina Synowiec[1] · Paulina Wigner[1] · Michal Bijak[4] · Monika Talarowska[5] · Piotr Galecki[6] · Janusz Szemraj[2] · Tomasz Sliwinski[1]

Abstract

The purpose of the preliminary study was to determine whether the occurrence of certain SNPs of genes encoding *IL-1α*, *IL-1β*, and *TNF-α* is associated with the development of depression. Five polymorphisms were selected: i.e. c.-1560G > C—*IL-1β* (rs1143623), c. -118 C > T—*IL-1β* (rs1143627), c.340G > T—*IL-1α* (rs17561), c.-1211T > C—*TNF-α* (rs1799964) and c.-488G > A—*TNF-α* (rs1800629). These were analyzed using TaqMan probes. The genotypes of the analyzed polymorphisms were found to be associated with disease severity and may affect the effectiveness of antidepressant therapy. In addition, the gene–gene analysis confirmed that combined genotypes of investigated SNPs may modulate the risk of depression.

Keywords Major depressive disorder · Inflammation · Single nucleotide polymorphism · Cytokine

Introduction

The expression of pro-inflammatory cytokine genes can be modulated by the presence of single nucleotide polymorphisms (SNPs) within them (Martin et al. 2015). Such modulation may be related with age of onset, severity of episodes and suicidal tendencies in major depressive disorder (MDD) (Kim et al. 2013; Luckhoff et al. 2014; Omrani et al. 2009). The present paper examines the effect of the SNPs c.-1560G > C—*IL-1β* (rs1143623), c. -118 C > T—*IL-1β* (rs1143627), c.340G > T—*IL-1α* (rs17561), c.-1211T > C—*TNF-α* (rs1799964) and c.-488G > A—*TNF-α* (rs1800629). All are located in the coding, promotor or regulatory region of the genes, and hence can affect mRNA stability, degradation and expression, resulting in changes in the activity of the final protein product (Prokunina and Alacron-Riquelme 2004; Roden et al. 2006).

Materials and Methods

Subjects

The study included 270 patients with depression hospitalized at the Department of Adult Psychiatry of the Medical University of Lodz, and 231 volunteers without health problems, selected randomly (Table 2). The inclusion and exclusion criteria, diagnosis and severity assessment were performed as described previously (Czarny et al. 2018). The study protocol was approved by the Bioethics Committee of the Medical University of Lodz (No. RNN/70/14/KE).

✉ Tomasz Sliwinski
tomasz.sliwinski@biol.uni.lodz.pl

1. Laboratory of Medical Genetics, Faculty of Biology and Environmental Protection, University of Lodz, 141/143 Pomorska Street, 90-236 Lodz, Poland

2. Department of Medical Biochemistry, Medical University of Lodz, Lodz, Poland

3. Department of Haemostatic Disorders, Medical University of Lodz, Lodz, Poland

4. Biohazard Prevention Centre, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland

5. Department of Personality and Individual Differences, Institute of Psychology, University of Lodz, Lodz, Poland

6. Department of Adult Psychiatry, Medical University of Lodz, Lodz, Poland

SNP Selection and Genotyping

To this study we selected polymorphisms, located in the coding or regulatory region of the genes, i.e.: c.-1560G > C—*IL-1β*, c. -118 C > T—*IL-1β*, c.340G > T—*IL-1α*, c.-1211T > C—*TNF-α*, and c.-488G > A—*TNF-α*. DNA isolation, and SNP selection and genotyping were conducted as described previously (Czarny et al. 2018).

Statistical Analysis

The descriptive statistics are shown as means ± SD or as medians with interquartile ranges. Normality of the studied group was verified with the Shapiro–Wilk test, homogeneity of variance was checked with Brown–Forsythe test. Accordingly, either the unpaired Student's *t* test or Mann–Whitney *U* test was used. Some bivariate and multivariate analyses were performed using resampling with replacement (the bootstrap-boosted versions of the tests, 10,000 iterations) to make sure that the revealed differences were not detected by pure chance.

An unconditional multiple logistic regression model was used to calculate the associations between the studied polymorphisms and the occurrence of disease. The results are shown as odds ratios (ORs) with 95% confidence interval (±95% CI). Since women tend to display a greater risk of depressive disorders then men, the OR values were also adjusted for sex (Clerci et al. 2009). In addition, the significant outcomes were further validated with the use of two approaches: the bootstrap-boosted multiple logistic regression (resampling with replacement, 10,000 iterations) and the cross-validated logistic regression (corresponding to the *d*-jackknife technique), with the patient group acting as the modelled class. This was intended to overcome any possible bias related to relatively low sample sizes. The goodness of fit of logistic regression models showing a significant degree of discrimination between controls and patients was estimated with Hosmer–Lemeshow test.

The analysis of the collected data was performed in Statistica 12 (Statsoft, USA), SigmaPlot 11.0 (Systat Software Inc., USA), Resampling Stats Add-in for Excel v.4 (Arlington, USA) and StudSize3.02 (CreoStat HB, Sweden).

Results

The distribution of genotypes and alleles was in agreement with the Hardy–Weinberg equilibrium.

To investigate the impact of the studied SNPs on the effectiveness of antidepressant therapy, patients were divided into two groups: one with a Hamilton Rating Scale score less than 7 points after treatment (marked as effective therapy) and another with more than 7 points (marked as ineffective therapy). For rs1799964, it was found that the T/T genotype and the T allele were associated with low effectiveness of pharmacotherapy, and the C/T genotype and C allele with positive response to the treatment (Table 1).

Patients with G/C and C/C genotypes of rs1143623 demonstrated different levels of disease severity based on the Hamilton Depression Rating Scale (Fig. 1).

The combined genotypes of rs1143623–rs1799964, rs1143627 rs17561 and rs1143627–rs1799964 decreased the risk of depression occurrence, while rs1143627–rs1800629 increased this risk (Table 2).

Discussion

The study describes the genotypes of five SNPs located in *IL-1β*, *IL-1α* and *TNF-α*. It is the first to confirm the association between gene–gene interaction and the development of major depressive disorder (MDD). It is also the first study to show a link between the presence of SNPs and the effectiveness of depression treatment, particularly in case of rs1799964, in which the T/T genotype and the T allele were associated with low effectiveness, and the C/T genotype and C allele, associated with a positive treatment response. Exact mechanism of this phenomenon has not been elucidated. However, T allele of mentioned SNP is associated with higher expression of *TNF-α*, that act pro-inflammatory and could interfere with mechanism of antidepressants action. Moreover, C/T genotype and C allele are associated with decreased serum TNF-α level and thus reduction of inflammation, which may act synergistically with the anti-inflammatory mechanism of action of antidepressants (Cui et al. 2012). This discovery could contribute to the selection of effective, personalized pharmacotherapy. We also found that carriers of C/C genotype of rs1143623 are exposed to more severe depressive episode than G/C carriers. It is established that higher level of cytokines is related with intensification of depression symptoms. Therefore, allele G of the SNP, which is associated with decreased pro-inflammatory *IL-1β* expression could predict less severe disease manifestation (Chen et al. 2006).

Our findings confirm that the G/C-T/C genotype of rs1143623–rs1799964 decreased the risk of MDD. Interestingly, rs1143623 was found to affect the activity of the *IL-1β* promoter; the presence of the minor G allele decreased expression of the gene, possibly by disturbing the GATA motif (Chen et al. 2006; Kapelski et al. 2016).

Table 1 Impact of c.-1560G > C—*IL-1β* (rs1143623), c. -118 C > T—*IL-1β* (rs1143627), c.340G > T—*IL-1α* (rs17561), c.-1211 T > C—*TNF-α* (rs1799964) and c.-488G > A—*TNF-α* (rs1800629) polymorphisms on effectiveness of the antidepressant treatment

Genotype/Allele	Responsive* patients		Unresponsive* patients		Crude OR (95% CI)*	p
	Number	Frequency	Number	Frequency		
c.-1560G > C—IL-1β (rs1143623)						
G/G	13	0.087	8	0.110	1.297 (0.512–3.284)	0.583
G/C	52	0.347	18	0.247	0.617 (0.329–1.158)	0.132
C/C	85	0.567	47	0.644	1.382 (0.776–2.463)	0.272
$\chi^2 = 0.333; p = 0.564$						
G	78	0.260	34	0.233	0.882 (0.574–1.355)	0.566
C	222	0.740	112	0.767	1.134 (0.738–1.742)	0.566
c.-118C > T—IL-1β (rs1143627)						
C/C	71	0.473	34	0.466	0.970 (0.554–1.699)	0.915
T/C	59	0.393	27	0.369	0.905 (0.508–1.612)	0.735
T/T	20	0.133	12	0.164	1.279 (0.588–2.783)	0.536
$\chi^2 = 0.144; p = 0.704$						
T	201	0.667	95	0.651	0.927 (0.627–1.370)	0.704
C	99	0.333	51	0.349	1.079 (0.730–1.595)	0.704
c.340G > T—IL-1α (rs17561)						
G/G	79	0.527	38	0.521	0.976 (0.557–1.708)	0.932
G/T	58	0.387	29	0.397	1.045 (0.590–1.853)	0.879
T/T	13	0.087	6	0.082	0.944 (0.344–2.592)	0.911
$\chi^2 = 0.0003; p = 0.986$						
G	216	0.720	105	0.719	0.996 (0.646–1.536)	0.986
T	84	0.280	41	0.281	1.004 (0.651–1.548)	0.986
c.-1211 T > C—TNF-α (rs1799964)						
T/T	**94**	**0.627**	**60**	**0.822**	**2.750 (1.386–5.454)**[0.700]	**0.004**
					[b]**2.750 (1.386–5.454)**	**0.004**
					[cv]**2.750 (1.386–5.454)**	**0.004**
C/T	**45**	**0.300**	**12**	**0.164**	**0.459 (0.226–0.934)**[0.700]	**0.032**
					[b]**0.459 (0.226–0.934)**	**0.032**
					[cv]**0.459 (0.226–0.934)**	**0.032**
C/C	11	0.073	1	0.014	0.176 (0.022–1.386)	0.099
$\chi^2 = 10.498; p = 0.001$						
T	**233**	**0.777**	**132**	**0.904**	**2.474 (1.358–4.507)**[0.700]	**0.003**
					[b]**2.474 (1.358–4.507)**	**0.003**
					[cv]**2.474 (1.358–4.507)**	**0.003**
C	**67**	**0.223**	**14**	**0.096**	**0.404 (0.222–0.736)**[0.700]	**0.003**
					[b]**0.404 (0.222–0.736)**	**0.003**
					[cv]**0.404 (0.222–0.736)**	**0.003**
c.-488G > A—TNF-α rs1800629						
A/A	2	0.013	1	0.014	1.028 (0.092–1.523)	0.982
G/A	44	0.293	19	0.260	0.848 (0.451–1.592)	0.607
G/G	104	0.693	53	0.726	1.172 (0.630–2.180)	0.616
$\chi^2 = 0.215; p = 0.643$						
A	48	0.160	21	0.144	0.873 (0.489–1.557)	0.644
G	252	0.840	125	0.856	1.146 (0.642–2.04)	0.644

*Responsive patients are patients, who have responded positively to therapy of SSRIs, while unresponsive patients have not responded to this therapy; 'adjusted OR' means OR adjusted for sex; for significant comparisons the superscript [b] means the bootstrap-boosted OR (resampling with replacement, 10,000 iterations); [cv] means the cross-validated OR. Statistical power (1-β) for significant comparisons given in superscripts

$p < 0.05$ along with corresponding ORs are in bold
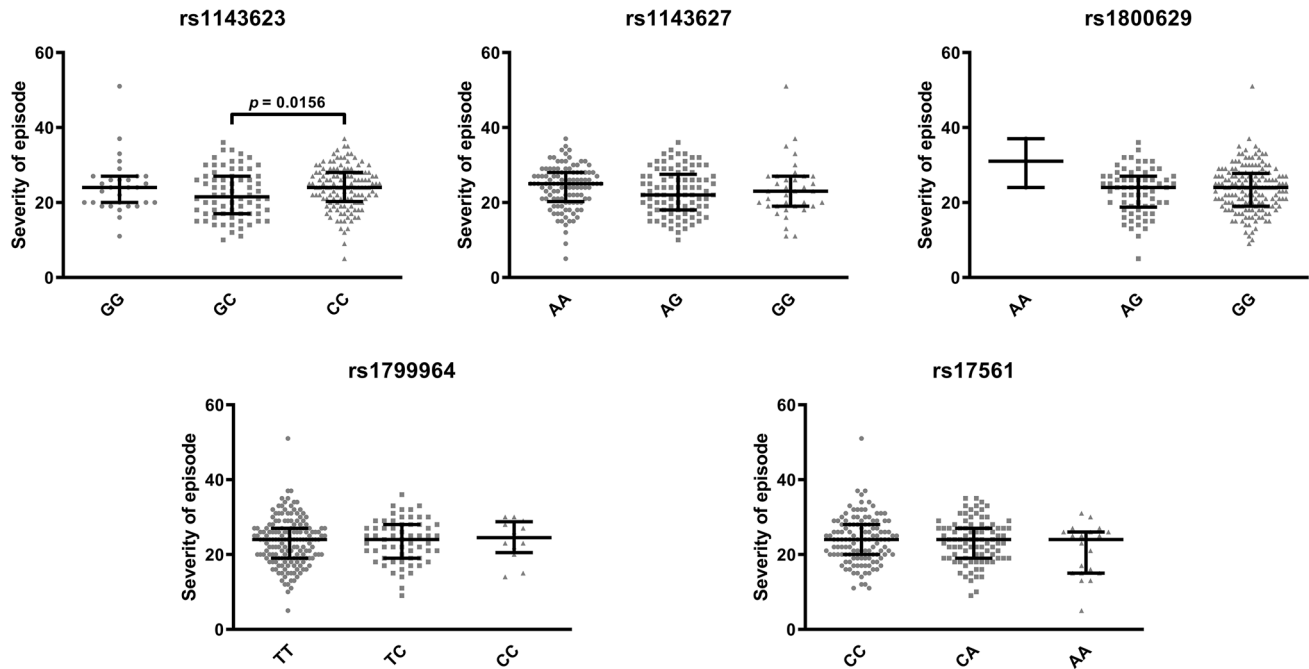
Fig. 1 Distribution of the severity of episode (before therapy) and single nucleotide polymorphisms of genes encoding pro-inflammatory cytokines c.-1560G > C—IL-1β (rs1143623), c. -118 C > T—IL-1β (rs1143627), c.340G > T—IL-1α (rs17561), c.-1211T > C—TNF-α (rs1799964) and c.-488G > A—TNF-α (rs1800629). The horizontal lines denote the median, while the whiskers show the interquartile range. Significance of differences estimated with Kruskal-Wallis, followed by the post-hoc multiple comparison Conover-Inman test; the Benjamini-Hochberg correction was used to validate the significant values of the post-hoc P for multiple variables: $P = 0.0477$, c.-1560G > C—IL-1β (rs1143623), $P = 0.1466$, c. -118 C > T—IL-1β (rs1143627), $P = 0.6597$, c.340G > T—IL-1α (rs17561), $P = 0.5248$, c.-1211T > C—TNF-α (rs1799964), $P = 0.2026$, c.-488G > A—TNF-α (rs1800629)

Rs1799964 is located in the promoter region and its T/C and C/C genotypes were associated with decreased serum TNF-α levels. This study also reported higher gene expression with the T allele, compared to allele C (Cui et al. 2012). Interestingly, the risk of depression was reduced in cases with the T/C–C/T genotype of rs1799964–rs1143627, with rs1143627 being located in the TATA-box motif of the promoter region (El-Omar et al. 2000). Depression was also less likely in the G/T-C/T genotype of rs17561–rs1143627. Interestingly, rs17561 was associated with ovarian cancer risk (White et al. 2012). This is the first study examining the association between rs1143627 or rs17561 and MDD.

The G/G-C/C combined genotype of rs1800629–rs1143627 was found to decrease the risk of depression. In addition, allele A of rs1800629 was found to be associated with the occurrence of depression (Jun et al. 2003) and with earlier age of onset (Luckhoff et al. 2014). However, the G/G genotype was correlated with suicidal attempts in depression (Kim et al. 2013). Accordingly, this variant was recorded to be more frequent in subjects who had attempted suicide than in a control group (Omrani et al. 2009).

The main limitation of our preliminary work is its relatively small sample size. However, to minimise the risk of

Table 2 Characteristic of the investigated subjects and gene–gene interactions of c.-1560G>C—IL-1β (rs1143623), c. -118 C>T—IL-1β (rs1143627), c.340G>T—IL-1α (rs17561), c.-1211 T>C—TNF-α (rs1799964) and c.-488G>A—TNF-α (rs1800629) polymorphism and the risk of depression

Group	Control (n=231)		Depression (n=270)					
Sex (M/F)	115/116		138/132					
Age (Mdn [Q_1; Q_3])	55 [47; 63]		52 [44; 57]					
Age of onset (Mdn [Q_1; Q_3])	–		36 [28; 45]					
HRDS1 (Mdn [Q_1; Q_3])	–		24 [19; 27.75]					
Combined genotype	Number	Frequency	Number	Frequency	Crude OR (95% CI)	p	Adjusted OR (95%CI)	p
c.-1560G>C—IL-1β (rs1143623) and- c.340G>T—IL-1α (rs17561)								
G/G-G/G	0	0	0	0	–	–	–	–
G/G-G/T	0	0	0	0	–	–	–	–
G/G-T/T	0	0	0	0	–	–	–	–
G/C-G/G	47	0.203	52	0.193	0.934 (0.601–1.451)	0.761	0.933 (0.601–1.450)	0.759
G/C-G/T	35	0.152	28	0.104	0.648 (0.381–1.102)	0.109	0.648 (0.381–1.102)	0.110
G/C-T/T	6	0.026	6	0.022	0.852 (0.271–2.679)	0.784	0.853 (0.271–2.686)	0.786
C/C-G/G	63	0.273	91	0.337	1.356 (0.923–1.990)	0.120	1.357 (0.924–1.994)	0.119
C/C-G/T	62	0268	77	0.285	1.087 (0.734–1.611)	0.676	1.087 (0.734–1.611)	0.677
C/C-T/T	18	0.078	16	0.059	0.745 (0.371–1.497)	0.409	0.745 (0.371–1.497)	0.408
c.-1560G>C—IL-1β (rs1143623)—c.-1211 T>C—TNF-α (rs1799964)								
G/G-T/T	0	0	0	0	–	–	–	–
G/G-T/C	0	0	0	0	–	–	–	–
G/G-C/C	0	0	0	0	–	–	–	–
G/C-T/T	57	0.247	61	0.226	0.891 (0.589–1.347)	0.584	0.891 (0.589–1.347)	0.584
G/C-T/C	**29**	**0.126**	**19**	**0.070**	**0.529 (0.288–0.972)**[0.613]	**0.040**	**0.529 (0.288–0.972)**[0.613]	**0.040**
					[b]**0.524 (0.317–0.867)**	**0.035**	[b]**0.524 (0.316–0.867)**	**0.035**
					[c,y]**0.529 (0.288–0.972)**	**0.040**	[c,y]**0.529 (0.288–0.972)**	**0.040**
G/C-C/C	2	0.009	6	0.022	2.602 (0.520–13.019)	0.244	2.601 (0.520–13.016)	0.244
C/C-T/T	91	0.394	124	0.459	1.307 (0.915–1.866)	0.141	1.307 (0.915–1.866)	0.141
C/C-T/C	47	0.203	52	0.193	0.934 (0.601–1.451)	0.761	0.934 (0.601–1.452)	0.763
C/C-C/C	5	0.022	8	0.030	1.380 (0.445–4.279)	0.577	1.379 (0.444–4.280)	0.578
c.-1560G>C—IL-1β (rs1143623)—c.-488G>A—TNF-α rs1800629								
G/G-G/G	0	0	0	0	–	–	–	–
G/G-G/A	0	0	0	0	–	–	–	–
G/G-A/A	0	0	0	0	–	–	–	–
G/C-G/G	64	0.277	63	0.233	0.794 (0.531–1.189)	0.263	0.794 (0.531–1.188)	0.262
G/C-G/A	21	0.091	22	0.081	0.887 (0.475–1.658)	0.707	0.887 (0.474–1.658)	0.706
G/C-A/A	3	0.013	1	0.004	0.283 (0.029–2.735)	0.275	0.283 (0.029–2.740)	0.276
C/C-G/G	96	0.416	126	0.467	1.230 (0.863–1.754)	0.251	1.230 (0.863–1.754)	0.252

Table 2 (continued)

Combined genotype	Number	Frequency	Number	Frequency	Crude OR (95% CI)	p	Adjusted OR (95%CI)	p
C/C-G/A	45	0.195	55	0.204	1.057 (0.681–1.642)	0.804	1.058 (0.681–1.643)	0.802
C/C-A/A	2	0.009	3	0.011	1.287 (0.213–7.767)	0.784	1.288 (0.213–7.774)	0.783
c.-118C>T—IL-1β (rs1143627)- c.340G>T—IL-1α (rs17561)								
C/C-G/G	14	0.061	21	0.078	1.307 (0.649–2.633)	0.453	1.307 (0.649–2.634)	0.453
C/C-G/T	8	0.035	17	0.063	1.873 (0.793–4.424)	0.152	1.873 (0.793–4.426)	0.153
C/C-T/T	1	0.004	3	0.011	2.584 (0.267–25.015)	0.412	2.594 (0.268–25.146)	0.411
C/T-G/G	53	0.229	65	0.241	1.065 (0.703–1.612)	0.766	1.065 (0.703–1.612)	0.757
C/T-G/T	**45**	**0.195**	**32**	**0.119**	**0.558 (0.341–0.913)**[0.679]	**0.020**	**0.558 (0.341–0.913)**[0.679]	**0.020**
					[b]**0.558 (0.371–0.840)**	**0.019**	[b]**0.558 (0.369–0.839)**	**0.019**
					[cv]**0.341..558 (0. 0.913)**	**0.020**	[cv]**0.558 (0.341–0.913)**	**0.020**
C/T-T/T	6	0.026	5	0.019	0.708 (0.213–2.349)	0.872	0.708 (0.213–2.357)	0.574
T/T-G/G	43	0.186	57	0.211	1.170 (0.52–1.820)	0.486	1.181 (0.752–1.822)	0.484
T/T-G/T	44	0.190	56	0.207	1.112 (0.716–1.728)	0.637	1.112 (0.716–1.729)	0.636
T/T-T/T	17	0.074	14	0.052	0.688 (0.332–1.429)	0.316	0.687 (0.331–1.428)	0.315
c.-118C>T—IL-1β (rs1143627)- c.-1211 T>C—TNF-α (rs1799964)								
C/C-T/T	19	0.082	29	0.107	1.343 (0.732–2.464)	0.342	1.342 (0.731–2.464)	0.342
C/C-T/C	4	0.017	11	0.041	2.410 (0.757–7.674)	0.137	2.410 (0.757–7.675)	0.137
C/C-C/C	0	0	1	0.004	–	–	–	–
C/T-T/T	65	0.281	70	0.259	0.894 (0.602–1.327)	0.578	0.894 (0.602–1.327)	0.578
C/T-T/C	**37**	**0.161**	**25**	**0.093**	**0.537 (0.312–0.924)**[0.669]	**0.024**	**0.537 (0.313–0.923)**[0.669]	**0.0.24**
					[b]**0.533 (0.340–0.836)**	**0.021**	[b]**0.533 (0.340–0.835)**	**0.021**
					[cv]**0.537 (0.313–0.923)**	**0.024**	[cv]**0.537 (0.313–0.923)**	**0.024**
C/T-C/C	2	0.009	7	0.026	3.048 (0.627–14.817)	0.167	3.046 (0.626–14.814)	0.168
T/T-T/T	64	0.277	86	0.319	1.220 (0.830–1.793)	0.313	1.220 (0.829–1.793)	0.313
T/T-T/C	35	0.152	35	0.130	0.834 (0.503–1.383)	0.482	0.834 (0.503–1.384)	0.483
T/T-C/C	5	0.022	6	0.022	1.027 (0.309–3.411)	0.965	1.026 (0.309–3.409)	0.966
c.-1211T>C—TNF-α (rs1799964)- c.340G>T—IL-1α (rs17561)								
T/T-G/G	67	0.290	96	0.356	1.350 (0.925–1.972)	0.119	1.351 (0.926–1.971)	0.119
T/T-G/T	62	0.268	72	0.267	0.991 (0.667–1.474)	0.965	0.991 (0.666–1.474)	0.963
T/T-T/T	19	0.082	17	0.063	0.750 (0.380–1.479)	0.406	0.750 (0.380–1.479)	0.406
T/C-G/G	37	0.160	36	0.133	0.807 (0.491–1.26)	0.397	0.807 (0.491–1.326)	0.397
T/C-G/T	34	0.147	30	0.111	0.724 (0.428–1.225)	0.229	0.724 (0.428–1.226)	0.230
T/C-T/T	5	0.022	5	0.018	0.853 (0.244–2.983)	0.803	0.853 (0.244–1.982)	0.803
C/C-G/G	6	0.026	11	0.041	1.593 (0.580–4.376)	0.367	1.592 (0.579–4.375)	0.367
C/C-G/T	1	0.004	3	0.011	2.584 (0.267–25.015)	0.412	2.582 (0.266–25.044)	0.413

Table 2 (continued)

Combined genotype	Number	Frequency	Number	Frequency	Crude OR (95% CI)	p	Adjusted OR (95%CI)	p
C/C-T/T	0	0	0	0	–	–	–	–
c.-118C>T—IL-1β (rs1143627)—c.-488G>A—TNF-α (rs1800629)								
C/C-G/G	**13**	**0.056**	**29**	**0.107**	**2.026 (1.027–3.997)$^{0.405}$** ^{b}**2.020 (1.153–3.539)** ^{cv}**2.026 (1.027–3.997)**	**0.042** **0.039** **0.042**	**2.026 (1.026–3.997)$^{0.405}$** ^{b}**2.020 (1.152–3.540)** ^{cv}**2.026 (1.027–3.997)**	**0.042** **0.039** **0.042**
C/C-G/A	9	0.039	10	0.037	0.949 (0.379–2.376)	0.911	0.945 (0.378–2.376)	0.910
C/C-A/A	1	0.004	2	0.007	1.716 (0.155–19.052)	0.660	1.465 (0.128–16.759)	0.759
C/T-G/G	74	0.320	79	0.293	0.878 (0.600–1.284)	0.501	0.881 (0.601–1.292)	0.517
C/T-G/A	27	0.117	23	0.085	0.704 (0.391–1.264)	0.240	0.670 (0.370–1.211)	0.185
C/T-A/A	3	0.013	0	0	–	–	–	–
T/T-G/G	73	0.316	81	0.300	0.928 (0.634–1.357)	0.699	0.938 (0.640–1.375)	0.743
T/T-G/A	30	0.130	44	0.163	1.304 (0.790–2.154)	0.299	1.310 (0.792–2.167)	0.293
T/T-A/A	1	0.004	2	0.007	1.716 (0.155–19.052)	0.660	1.816 (0.164–20.167)	0.627
c.-488G>A—TNF-α (rs1800629)- c.340G>T—IL-1α (rs17561)								
G/G-G/G	76	0.329	103	0.381	1.258 (0.870–1.818)	0.222	1.258 (0.871–1.819)	0.222
G/G-G/T	64	0.277	71	0.263	0.931 (0.0627–1.383)	0.723	0.931 (0.627–1.382)	0.722
G/G-T/T	20	0.087	15	0.056	0.621 (0.310–1.242)	0.178	0.620 (0.309–1.241)	0.177
G/A-G/G	34	0.147	38	0.141	0.949 (0.576–1.565)	0.838	0.949 (0.575–1.565)	0.837
G/A-G/T	28	0.121	32	0.119	0.975 (0.568–1.674)	0.926	0.975 (0.568–1.674)	0.926
G/A-T/T	4	0.017	7	0.026	1.510 (0.437–5.226)	0.515	1.520 (0.437–5.282)	0.510
A/A-G/G	0	0	2	0.007	–	–	–	–
A/A-G/T	5	0.022	2	0.007	0.337 (0.065–1.755)	0.197	0.337 (0.065–1.756)	0.197
A/A-T/T	0	0	0	0	–	–	–	–

M means male; *F* means Female

Mdn median, *Q1* first quartile, *Q3* third quartile, *HRDS1* points in Hamilton Depression Rating Scale measured before antidepressant treatment

*'Adjusted OR' means OR adjusted for sex; for significant comparisons the superscript ^{b} means the bootstrap-boosted OR (resampling with replacement, 10,000 iterations); ^{cv} means the cross-validated OR. Statistical power (1-β) for significant comparisons given in superscripts

$p < 0.05$ along with corresponding ORs are in bold

obtaining false positive results, two resampling approaches were performed. Another limitation was the ethnic homogeneity of the studied group, which reduces the potential to extrapolate the results to other ethnic groups. Therefore, these results should be interpreted with caution and considered preliminary.

Conclusion

The SNPs of genes encoding pro-inflammatory cytokines may have impact on the risk and treatment of depression.

Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All procedures was approved by the Bioethics Committee of the Medical University of Lodz (No. RNN/70/14/KE).

Informed Consent Informed consent was obtained from all individual participants included in the study and was approved by the Bioethics Committee of the Medical University of Lodz (No. RNN/70/14/KE).

References

Chen H, Wilkins LM, Aziz N et al (2006) Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. Hum Mol Genet 15(4):519–529

Clerici M, Arosio B, Mundo E et al (2009) Cytokine polymorphisms in the pathophysiology of mood disorders. CNS Spectrosc 14(8):419–425

Cui G, Wang H, Li R, Zhang L et al (2012) Polymorphism of tumor necrosis factor alpha (TNF-alpha) gene promoter, circulating TNF-alpha level, and cardiovascular risk factor for ischemic stroke. J Neuroinflamm 10(9):235

Czarny P, Wigner P, Strycharz J et al (2018) Single-nucleotide polymorphisms of uracil-processing genes affect the occurrence and the onset of recurrent depressive disorder. PeerJ 6:e5116

El-Omar EM, Carrington M, Chow WH et al (2000) Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 404(6776):398–402

Jun TY, Pae CU, Chae JH et al (2003) Possible association between -G308A tumor necrosis factor-alpha gene polymorphism and major depressive disorder in the Korean population. Psychiatr Genet 13(3):179–181

Kapelski P, Skibinska M, Maciukiewicz M et al (2016) An association between functional polymorphisms of the interleukin 1 gene complex and schizophrenia using transmission disequilibrium test. Arch Immunol Ther Exp 64(1):161–168

Kim YK, Hong JP, Hwang JA et al (2013) TNF-alpha -308G%3eA polymorphism is associated with suicide attempts in major depressive disorder. J Affect Disord 150(2):668–672

Lückhoff HK, Van Rensburg SJ, Botha K et al (2014) The pro-inflammatory TNFA -308GA (rs1800629) polymorphism is associated with an earlier age at onset in patients with major depressive disorder. J Psychiatry 17(3):1000124

Martin C, Tansey KE, Schalkwyk LC, Powell TR (2015) The inflammatory cytokines: molecular biomarkers for major depressive disorder? Biomark Med 9(2):169–180

Omrani MD, Bushehri B, Bagheri M et al (2009) Role of IL-10-1082, IFN-gamma +874, and TNF-alpha -308 genes polymorphisms in suicidal behavior. Arch Suicide Res 13:330–339

Prokunina L, Alarcón-Riquelme ME (2004) Regulatory SNPs in complex diseases: their identification and functional validation. Expert Rev Mol Med 6(10):1–15

Roden DM, Altman RB, Benowitz NL et al (2006) Pharmacogenetics research network Pharmacogenomics: challenges and opportunities. Ann Intern Med 145(10):749–757

White KL, Schildkraut JM, Palmieri RT et al (2012) Ovarian Cancer Association Consortium Ovarian cancer risk associated with inherited inflammation-related variants. Cancer Res 72(5):1064–1069

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Novel association between TGFA, TGFB1, IRF1, PTGS2 and IKBKB single-nucleotide polymorphisms and occurrence, severity and treatment response of major depressive disorder

Katarzyna Bialek[1], Piotr Czarny[2], Cezary Watala[3], Paulina Wigner[1], Monika Talarowska[4], Piotr Galecki[5], Janusz Szemraj[2] and Tomasz Sliwinski[1]

[1] Laboratory of Medical Genetics, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland
[2] Department of Medical Biochemistry, Medical University of Lodz, Lodz, Poland
[3] Department of Haemostatic Disorders, Medical University of Lodz, Lodz, Poland
[4] Institute of Psychology, Department of Personality and Individual Differences, University of Lodz, Lodz, Poland
[5] Department of Adult Psychiatry, Medical University of Lodz, Lodz, Poland

ABSTRACT

Background: Activation of the immune system might affect the severity of depressive episodes as well as response to the antidepressant treatment. The purpose of this study was to investigate whether the occurrence of variant alleles of analyzed SNPs are involved in prevalence and progression of depression. Moreover, selected genes and SNPs have not been investigated in context of the disease severity and treatment. Therefore, six polymorphisms were selected: g.41354391A>G-*TGFB1* (rs1800469), g.132484229C>A-*IRF* (rs2070729), g.186643058A>G-*PTGS2* (rs5275), g.186640617C>T-*PTGS2* (rs4648308), g.70677994G>A-*TGFA* (rs2166975) and g.42140549G>T–*IKBKB* (rs5029748).

Methods: A total of 360 (180 patients and 180 controls) DNA samples were genotyped using TaqMan probes.

Results: We observed that A/G of the rs2166975 *TGFA*, A/C of rs2070729 *IRF1* and G/T of rs5029748 *IKBKB* were associated with an increased risk of depression development while the T/T of rs5029748 *IKBKB*, T/T of rs4648308 *PTGS2* and G/G of rs2166975 *TGFA* reduced this risk. We also stratified the study group according to gender and found that genotype A/G and allele G of the rs2166975 *TGFA*, G/T of rs5029748 *IKBKB* as well as C allele of rs4648308 *PTGS2*, homozygote A/A and allele A of rs5275 *PTGS2* were associated with increased risk of depression development in men while homozygote G/G of rs5275 *PTGS2* decreased this risk. Moreover, C/T of rs4648308 *PTGS2* and A/G of rs5275 *PTGS2* was positively correlated with the risk of the disease occurrence in women. Furthermore, a gene–gene analysis revealed a link between studied polymorphisms and depression. In addition, A/A of rs1800469 *TGFB1* was associated with earlier age of onset of the disease while G/G of this SNP increased severity of the depressive episode. Interestingly, A/C of rs2070729 *IRF1* and T/T of rs5029748 *IKBKB* may modulate the

effectiveness of selective serotonin reuptake inhibitors therapy. In conclusion, studied SNPs may modulate the risk of occurrence, age of onset, severity of the disease and response to the antidepressant treatment.

INTRODUCTION

Depression (Major depressive disorder, MDD) is one of the most frequently diagnosed mental diseases. According to World Health Organization, about 350 million people suffer from this disorder all over the world (*WHO, 2018*). Despite the importance of the problem, pathogenesis of depression is not fully understood. However, there is a growing body of evidence suggesting that immune system impairment and dysregulation is associated with the pathophysiology of MDD. In particular, the "cytokine hypothesis" is widely accepted as one of the mechanisms for the development of depression (*Capuron & Miller, 2011*). This theory postulates that MDD is a result of elevated expression of pro-inflammatory cytokines, which act as neuromodulators as well as main agents in mediation of the neuroendocrine, neurochemical and behavioral features of the disease (*Schiepers, Wichers & Maes, 2005*). Some evidence confirmed link between inflammation and depression. Primarily, MDD patients exhibit increased levels of cytokines and other pro-inflammatory markers (*Capuron & Miller, 2011*). Additionally, medical conditions connected with increased inflammatory response are associated with greater risk of MDD developing (*Capuron & Miller, 2011*).

One of the cytokine class strongly associated with depression are interferons (IFN), cluster of signaling proteins involved in immune response. More than twenty different IFN proteins have been identified so far and divided into classes. IFN proteins are able to activate immune cells, that is, natural killer cells (NK cells) and macrophages (*Pinto & Andrade, 2016*). For instance, IFN-α is implicated in modulation of mood, behavior and sleep-wake cycle, partially by its ability to activate the pro-inflammatory cytokine network including, interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) (*Zahiu & Mihai, 2014*). IFN and IFN-inducible genes, involved in immunity and inflammation, are transcriptionally regulated by interferon regulatory factor 1 (IRF1) (*Tamura et al., 2008*). IRF1 was a first identified transcription factor in IFN system and as a member of interferon regulatory factor family, plays important role in controlling expression of aforementioned genes (*Kröger et al., 2002*). Besides this, IRF1 promotes inflammatory cytokine release and regulates expression of interleukin 12 (IL-12) and interleukin 15 (IL-15), which are involved in MDD (*Tamura et al., 2008*).

Besides cytokine theory, various inflammatory pathways are thought to be activated in course of depression, including activation of the NF-kB (nuclear factor-kB), what leads to increased levels of pro-inflammatory cytokines (*Bierhaus et al., 2003*; *Pace et al., 2006*). NF-kB is a ubiquitous transcriptional factor that regulates expression of genes involved in

pleiotropic functions, including pro-inflammatory cytokines and co-stimulatory molecules (*Takeda & Akira, 2007*; *Krakauer, 2008*; *Zhang, Lenardo & Baltimore, 2017*). Inactive NF-kB molecules retain in the cytoplasm by interaction with IkB proteins, allowing to immediate activation in response to adequate impulse (*Napetschnig & Wu, 2013*). Canonical signaling of NF-kB is activated by IkB kinase (IKK complex), consisting of three subunits, each encoded by separate gene, that is, IKK-a (Inhibitor of nuclear factor kappa-B kinase subunit alpha) encoded by *CHUK* gene, IKK-B (inhibitor of nuclear factor kappa-B kinase subunit beta) by *IKBKB* gene and IKK-g (inhibitor of nuclear factor kappa-B kinase subunit gamma) by *IKBKG*. The activation of IKK is induced by phosphorylation of serine residues in catalytic subunits of kinase complex (*Napetschnig & Wu, 2013*; *Karin & Ben-Neriah, 2000*; *Cardinez et al., 2018*). Therefore, defective expression of NF-kB as the pro-inflammatory transcription factor, caused by alterations in *IKBKB* gene, may play a role in the development of depression (*Napetschnig & Wu, 2013*).

Transforming growth factors (TGF) constitute of two classes of polypeptide growth factors, namely TGFA (transforming grow factor α) and TGFB (transforming grow factor β). Important functions of these cytokines are embryonic development and regulation of specific reactions of immune system by their ability to induce T regulatory cells (Treg) (*Kissin et al., 2002*; *Yamagiwa et al., 2001*). TGFA is a ligand for epidermal growth factor receptor, which stimulates cell migration and proliferation. These gene and protein have been associated with many types of cancers and other diseases (*Ten Dijke & Hill, 2004*). Another piece of evidence confirmed that TGFB, an anti-inflammatory cytokine, plays role in brain inflammation as well as in peripheral immune response. Namely, TGFB is mainly involved in regulating inflammatory response by induction of differentiation of CD4^{+} T cells (*Nam et al., 2008*; *Passos et al., 2010*). Another essential function of the protein is cell to cell signaling, and thus controlling of cell growth and differentiation (*Ten Dijke & Hill, 2004*). In addition, TGFB is able to exert neuroprotective effects in many neurodegenerative disorders (*Vivien & Ali, 2006*). Information about its role in depression are contradictory. On the one hand, in animal model of depression, the cytokine level is increased and causes imbalance between Treg and Th17 cells (*Hong et al., 2013*). On the other hand, some studies reported that levels of TGFB in depressed patients are lower than in healthy control group (*Musil et al., 2011*; *Sutcigil et al., 2007*). Moreover, TGFB alone is sufficient to stimulate production of pro-inflammatory cytokines for example, IL-1 and TNF-α (*Kunzmann et al., 2003*). The protein is also able to induce expression of prostaglandin-endoperoxide synthase 2 (PTGS2; cyclooxygenase-2—COX-2) encoded by *PTGS2* gene, which is involved in pathogenesis of MDD. PTGS2 besides contribution to processes related to inflammation, also participates in the production of free radicals, which is partly utilized by PTGS2 itself (*Aktan, 2004*; *Hansson, Olsson & Nauseef, 2006*). Moreover, COX-2 catalyzes conversion of arachidonic acid (AA) to prostaglandins (PGs), which further intensify inflammation and neurodegenerative processes in central nervous system (CNS) (*Minghetti, 2004*). In response to growth factors, cytokines and other inflammatory molecules, PTGS2 is immediately expressed and is responsible for the production of prostanoid in both acute and chronic inflammatory conditions

(*Breyer et al., 2001*; *Shi et al., 2010*). Additionally, in animal model of depression increased expression of PTGS2 was observed in brain regions (*Cassano et al., 2006*).

The evidence suggests that MDD may be associated with impairment of immune system, caused by defective activity of aforementioned genes. Moreover, genetic factors may play an essential role in development of depression, since genome-wide association studies (GWAS) found several regions significantly associated with MDD (*Shyn et al., 2011*; *Wray et al., 2018*). Therefore, the present study examines the prospective relationship between the occurrence, age of onset, severity or antidepressant treatment efficacy of MDD and appearance of single nucleotide polymorphism (SNP) located in inflammatory-related genes, that is, g.132484229C>A of *IRF1* (rs2070729, located on 5q31.1), g.186643058A>G of *PTGS2* (rs5275, located on 1q31.1), g.186640617C>T of *PTGS2* (rs4648308, located on 1q31.1), g.70677994G>A of *TGFA* (rs2166975, located on 2p13.3), g.41354391A>G of *TGFB1* (rs1800469, located on 19q13.2) and g.42140549G>T of *IKBKB* (rs5029748, located on 8p11.21). Selected SNPs are located within immune genes participating in inflammatory-related signaling pathways. Therefore, they could affect gene expression and protein function and thus contribute to immune disruptions leading to increased risk of MDD.

MATERIALS AND METHODS

Subjects

The study included a total of 360 participants randomly selected. A group of 180 patients with depression hospitalized at the Department of Adult Psychiatry of the Medical University of Lodz and 180 volunteers without health problems, selected randomly (Table 1). Participants who took part in the experiment were native, not-related Poles. Patients were included based on the criteria set out in ICD-10 (F32.0–7.32.2, F33.0–F33.8). Medical and psychiatric records were obtained in accordance with ICD-10 criteria, using the Standardized Composite International Diagnostic Interview (CIDI). The depression' severity was evaluated using the 21-item Hamilton Depression Rating Scale (HDRS-21). The exclusion criteria included: axis I and II disorders other than MDD, chronic somatic diseases, autoimmune disorders (psoriasis, rheumatoid arthritis, chronic obstructive pulmonary disease, cancer, chronic kidney disease, systemic lupus erythematosus, type 1 diabetes, hepatitis B and C virus and HIV infection), neuroinflammatory and neurodegenerative disorders (including multiple sclerosis, Alzheimer's disease, Parkinson's disease) and central nervous system damage. Furthermore, subjects with familial incidence of mental diseases, other than MDD did not participate in the experiment. Psychiatric examination was conducted by the same psychiatrist, before the subjects were included in the experiment and after 8 weeks of pharmacotherapy with selective serotonin reuptake inhibitor (SSRI). Control group included selected randomly, volunteers with negative history of mental disorders. Participation in the experiment was voluntary. Controls and patients who did not agree to participate in the study were excluded. The purpose of the study was clearly presented, participants were assured that their personal information would be kept confidential. All of the subjects agreed by giving

Table 1 Characteristic of studied population. M means male; F means Female Mdn—median; Q1—first quartile; Q3—third quartile HRDS1—points in Hamilton Depression Rating Scale measured before antidepressant treatment.

Group	Control ($n = 180$)	Patients ($n = 180$)
Sex (M/F)	93/87	91/89
Age (Mdn (Q_1; Q_3))	57 (50; 65)	51 (44; 56)
Age of onset (Mdn (Q_1; Q_3))	–	34 (28; 43)
HRDS1 (Mdn (Q_1; Q_3))	–	24 (19; 27)
Treatment efficacy		
Responsive (reduction from baseline of ≥ 50% in the total score)		93%
Remission (total HRDS1 score ≤7)		66%

Table 2 Characteristic of studied polymorphisms.

Gene	rs number	Polymorphis	Localization	Minor allele freqency
TGFA	rs2166975	g.70677994G>A	Exon 5	A = 0.256
TGFB	rs1800469	g.41354391A>G	5′ of *TGFB* gene	A = 0.312
IRF1	rs2070729	g.132484229C>A	Intron 9	A = 0.465
IKBKB	rs5029748	g.42140549G>T	Intron 2	T = 0.259
PTGS2	rs5275	g.186643058A>G	3′ UTR of *PTGS2* gene	G = 0.310
	rs4648308	g.186640617C>T	3′ of *PTGS2* gene	T = 0.142

their written consent to participate in the experiment according to the protocol approved by the Bioethics Committee of the Medical University of Lodz (No. RNN/70/14/KE).

SNP selection

Selection of the studied polymorphisms was performed using the public domain of the database for single nucleotide polymorphisms of the National Center for Biotechnology (NCBI dbSNP, www.ncbi.nlm.nih.gov/snp/) (Bethesda, Montgomery County, MD, USA). The criteria used for the SNPs' selection were that the minor allele frequency is greater than 0.05 in the European population, and that they are located in the coding or regulatory region of the genes and may have functional meaning for transcription and protein function. Detailed information about selected polymorphisms are presented in Table 2.

DNA isolation

Genomic DNA was isolated from venous blood in accordance with the manufacturer instructions. Blood samples were collected from control group and patients with MDD. Blood Mini Kit (A&A Biotechnology, Gdynia, Poland) was used to extract nucleic acid. The purity of and concentration of the DNA was measured spectrophotometrically by calculating the ratio between absorbance at 260 nm and 280 nm, using Picodrop™ (Picodrop Limited, Astranet Systems Ltd., Cambridge, UK). Samples were stored at −20 °C until use.

Genotyping

The investigated SNPs were genotyped using a TaqMan SNP Genotyping Assay (Thermo Fisher Scientific, Waltham, MA, USA), and a 2X Master Mix Takyon for Probe Assay—No ROX (Eurogentec, Liège, Belgium). Reactions were conducted in accordance with the manufacturer's instruction. Real-time PCR were performed with a Bio-Rad CFX96 Real-Time PCR Detection System, and analyzed in CFX Manager Software (Bio-Rad Laboratories Inc., Hercules, CA, USA).

Statistical analysis

The collected data were analyzed in Statistica 12 (Statsoft, Tulsa, OK, USA), SigmaPlot 14.0 (Systat Software Inc., San Jose, CA, USA), Resampling Stats Add-in for Excel v.4 (Arlington, TX, USA) and StudSize3.02 (CreoStat HB, Florunda, Sweden). The descriptive statistics are shown as medians with interquartile ranges. Normality of the studied group was verified with the Shapiro–Wilk test, homogeneity of variance was checked with Brown–Forsythe test. Accordingly, either the unpaired Student's t test or Mann–Whitney U test was used. To calculate the associations between studied polymorphisms and the occurrence of a disease an unconditional multiple logistic regression model was used. The results are shown as odds ratio (OR) with 95% confidence interval (95% CI). The OR values were adjusted for the potential confounders, including age and sex. We also stratified results into male and female group and evaluated correlation between case/control for each polymorphism. In addition, in order to strengthen that the revealed differences were not detected by a pure chance the significant outcomes were further validated with the use of two approaches: the bootstrap-boosted multiple logistic regression (resampling with replacement, 10,000 iterations) and the cross-validated logistic regression (corresponding to the d-jackknife technique), with the patient group acting as the modeled class. This was intended to overcome any possible bias related to relatively low sample sizes. The goodness of fit of logistic regression models showing a significant degree of discrimination between controls and patients was estimated with Hosmer–Lemeshow test.

Efficiency of the treatment was calculated using the formula as described before (*Czarny et al., 2019*):

$$TE = \frac{(\text{HAM-D}_0 - \text{HAM-D}_E) \times 100\%}{\text{HAM-D}_0}$$

TE-treatment efficiency; HAM-D$_0$—score before therapy; HAM-D$_E$—score after therapy.

RESULTS

Single nucleotide polymorphisms of genes encoding IRF1, IKBKB, TGFA, TGFB and PTGS2 as a risk of MDD

The distribution of genotypes and alleles in both depressed and control groups was in agreement with Hardy–Weinberg equilibrium. Results are presented in Table 3.
The results demonstrated that the A/G genotype of the g.70677994G>A (rs2166975)

Table 3 Distribution of genotypes and alleles of rs1800469 (*TGFB1*), rs2070729 (*IRF1*), rs5275 (*PTGS2*), rs4648308 (*PTGS2*), rs2166975 (*TGFA*), rs5029748 (*IKBKB*) and the risk of depression occurrence.

Genotype/Allele	Control		Depression		Crude OR (95% CI)	p	Adjusted OR (95% CI)*	p
	Number	Frequency	Number	Frequency				
g.41354391A>G of *TGFB1* (rs1800469)								
A/A	23	0.128	20	0.117	0.853 [0.451–1.616]	0.626	0.739 [0.367–1.49]	0.398
A/G	71	0.394	76	0.428	1.231 [0.623–2.432]	0.550	1.197 [0.762–1.879]	0.435
G/G	86	0.478	84	0.483	1.123 [0.575–2.196]	0.734	0.949 [0.609–1.479]	0.818
$\chi^2 = 0.403$; $p = 0.818$								
A	117	0.325	116	0.322	0.987 [0.723–1.349]	0.937	0.961 [0.687–1.344]	0.815
G	243	0.675	244	0.678	1.013 [0.741–1.384]	0.937	1.041 [0.744–1.456]	0.815
g.70677994G>A of *TGFA* (rs2166975)								
A/A	27	0.142	15	0.081	0.530 [0.272–1.031]	0.062	0.576 [0.280–1.184]	0.133
A/G	**59**	**0.311**	**83**	**0.446**	[b]**1.814 [1.197–2.749]**	**0.005**	[b]**2.115 [1.341–3.336]**	**0.001**
					1.789 [1.173–2.728][0.692]	0.007	2.091 [1.323–3.304][0.893]	0.002
G/G	**104**	**0.547**	**88**	**0.473**	0.743 [0.495–1.114]	0.150	[b]**0.609 [0.392–0.946]**	**0.027**
							0.615 [0.395–0.957][0.691]	0.031
$\chi^2 = 8.627$; $p = 0.013$								
A	113	0.297	113	0.304	1.031 [0.755–1. 408]	0.848	1.173 [0.839–1.640]	0.351
G	267	0.703	259	0.696	0.970 [0.710–1.325]	0.848	0.853 [0.610–1.192]	0.351
g.132484229C>A of *IRF1* (rs2070729)								
A/A	37	0.209	36	0.193	0.902 [0.540–1.507]	0.694	0.883 [0.507–1.539]	0.661
A/C	**76**	**0.429**	**99**	**0.529**	[b]**1.409 [1.002–2.216]**	**0.048**	[b]**1.504 [0.963–2.348]**	**0.077**
					1.495 [0.989–2.261][0.457]	0.057	1.496 [0.957–2.337]	0.073
C/C	64	0.362	52	0.278	0.680 [0.437–1.059]	0.088	0.692 [0.429–1.115]	0.130
$\chi^2 = 4.006$; $p = 0.135$								
A	150	0.424	171	0.457	1.146 [0.855–1.536]	0.363	1.225 [0.893–1.681]	0.208
C	204	0.576	203	0.543	0.873 [0.651–1.170]	0.363	0.816 [0.595–1.120]	0.208
g.42140549G>T of *IKBKB* (rs5029748)								
G/G	108	0.587	100	0.559	0.891 [0.588–1.350]	0.586	0.928 [0.594–1.450]	0.743
G/T	**40**	**0.217**	**59**	**0.330**	[b]**1.787 [1.125–2.839]**	**0.014**	[b]**1.813 [1.072–3.066]**	**0.026**
					1.770 [1.108–2.829][0.551]	0.017	1.776 [1.080–2.921][0.556]	0.024
T/T	**36**	**0.196**	**20**	**0.112**	[b]**0.507 [0.272–0.945]**	**0.032**	[b]**0.450 [0.229–0.885]**	**0.021**
					0.517 [0.286–0.934][0.647]	0.029	0.461 [0.243–0.877][0.759]	0.018
$\chi^2 = 1.509$; $p = 0.470$								
G	256	0.696	259	0. 723	1.145 [0.830–1.578]	0.409	1.210 [0.857–1.707]	0.279
T	112	0.304	99	0.277	0.874 [0.634–1.204]	0.409	0.827 [0.586–1.167]	0.279
g.186643058A>G of *PTGS2* (rs5275)								
A/A	79	0.422	81	0.433	1.045 [0.693–1.574]	0.834	1.079 [0.696–1.674]	0.734
A/G	75	0.401	83	0.444	1.192 [0.790–1.797]	0.402	1.262 [0.812–1.961]	0.302
G/G	33	0.176	23	0.123	0.654 [0.368–1.164]	0.149	0.550 [0.295–1.024]	0.059
$\chi^2 = 1.848$; $p = 0.397$								
A	233	0.623	245	0.655	1.149 [0.853–1.549]	0.361	1.225 [0.890–1.688]	0.214
G	141	0.377	129	0.345	0.870 [0.675–1.173]	0.361	0.816 [0.593–1.124]	0.214

(Continued)

Table 3 (continued).

Genotype/Allele	Control		Depression		Crude OR (95% CI)	p	Adjusted OR (95% CI)*	p
	Number	Frequency	Number	Frequency				
g.186640617C>T of *PTGS2* (rs4648308)								
C/C	130	0.703	124	0.697	0.972 [0.620–1.522]	0.900	0.927 [0.575–1.496]	0.756
C/T	40	0.216	52	0.292	1.496 [0.929–2.409]	0.097	[b]1.673 [0.994–2.815]	0.052
							1.650 [0.991–2.745][0.438]	0.054
T/T	**14**	**0.076**	**2**	**0.011**	**[b]0.129 [0.027–0.631]**	**0.011**	**[b]0.103 [0.029–0.511]**	**0.003**
					0.139 [0.031–0.620][0.932]	0.010	0.110 [0.023–0.522][0.946]	0.005
χ^2 =10.61; p = 0.005								
C	300	0.815	300	0.843	1.2148 [0.824–1.790]	0.327	1.208 [0.799–1.828]	0.370
T	68	0.184	56	0.157	0.824 [0.559–1.214]	0.327	0.828 [0.547–1.252]	0.370

Notes:
* 'Adjusted OR' means OR adjusted for sex and age; for significant comparisons the superscript b means the bootstrap-boosted OR (resampling with replacement, 10,000 iterations); all OR values without bootstrap analysis were calculated using cross-validation algorithm.
Statistical power $(1 - \beta)$ (calculated at $\alpha = 0.05$) for significant comparisons given in superscripts.
$p < 0.05$ along with corresponding ORs are in bold.

polymorphism of the *TGFA* gene is associated with an increased risk of depression development, while G/G genotype decreased this risk. Furthermore, in case of *IRF1*, carriers of A/C genotype of the g.132484229C>A (rs2070729) have a greater chance of developing the disease. Moreover, the T/T homozygote of g.186640617C>T (rs4648308) of *PTGS2* gene is negatively correlated with risk of MDD development. Similarly, In the case of g.42140549G>T (rs5029748) polymorphism of *IKBKB*, we found that T/T homozygote decreased risk of MDD occurrence, while the heterozygote of the same gene variant decreased this risk.

Single-nucleotide polymorphisms of genes encoding IRF1, IKBKB, TGFA, TGFB and PTGS2 and MDD occurrence in male and female population

Since women show two-times higher risk of MDD occurrence compared to men, we decided to investigated the association between prevalence of the disease in stratified male/female population and all studied SNPs. Results are presented in Table 4. The results demonstrated that in the case of g.70677994G>A (rs2166975) polymorphism of the *TGFA*, the A/G genotype increased the risk of MDD in men, but not in women. Moreover, allele A of this SNP was associated with decreased chance of the disease, while allele G was strongly correlated with higher risk of MDD. Furthermore, in male population allele G and G/G homozygote of the g.186643058A>G (rs5275) of *PTGS2* decreased risk of depression while, allele A and A/A homozygote of the same polymorphism was associated with increased risk of the occurrence of the disease. Additionally, it was found that A/G genotype of this SNP was correlated with higher risk of MDD in the female group. Another SNP of *PTGS2* gene, g.186640617C>T (rs4648308) was associated with MDD risk in both studied groups. Precisely, C/T genotype was positively correlated with the risk of the occurrence of MDD in women. Similarly, allele C of the mentioned polymorphism

Table 4 Distribution of genotypes and alleles of rs2070729 (*IRF1*), rs5275 (*PTGS2*), rs4648308 (*PTGS2*), rs2166975 (*TGFA*), rs5029748 (*IKBKB*) and the risk of depression occurrence in male and female population.

Genotype/Allele	Control		Depression		Crude OR (95% CI)	p	Adjusted OR (95% CI)*	p
	Number	Frequency	Number	Frequency				
Male								
g.70677994G>A of *TGFA* (rs2166975)								
A/A	13	0.126	8	0.085	0.644 [0.254–1.641]	0.353	0.808 [0.302–2.164]	0.671
A/G	**34**	**0.330**	**45**	**0.479**	[b]1.843 [1.037–3.272]	**0.037**	[b]2.318 [1.222–4.400]	**0.010**
					1.864 [1.047–3.317][0.468]	0.034	2.280 [1.218–4.268][0.733]	0.009
G/G	56	0.544	41	0.436	0.649 [0.370–1.145]	0.132	[b]0.476 [0.250–0.905]	**0.024**
							0.480 [0.257–0.898][0.740]	0.022
$\chi^2 = 4.640$; $p = 0.098$								
A	60	0.291	61	0.709	[b]0.109 [0.062–0.189]	<0.001	[b]0.106 [0.060–0.186]	<0.001
					0.113 [0.065–0.195][0.992]	<0.001	0.109 [0.063–0.190][0.999]	<0.001
G	146	0.324	127	0.676	[b]9.005 [5.242–15.468]	<0.001	[b]9.281 [5.308–16.225]	<0.001
					8.861 [5.125–15.319][0.992]	<0.001	9.135 [5.260–15.867][0.999]	<0.001
g.42140549G>T of *IKBKB* (rs5029748)								
G/G	61	0.610	53	0.589	0.916 [0.510–1.644]	0.769	0.955 [0.514–1.776]	0.885
G/T	**19**	**0.190**	**30**	**0.333**	[b]2.153 [1.082–4.288]	**0.029**	[b]2.073 [1.016–4.300]	**0.049**
					2.132 [1.097–4.143][0.466]	0.026	2.063 [1.024–4.154][0.423]	0.049
T/T	**20**	**0.200**	**7**	**0.078**	[b]0.316 [0.118–0.849]	**0.022**	[b]0.295 [0.100–0.869]	**0.027**
					0.337 [0.135–0.841][0.758]	0.020	0.310 [0.116–0.830][0.799]	0.021
$\chi^2 = 8.788$; $p = 0.012$								
G	141	0.705	136	0.756	1.211 [0.817–1.796]	0.341	1.305 [0.826–2.061]	0.253
T	59	0.295	44	0.244	0.826 [0.557–1.225]	0.341	0.766 [0.485–1.210]	0.253
g.186643058A>G of *PTGS2* (rs5275)								
A/A	**40**	**0.392**	**48**	**0.505**	1.583 [0.896–2.796]	0.111	[b]2.073 [0.999–4.300]	**0.050**
							1.803 [0.982–3.309][0.464]	0.057
A/G	41	0.402	37	0.389	0.949 [0.534–1.687]	0.858	0.852 [0.462–1.575]	0.611
G/G	21	0.206	10	0.105	0.454 [0.201–1.028]	0.057	[b]0.427 [0.171–1.019]	**0.052**
							0.438 [0.186–1.032][0.599]	0.059
$\chi^2 = 4.593$; $p = 0.101$								
A	**121**	**0.593**	**133**	**0.700**	[b]1.588 [1.031–2.445]	**0.036**	[b]1.659 [1.064–2.586]	**0.025**
					1.601 [1.054–2.430][0.672]	0.027	1.664 [1.087–2.548][0.745]	0.019
G	**83**	**0.407**	**57**	**0.300**	[b]0.621 [0.399–0.968]	**0.035**	[b]0.603 [0.393–0.926]	**0.021**
					0.625 [0.412–0.949][0.608]	0.027	0.601 [0.393–0.920][0.666]	0.019
g.186640617C>T of *PTGS2* (rs4648308)								
C/C	65	0.663	67	0.736	1.417 [0.754–2.664]	0.276	1.335 [0.687–2.595]	0.394
C/T	25	0.255	24	0.264	1.046 [0.543–2.014]	0.892	1.128 [0.564–2.255]	0.734
T/T	8	0.082	0	0	–	–	–	–
$\chi^2 = 7.802$; $p = 0.020$								
C	**155**	**0.791**	**158**	**0.868**	[b]1.772 [0.996–3.162]	0.052	[b]1.744 [0.983–3.094]	**0.049**
					1.741 [1.004–3.019][0.848]	0.048	1.751 [1.007–3.040][0.854]	0.047
T	**41**	**0.209**	**24**	**0.132**	[b]0.567 [0.322–0.996]	**0.049**	[b]0.566 [0.315–0.999]	**0.049**
					0.574 [0.331–0.996][0.553]	0.048	0.571 [0.329–0.993][0.558]	0.047

(Continued)

Table 4 (continued).

Genotype/Allele	Control		Depression		Crude OR (95% CI)	p	Adjusted OR (95% CI)*	p
	Number	Frequency	Number	Frequency				
Female								
g.132484229C>A of *IRF1* (rs2070729)								
A/A	20	0.244	20	0.215	0.849 [0.417–1.730]	0.650	0.655 [0.297–1.437]	0.291
A/C	**32**	**0.390**	**48**	**0.516**	**1.667 [0.910–3.056]**	**0.096**	**[b]2.016 [1.025–3.966]**	**0.042**
							1.936 [1.003–3.738][0.508]	**0.049**
C/C	30	0.366	25	0.269	0.637 [0.334–1.216]	0.169	0.657 [0.328–1.318]	0.237
$\chi^2 = 2.975$; $p = 0.226$								
A	72	0.439	88	0.473	1.136 [0.756–1.706]	0.539	1.159 [0.730–1.840]	0.532
C	92	0.561	98	0.527	0.880 [0.586–1.322]	0.539	0.862 [0.544–1.370]	0.532
g.186643058A>G of *PTGS2* (rs5275)								
A/A	39	0.459	33	0.359	0.661 [0.359–1.211]	0.176	0.595 [0.309–1.143]	0.119
A/G	**34**	**0.400**	**46**	**0.500**	**1.500 [0.823–2.734]**	**0.183**	**1.962 [1.024–3.758]**	**0.042**
							1.952 [1.017–3.746][0.524]	**0.044**
G/G	12	0.141	13	0.141	1.001 [0.427–2.344]	0.998	0.718 [0.284–1.812]	0.483
$\chi^2 = 2.006$; $p = 0.356$								
A	112	0.659	112	0.609	0.810 [0.527–1.244]	0.336	0.833 [0.524–1.325]	0.441
G	58	0.341	72	0.391	1.235 [0.804–1.898]	0.336	0.816 [0.755–1.908]	0.441
g.186640617C>T of *PTGS2* (rs4648308)								
C/C	65	0.756	57	0.655	0.614 [0.315–1.195]	0.148	0.595 [0.294–1.205]	0.149
C/T	**15**	**0.174**	**28**	**0.322**	**[b]2.270 [1.100–4.684]**	**0.027**	**[b]2.574 [1.224–5.415]**	**0.013**
					2.246 [1.098–4.596][0.806]	**0.027**	**2.533 [1.178–5.449][0.587]**	**0.017**
T/T	6	0.070	2	0.023	0.314 [0.061–1.620]	0.163	0.211 [0.037–1.211]	0.081
$\chi^2 = 6.449$; $p = 0.039$								
C	145	0.843	142	0.816	0.843 [0.495–1.435]	0.530	0.853 [0.469–1.553]	0.603
T	27	0.157	32	0.184	1.186 [0.697–2.018]	0.530	1.172 [0.644–2.135]	0.603

Notes:
* 'Adjusted OR' means OR adjusted for sex and age; for significant comparisons the superscript b means the bootstrap-boosted OR (resampling with replacement, 10,000 iterations); all OR values without bootstrap analysis were calculated using cross-validation algorithm.
Statistical power $(1 - \beta)$ (calculated at $\alpha = 0.05$) for significant comparisons given in superscripts.
$p < 0.05$ along with corresponding ORs are in bold.

increased prevalence of the disease among men, while allele T decreased this risk. We also found that genotypes of g.42140549G>T (rs5029748) polymorphism of *IKBKB* gene were related with appearance of MDD in male population. Particularly, G/T genotype was connected with increased risk of depression, while T/T genotype of the same SNP decreased this risk.

Gene-gene interactions of IRF1, IKBKB, TGFA, TGFB and PTGS2 and the risk of MDD

In this research, we also studied whether the combined genotypes of investigated polymorphism are associated with appearance of MDD. Results are presented in Table 5. In reference to effect of combined genotypes, it was found that G/G-T/T genotypes of

Table 5 Gene-gene interactions of rs1800469 (*TGFB1*), rs2070729 (*IRF1*), rs5275 (*PTGS2*), rs4648308 (*PTGS2*), rs2166975 (*TGFA*), rs5029748 (*IKBKB*) and the risk of depression occurrence.

Combined genotype	Control (*n* = 180)		Depression (*n* = 180)		Crude OR (95% CI)	*p*	Adjusted OR (95% CI)*	*p*
	Number	Frequency	Number	Frequency				
g.41354391A>G of *TGFB1* (rs1800469)–g.70677994G>A of *TGFA* (rs2166975)								
A/G-A/G	24	0.126	35	0.186	1.592 [0.904–2.803]	0.106	[b]1.906 [1.032–3.518]	0.039
							1.898 [1.036–3.477][0.490]	0.038
g.70677994G>A of *TGFA* (rs2166975)–g.132484229C>A of *IRF1* (rs2070729)								
A/G-A/C	27	0.141	45	0.241	[b]1.951 [1.152–3. 305]	0.013	[b]2.117 [1.224–3.660]	0.007
					1.925 [1.136–3.262][0.554]	0.015	2.092 [1.193–3.660][0.667]	0.010
g.70677994G>A of *TGFA* (rs2166975)–g.186643058A>G of *PTGS2* (rs5275)								
G/G-G/G	22	0.115	8	0.043	[b]0.320 [0.127–0.807]	0.016	[b]0.223 [0.087–0.574]	0.002
					0.341 [0.148–0.788][0.828]	0.012	0.233 [0.094–0.579][0.940]	0.002
A/A-G/G	7	0.037	1	0.005	0.139 [0.017–1.141]	0.066	[b]0.167 [0.027–1.031]	0.054
							0.129 [0.014–1.159][0.805]	0.068
A/G-G/G	4	0.021	14	0.074	[b]3.581 [1.233–13.12]	0.026	[b]4.264 [1.416–12.839]	0.010
					3.761 [1.215–11.647][0.291]	0.022	4.137 [1.263–13.545][0.291]	0.019
g.70677994G>A of *TGFA* (rs2166975)–g.186640617C>T of *PTGS2* (rs4648308)								
G/G-T/T	12	0.063	1	0.005	[b]0.087 [0.013–0.638]	0.018	[b]0.057 [0.011–0.312]	0.001
					0.080 [0.010–0.620][0.942]	0.016	0.051 [0.006–0.420][0.948]	0.006
A/G-C/T	10	0.052	25	0.133	[b]3.005 [1.242–7.269]	0.015	[b]3.240 [1.442–7.280]	0.004
					2.776 [1.294–5.956][0.584]	0.009	3.115[1.397–6.944] [0.663]	0.005
g.70677994G>A of *TGFA* (rs2166975)–g.42140549G>T of *IKBKB* (rs5029748)								
G/G-T/T	23	0.120	9	0.048	[b]0.362 [0.156–0.840]	0.018	[b]0.286 [0.106–0.772]	0.013
					0.367 [0.165–0.816][0.801]	0.014	0.306 [0.131–0.719][0.882]	0.007
A/G-G/T	11	0.058	24	0.128	[b]2.393 [1.136–5.042]	0.022	[b]2.645 [1.184–5.910]	0.018
					2.395 [1.138–5.041][0.472]	0.021	2.621 [1.208–5.688][0.571]	0.015
g.132484229C>A of *IRF1* (rs2070729)–g.186643058A>G of *PTGS2* (rs5275)								
A/C-A/G	29	0.152	49	0.261	[b]2.077 [1.206–3.576]	0.008	[b]1.863 [1.022–3.394]	0.042
					1.969 [1.180–3.286][0.614]	0.009	1.844 [1.069–3.180][0.515]	0.028
g.132484229C>A of *IRF1* (rs2070729)–g.42140549G>T of *IKBKB* (rs5029748)								
A/C-G/T	16	0.084	29	0.154	[b]2.032 [1.036–3.989]	0.039	[b]1.918 [0.935–3.931]	0.075
					1.995 [1.044–3.810][0.402]	0.036	1.901 [0.958–3.774][0.362]	0.066
g.42140549G>T of *IKBKB* (rs5029748)–g.186643058A>G of *PTGS2* (rs5275)								
T/T-G/G	14	0.073	2	0.011	[b]0.131 [0.037–0.598]	0.008	[b]0.126 [0.027–0.589]	0.008
					0.136 [0.030–0.607][0.936]	0.009	0.132 [0.02–0.610][0.939]	0.009
G/T-A/G	16	0.084	31	0.165	[b]2.235 [1.114–4.487]	0.024	[b]1.933 [0.883–4.233]	0.008
					2.160 [1.138–4.098][0.512]	0.018	1.894 [0.968–3.704][0.357]	0.009
g.42140549G>T of *IKBKB* (rs5029748)–g.186640617C>T of *PTGS2* (rs4648308)								
G/T-C/T	4	0.021	19	0.101	[b]5.013 [1.531–18.121]	0.005	[b]4.164 [1.232–15.343]	0.035
					5.256 [1.753–15.760][0.291]	0.003	4.320 [1.390–13.428][0.286]	0.011

Notes:
* 'Adjusted OR' means OR adjusted for sex and age; for significant comparisons the superscript b means the bootstrap-boosted OR (resampling with replacement, 10,000 iterations); all OR values without bootstrap analysis were calculated using cross-validation algorithm.
Statistical power (1 − β) (calculated at α = 0.05) for significant comparisons given in superscripts.
p < 0.05 along with corresponding ORs are in bold.

g.70677994G>A (rs2166975)—*TGFA* and g.186640617C>T (rs4648308)—*PTGS2* was associated with decreased risk of depression occurrence, while A/G-C/T genotypes increased this risk. The A/G-A/C genotypes of g.70677994G>A (rs2166975)—*TGFA* and g.132484229C>A (rs2070729)—*IRF1* as well as A/G-A/G genotypes of g.70677994G>A (rs2166975) *TGFA* and g.41354391A>G (rs1800469)—*TGFB* also increased the risk of the disease. Furthermore, higher risk of MDD occurrence was associated with the G/T-A/G genotypes of g.42140549G>T (rs5029748)—*IKBKB* and g.186643058A>G (rs5275)—*PTGS2*, however the T/T-G/G genotypes reduced this risk. In the case of linked genotypes of g.70677994G>A (rs2166975)—*TGFA* and g.186643058A>G (rs5275)—*PTGS2*, we found that link between A/G-G/G of this genes was associated with higher risk of appearance of the MDD, while G/G-G/G as well as A/A-G/G genotypes decreased this chance. Similarly, A/G-G/T combined genotypes of g.70677994G>A (rs2166975)—*TGFA* and g.42140549G>T (rs5029748)—*IKBKB* increased risk of MDD but G/G-T/T genotypes of the same SNP were associated with lower risk of disease incidence. Moreover, carriers of A/C-A/G combined genotypes of g.132484229C>A (rs2070729)—I*RF1* and g.186643058A>G (rs5275)—*PTGS*, A/C-G/T of g.132484229C>A (rs2070729)—I*RF1* and g.42140549G>T (rs5029748)—*IKBKB* as well as G/T-C/T genotypes of g.42140549G>T (rs5029748)—*IKBKB* and g.186640617C>T (rs4648308)—*PTGS2* had a greater risk of MDD appearance.

Single-nucleotide polymorphisms of genes encoding IRF1, IKBKB, TGFA, TGFB, PTGS2 and the age of the first episode of MDD and the severity classification on the hamilton depression rating scale

To estimate whether the investigated polymorphisms may had an impact on the age of the first episode of MDD, patients were stratified in accordance to genotype and their age of onset was compared (Fig. 1). A significant difference was found between A/A and A/G genotypes as well as A/A and G/G genotypes of g.41354391A>G (rs1800469)—*TGFB1*. Carriers of A/A genotype had their first episode significantly earlier compared to other genotypes.

In the case of the impact of genotypes of the investigated SNPs on the episode severity measured using the Hamilton Depression Rating Scale (HDRS) (Fig. 2), significant differences was found between carriers of A/A and G/G genotypes of g.41354391A>G (rs1800469)—*TGFB1*.

Single-nucleotide polymorphisms of genes encoding IRF1, IKBKB, TGFA, TGFB, PTGS2 and effectiveness of depression treatment

We also evaluated impact of the studied polymorphisms on the effectiveness of antidepressant treatment with selective serotonin reuptake inhibitor (SSRI) (Fig. 3). Regarding the effect of investigated SNPs on treatment efficiency, differences was found between A/A and A/C genotypes of g.132484229C>A (rs2070729)—*IRF1* as well as G/G and T/T genotypes of g.42140549G>T (rs5029748)—*IKBKB*.
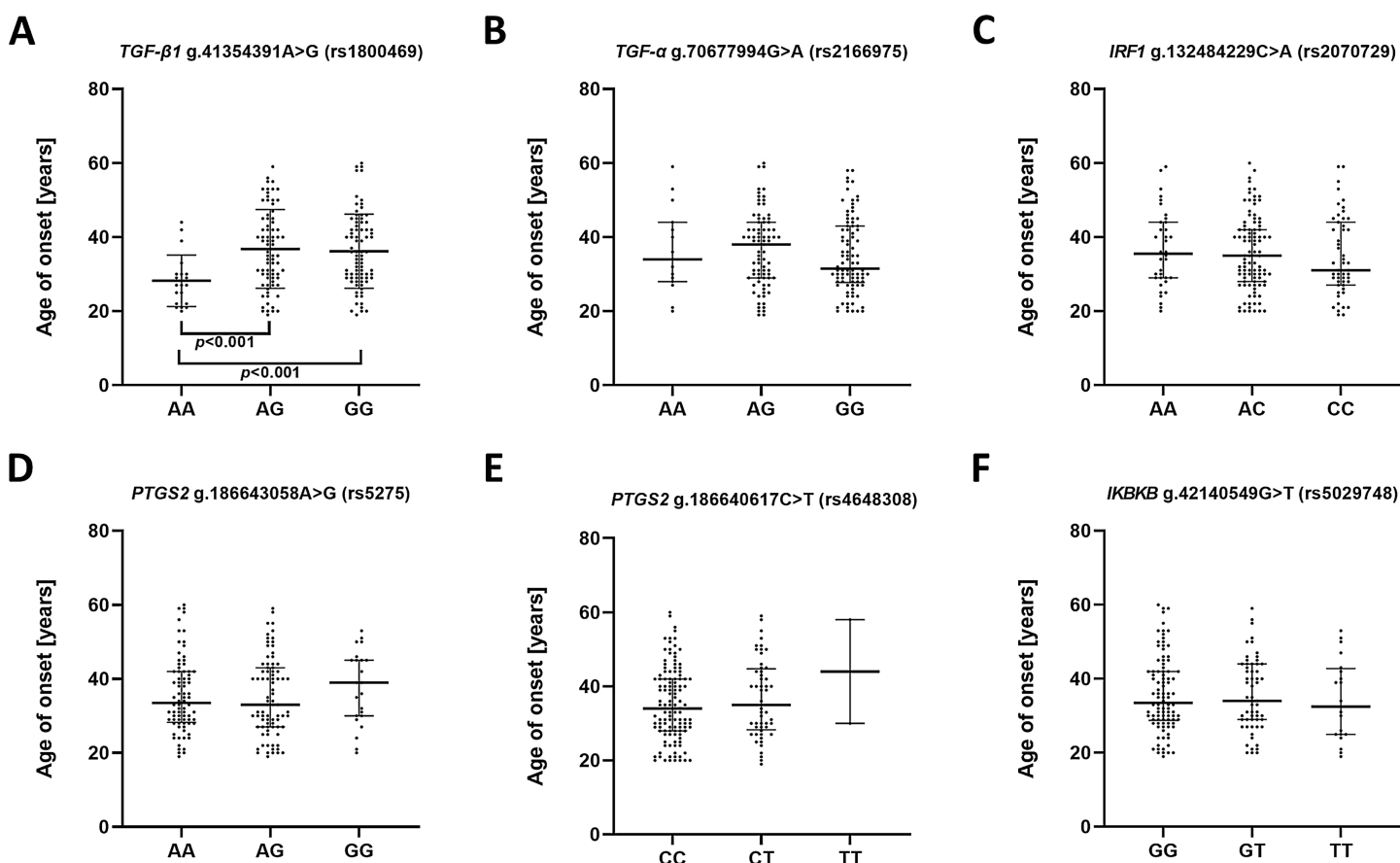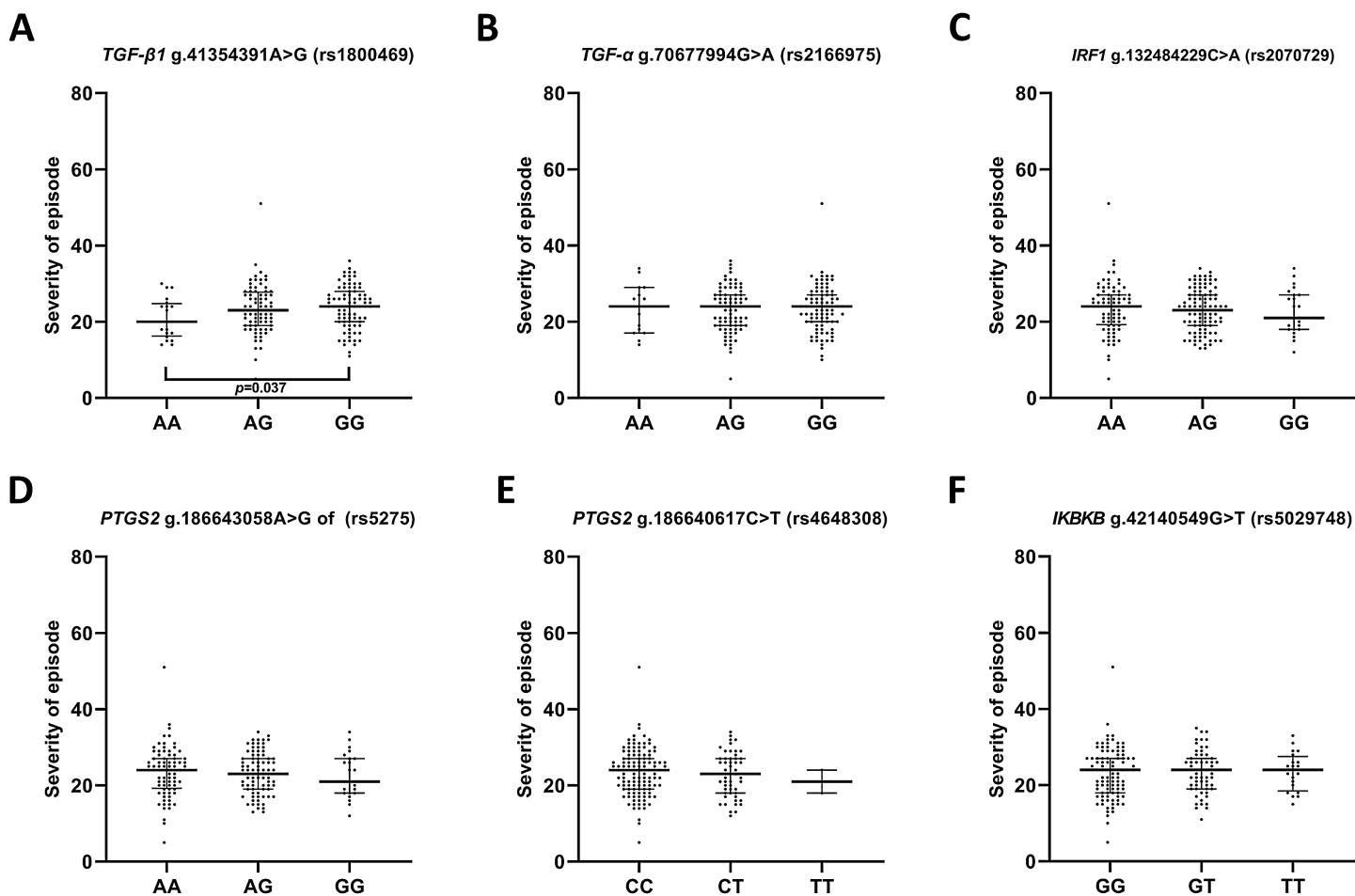
Figure 1 Impact of single-nucleotide polymorphisms localized in inflammatory genes on the age of the first episode of MDD. (A) *TGFB1* g.41354391A>G (rs1800469) (B) *TGFA* g.70677994G>A (rs2166975) (C) *IRF1* g.132484229C>A (rs2070729) (D) *PTGS2* g.186643058A>G (rs5275) (E) *PTGS2* g.186640617C>T (rs4648308) (F) *IKBKB* g.42140549G>T (rs5029748). Results are presented as scatter dot plots. The horizontal lines denote the median, while the whiskers show the inter-quartile range.
Full-size ◲ DOI: 10.7717/peerj.8676/fig-1

DISCUSSION

There is strong amount of evidence that inflammation is undeniably associated with major depressive disorder. Moreover, it was confirmed that some inflammatory genes and presence of their genetics variants play important role in MDD development. Additionally, several loci/chromosomal regions connected with MDD were mapped by genome-wide linkage analysis, that is, 1q32.1, 2p25.1, 3p21.1, 3p26.1, 3q26.1, 6p22.3, 8q22.2, 8q22.3, 8q12.1, 8q23.3, 11p14.2-p14.3, 13q31.1–q31.3, 15q25.2 and 19q12 (*McGuffin et al., 2005*; *Shyn et al., 2011*; *Sullivan et al., 2013*). Selected candidate genes in current study are located in proximity to the above mentioned regions of chromosomes. In this research, we genotyped six polymorphic variants of *TGFA, TGFB1, IRF1* and *PTGS2* genes; and to our knowledge, none of this SNPs have been studied in the context of severity and treatment response in depression before. However, these SNPs were included in GWAS but only one of them, that is, rs2070729, had *p* value below 0.05.

The first of investigated polymorphisms in this study was g.70677994G>A (rs2166975)—*TGFA*. The SNP is localized on 2p13.3 and it is responsible for synonymous change

Figure 2 Distribution of the severity of episode (before therapy) and single nucleotide polymorphisms localized in inflammatory genes. Severity of current episode according to 21-item Hamilton Depression Rating Scale (HAM-D) (A) *TGFB1* g.41354391A>G (rs1800469) (B) *TGFA* g.70677994G>A (rs2166975) (C) *IRF1* g.132484229C>A (rs2070729) (D) *PTGS2* g.186643058A>G (rs5275) (E) *PTGS2* g.186640617C>T (rs4648308) (F) *IKBKB* g.42140549G>T (rs5029748). Results are presented as scatter dot plots. The horizontal lines denote the median, while the whiskers show the inter-quartile range.   Full-size ◩ DOI: 10.7717/peerj.8676/fig-2

Val159Val. This terminal amino acid is present in the precursor protein and is necessary for glycosylation during protein maturation as well as protein localization to the cell surface (*Briley et al., 1997*). In our study, we were the first to show a link between rs2166975 polymorphism of *TGFA* and depression. The results confirmed that A/G genotype of rs2166975 is more frequently distributed in patients suffering from depression. Interestingly, the same genotype increased the risk of MDD only in man population. In the case of the gene–gene interactions between polymorphism of *TGFA* and other SNPs, analysis confirmed that A/G-A/C combined genotypes of rs2166975—*TGFA* and rs207072—*IRF1* are associated with higher chance to develop MDD. In addition, A/G-G/G genotypes of rs2166975—*TGFA* and rs5275—*PTGS2* is associated with higher risk of MDD, while G/G-G/G homozygotes decreased this chance. It was indicated that rs2166975 in *TGFA* gene, showed association with the risk of cleft palate (*Morkūnienė et al., 2007*). Furthermore, another study confirmed, using transmission disequilibrium test, that minor allele of
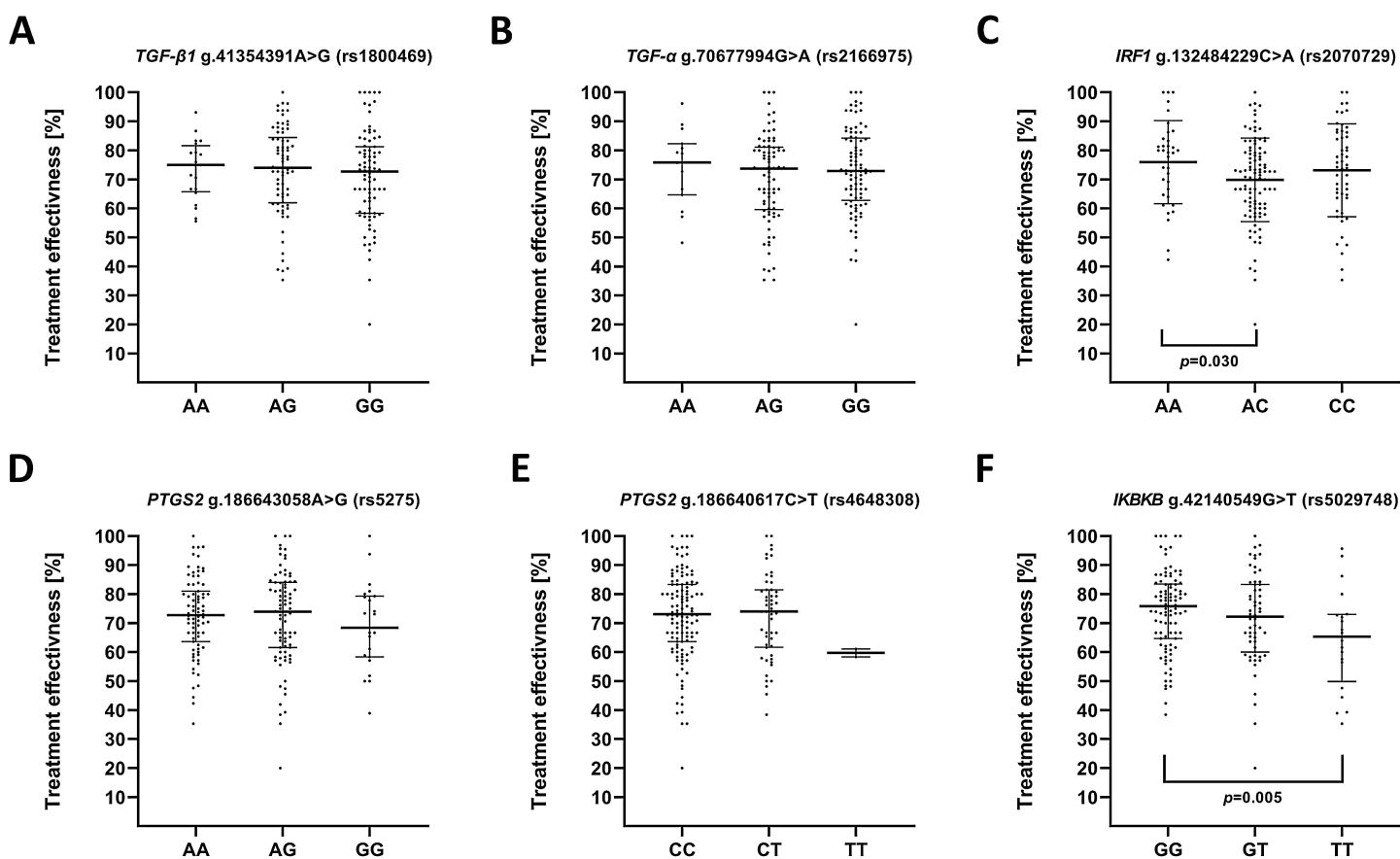
Figure 3 Impact of single-nucleotide polymorphisms localized in inflammatory genes on the effectiveness of the treatment. Treatment effectiveness expressed as percentage of HAM-D decline after therapy. (A) *TGFB1* g.41354391A>G (rs1800469) (B) *TGFA* g.70677994G>A (rs2166975) (C) *IRF1* g.132484229C>A (rs2070729) (D) *PTGS2* g.186643058A>G (rs5275) (E) *PTGS2* g.186640617C>T (rs4648308) (F) *IKBKB* g.42140549G>T (rs5029748). Results are presented as scatter dot plots. The horizontal lines denote the median, while the whiskers show the inter-quartile range. Full-size 🖼 DOI: 10.7717/peerj.8676/fig-3

rs2166975 was over-transmitted to cleft-palate cases (*Carter et al., 2010*). Although there are no studies investigating role of rs2166975 polymorphism in depression or any other psychiatric disorders, our results suggest important role of investigated polymorphism in pathophysiology and course of depression.

The second studied SNP, g.41354391A>G (rs1800469) of *TGFB1*, is located on 19q13.2 in the proximal negative regulatory region of the gene. The human TGFB1 protein is considered to be one of the immunosuppressive cytokines, which plays crucial role in CNS development (*Sousa Vde et al., 2004*). It is responsible for such functions as astrocyte differentiation, synaptogenesis and neuronal migration (*De Sampaio e Spohr et al., 2002*; *Sousa Vde et al., 2004*; *Feng & Ko, 2008*; *Siegenthaler & Miller, 2004*). Our results show that rs1800469 polymorphism is associated with both severity of depressive episodes and age of the onset of the disease. Precisely, carriers of G/G genotype are characterized by more severe episodes than A/A genotype carriers, which may correlate with increased concentrations of TGFB1. Moreover, a significant difference in the age of the first episode of MDD was found between A/A and A/G genotypes, as well as A/A and G/G genotypes

of rs1800469—*TGFB1*. In accordance to our findings, TGFB levels were found to be increased in people suffering from MDD (*Davami et al., 2016*; *Kim et al., 2007*; *Kim et al., 2008*) as well as in Chronic HBV-Infected Patients (CHB) with mild depression symptoms (*Bahramabadi et al., 2017*). It has been reported that rs1800469, is not associated with neither Alzheimer's disease risk (*Chang et al., 2013*) nor Schizophrenia (*Kapelski et al., 2015*). However rs1800469 of *TGFB1* is associated with altered plasma levels of TGFB1, which may modulate a susceptibility to MDD (*Shah et al., 2006*; *Wang et al., 2008*). Data suggest that, allele G is associated with lower expression of *TGFB1* (*Shah et al., 2006*). On the other hand, another study confirmed that genotypes A/G and G/G was correlated with increased plasma TGFB1 concentrations, indicating that G allele is associated with higher production of the protein (*Wang et al., 2008*). It was found that other SNPs of *TGFB1* could be associated with MDD. In the case of rs1800470 (codon 10), genotype T/T is significantly more frequently distributed in depressed patients (*Mihailova et al., 2016*). Moreover, another study revealed that C/C genotype of the same SNP is positively correlated with higher risk of depression development and more severe episodes of the disease (*Caraci et al., 2012*). Although TGFB1 is considered to play important role in psychoneuroimmunology, there is only few research about its association with mental disorders, and interestingly there is no other studies investigated role of mentioned rs1800469 in MDD.

In our study we also investigated whether SNPs in *PTGS2* gene are involved in MDD development. As mentioned in Introduction, PTGS2 participates in inflammatory processes partly related with neurodegeneration in CNS (*Minghetti, 2004*). There is evidence demonstrating that rs20417 polymorphism of *PTGS2* may play a role in MDD. Precisely, presence of G allele is strongly associated with increased risk of depression development (*Gałecki et al., 2010*). However, we have not included this polymorphism in our study. Instead, we explored g.186640617C>T (rs4648308) polymorphism located on 1q31.1. There are evidence of its involvement in depression. Precisely, allele T and C/T genotype (in positive strand allele A and G/A genotype) of mentioned SNP are associated with significantly increased risk of IFN-α-induced depression (*Su et al., 2010*). Part of our result are consistent with this findings, namely, we found that C/T heterozygote increased risk of MDD in woman, as well as the C allele increased this chance in man group. On the contrary, we also reported that T/T genotype carriers of this SNP are less likely to develop depression in general population. Similarly, in man group allele T was also negatively correlated with depression prevalence.

Second polymorphism of *PTGS2* gene, g.186643058A>G (rs5275) located on 1q31.1, is a functional SNP, which modulates expression of PTGS2. We were first to found that allele G is connected with higher chance of MDD occurrence. Additionally, it is confirmed that this SNP is associated with severe pain in lung cancer patients. Namely, A/A and A/G (in forward strand T/T and T/C) carriers experience more severe pain than G/G carriers (*Reyes-Gibby et al., 2009*; *Reyes-Gibby et al., 2013*). However, *Mendlewicz et al. (2012)* found no association between *PTGS2* rs5275 polymorphism and treatment response and remission of MDD. Still, there are no other studies investigated aforementioned SNPs in *PTGS2* gene in context of MDD.

Another SNP candidate in our research was g.42140549G>T (rs5029748) of *IKBKB* gene. It is located on 8p11.21, in intronic region of the gene, thus do not cause amino acid substitution. We were first to analyze the mentioned polymorphism as a risk factor for MDD. Our main finding relates to the connection between this SNP and effectiveness of depression treatment. Namely, we demonstrated differences in SSRI response between carriers of G/G and T/T genotypes. Moreover, presence of G/T genotype of rs5029748 is associated with increased risk of MDD development either in general or man population, while the T/T homozygote of the same gene variant reduces this risk in the same studied groups. In addition, carrier of combined G/T-A/G genotypes of rs5029748—*IKBKB* and rs5275—*PTGS2* are more likely to develop MDD, while T/T-G/G genotype showed protective effect. Moreover A/G-G/T genotype of rs5029748 *IKBKB* and rs2166975—*TGFA*, increased risk of depression but G/T-T/T are associated with lower risk of disease. The trend of increasing risk of depression prevalence is also present in the case of linked genotypes of rs5029748—IKBKB and rs4648308—PTGS2. Some studies revealed association between aforementioned SNP and risk of colorectal or colon cancer (*Seufert et al., 2013*; *Curtin et al., 2013*). Precisely, minor allele T of rs5029748, was associated with decreased risk of colon cancer (*Curtin et al., 2013*). Although our result showed that single-nucleotide polymorphism of *IKBKB* may play significant role in MDD, they have not been investigated in pathogenesis of the disease before.

The g.132484229C>A (rs2070729)—*IRF1* polymorphism was the last studied SNP in this article. It is located on 5q31.1 in intronic gene region. The SNP is associated with susceptibility to hepatitis C virus (HCV) infection (*Fortunato et al., 2008*). What is more, allele C of this SNP is linked to higher vulnerability HIV-1 acquisition (*Lingappa et al., 2011*). To our best knowledge, we were first to analyze role of rs2070729 in MDD. Regarding the effect of investigated SNP on treatment efficiency, data in our study showed significant differences in antidepressant response between A/A and A/C genotypes of rs2070729—*IRF1*, A/A carriers were more likely to better treatment response. Exact explanation of this mechanism has not been elucidated yet in previous research. However, since A allele is a minor one in European population, we speculate that it might be associated with decreased expression of IRF1 and thus reduction of inflammatory cytokine release. Therefore, together with anti-inflammatory properties of antidepressants it could enhance the their effect. We also found that carriers of A/C genotype of rs2070729—*RF1* were linked with A/G of rs5275—*PTGS* or G/T of rs5029748—*IKBKB* had a greater risk of MDD appearance. These results suggest that SNP in *IRF1* gene may have impact in depression development.

Our preliminary study has several potential limitations. Firstly, the sample size was relatively small. Nevertheless, two resampling approaches were performed so as to minimize the risk of obtaining false positive results. Another limitation was the homogenic ethnicity of studied group. This could reduce the potential to extrapolate the results to other ethnic groups. Furthermore, it must be emphasized that there is limited data on the impact of these SNPs on the level of mRNA and protein expression/activity. Consequently, presented results should be considered preliminary and interpreted with caution.

CONCLUSIONS

The single-nucleotide polymorphisms located in *IRF1, IKBKB, TGFA, TGFB1, PTGS2* genes modulate the risk of occurrence, age of onset, severity of the disease and response to the antidepressant treatment. Our result suggest that inflammatory pathways, in which studied genes are involved may be at least partially implicated in etiology of MDD. Moreover, discovery about impact of *IRF1* and *IKBKB* SNPs on treatment response could contribute to the discovery of effective, personalized pharmacotherapy. However, future studies should elucidate the implication of the studied polymorphisms in biological functions, for example, mRNA and protein expression, protein activity. On the whole, our results might cast a new light on the pathogenesis of major depressive disorders.

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Katarzyna Bialek conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Piotr Czarny conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Cezary Watala analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Paulina Wigner conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Monika Talarowska performed the experiments, analyzed the data, authored or reviewed drafts of the paper, diagnosis of the patients, and approved the final draft.
- Piotr Galecki conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, diagnosis of the patients, and approved the final draft.

- Janusz Szemraj conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Tomasz Sliwinski conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Human Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Protocol of the study was approved by the Bioethics Committee of the Medical University of Lodz (No. RNN/70/14/KE).

Data Availability

The following information was supplied regarding data availability:

The raw data is available in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.8676#supplemental-information.

REFERENCES

Aktan F. 2004. iNOS-mediated nitric oxide production and its regulation. *Life Sciences* **75(6)**:639–653 DOI 10.1016/j.lfs.2003.10.042.

Bahramabadi R, Fathollahi MS, Hashemi SM, Arababadi MS, Yousefi-Daredor H, Bidaki R, Khaleghinia M, Bakhshi MH, Yousefpoor Y, Torbaghan YE, Arababadi MK. 2017. Serum levels of IL-6, IL-8, TNF-α, and TGF-β in chronic HBV-infected patients: effect of depression and anxiety. *Laboratory Medicine* **49(1)**:41–46.

Bierhaus A, Wolf J, Andrassy M, Rohleder N, Humpert PM, Petrov D, Ferstl R, Von Eynatten M, Wendt T, Rudofsky G, Joswig M, Morcos M, Schwaninger M, McEwen B, Kirschbaum C, Nawroth PP. 2003. A mechanism converting psychosocial stress into mononuclear cell activation. *Proceedings of the National Academy of Sciences of the United States of America* **100(4)**:1920–1925 DOI 10.1073/pnas.0438019100.

Breyer RM, Bagdassarian CK, Myers SA, Breyer MD. 2001. Prostanoid receptors: subtypes and signaling. *Annual Review of Pharmacology and Toxicology* **41(1)**:661–690 DOI 10.1146/annurev.pharmtox.41.1.661.

Briley GP, Hissong MA, Chiu ML, Lee DC. 1997. The carboxyl-terminal valine residues of proTGF alpha are required for its efficient maturation and intracellular routing. *Molecular Biology of the Cell* **8(8)**:1619–1631 DOI 10.1091/mbc.8.8.1619.

Capuron L, Miller AH. 2011. Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacology & Therapeutics* **130(2)**:226–238 DOI 10.1016/j.pharmthera.2011.01.014.

Caraci F, Bosco P, Signorelli M, Spada RS, Cosentino FI, Toscano G, Bonforte C, Muratore S, Prestianni G, Panerai S, Giambirtone MC, Gulotta E, Romano C, Salluzzo MG, Nicoletti F, Copani A, Drago F, Aguglia E, Ferri R. 2012. The CC genotype of transforming growth factor-β1 increases the risk of late-onset Alzheimer's disease and is associated with AD-related depression. *European Neuropsychopharmacology* **22(4)**:281–289 DOI 10.1016/j.euroneuro.2011.08.006.

Cardinez C, Miraghazadeh B, Tanita K, Da Silva E, Hoshino A, Okada S, Chand R, Asano T, Tsumura M, Yoshida K, Ohnishi H, Kato Z, Yamazaki M, Okuno Y, Miyano S, Kojima S, Ogawa S, Andrews TD, Field MA, Burgio G, Morio T, Vinuesa CG, Kanegane H, Cook MC. 2018. Gain-of-function IKBKB mutation causes human combined immune deficiency. *Journal of Experimental Medicine* **215(11)**:2715–2724 DOI 10.1084/jem.20180639.

Carter TC, Molloy AM, Pangilinan F, Troendle JF, Kirke PN, Conley MR, Orr DJ, Earley M, McKiernan E, Lynn EC, Doyle A, Scott JM, Brody LC, Mills JL. 2010. Testing reported associations of genetic risk factors for oral clefts in a large Irish study population. *Birth Defects Research. Part A Clinical and Molecular Teratology* **88(2)**:84–93.

Cassano P, Hidalgo A, Burgos V, Adris S, Argibay P. 2006. Hippocampal upregulation of the cyclooxygenase-2 gene following neonatal clomipramine treatment (a model of depression). *Pharmacogenomics Journal* **6(6)**:381–387 DOI 10.1038/sj.tpj.6500385.

Chang W-W, Zhang L, Jin Y-L, Yao Y-S. 2013. Meta-analysis of the transforming growth factor-β1 polymorphisms and susceptibility to Alzheimer's disease. *Journal of Neural Transmission* **120(2)**:353–360 DOI 10.1007/s00702-012-0850-7.

Curtin K, Wolff RK, Herrick JS, Abo R, Slattery ML. 2013. Exploring multilocus associations of inflammation genes and colorectal cancer risk using hapConstructor. *BMC Medical Genetics* **11**:170.

Czarny P, Wigner P, Strycharz J, Swiderska E, Synowiec E, Szatkowska M, Sliwinska A, Talarowska M, Szemraj J, Su KP, Maes M, Sliwinski T, Galecki P. 2019. Mitochondrial DNA copy number, damage, repair and degradation in depressive disorder. *World Journal of Biological Psychiatry* **13**:1–11 DOI 10.1080/15622975.2019.1588993.

Davami MH, Baharlou R, Ahmadi Vasmehjani A, Ghanizadeh A, Keshtkar M, Dezhkam I, Atashzar MR. 2016. Elevated IL-17 and TGF-β serum levels: a positive correlation between T-helper 17 cell-related pro-inflammatory responses with major depressive disorder. *Basic and Clinical Neuroscience* **7(2)**:137–142.

De Sampaio e Spohr TC, Martinez R, Da Silva EF, Neto VM, Gomes FC. 2002. Neuro-glia interaction effects on GFAP gene: a novel role for transforming growth factor-beta1. *European Journal of Neuroscience* **16(11)**:2059–2069.

Feng Z, Ko C-P. 2008. Schwann cells promote synaptogenesis at the neuromuscular junction via transforming growth factor-β1. *Journal of Neuroscience* **28(39)**:9599–9609 DOI 10.1523/JNEUROSCI.2589-08.2008.

Fortunato G, Calcagno G, Bresciamorra V, Salvatore E, Filla A, Capone S, Liguori R, Borelli S, Gentile I, Borrelli F, Borgia G, Sacchetti L. 2008. Multiple sclerosis and hepatitis C virus infection are associated with single nucleotide polymorphisms in interferon pathway genes. *Journal of Interferon & Cytokine Research* **28(3)**:141–152 DOI 10.1089/jir.2007.0049.

Gałecki P, Florkowski A, Bieńkiewicz M, Szemraj J. 2010. Functional polymorphism of cyclooxygenase-2 gene (G-765C) in depressive patients. *Neuropsychobiology* **62(2)**:116–120 DOI 10.1159/000317284.

Hansson M, Olsson I, Nauseef WM. 2006. Biosynthesis, processing, and sorting of human myeloperoxidase. *Archives of Biochemistry and Biophysics* **445(2)**:214–224 DOI 10.1016/j.abb.2005.08.009.

Hong M, Zheng J, Ding Z-Y, Chen J-H, Yu L, Niu Y, Hua Y-Q, Wang L-L. 2013. Imbalance between Th17 and Treg cells may play an important role in the development of chronic unpredictable mild stress-induced depression in mice. *Neuroimmunomodulation* **20(1)**:39–50 DOI 10.1159/000343100.

Kapelski P, Skibinska M, Maciukiewicz M, Wilkosc M, Frydecka D, Groszewska A, Narozna B, Dmitrzak-Weglarz M, Czerski P, Pawlak J, Rajewska-Rager A, Leszczynska-Rodziewicz A, Slopien A, Zaremba D, Twarowska-Hauser J. 2015. Association study of functional polymorphisms in interleukins and interleukin receptors genes: IL1A, IL1B, IL1RN, IL6, IL6R, IL10, IL10RA and TGFB1 in schizophrenia in Polish population. *Schizophrenia Research* **169(1–3)**:1–9 DOI 10.1016/j.schres.2015.10.008.

Karin M, Ben-Neriah Y. 2000. Phosphorylation meets ubiquitination: the control of NF-κB activity. *Annual Review of Immunology* **18**:621–663.

Kim Y-K, Lee S-W, Kim S-H, Shim S-H, Han S-W, Choi S-H, Lee B-H. 2008. Differences in cytokines between non-suicidal patients and suicidal patients in major depression. *Progress in Neuro-Psychopharmacological and Biological Psychiatry* **32(2)**:356–361 DOI 10.1016/j.pnpbp.2007.08.041.

Kim Y-K, Na K-S, Shin K-H, Jung H-Y, Choi S-H, Kim J-B. 2007. Cytokine imbalance in the pathophysiology of major depressive disorder. *Progress in Neuro-Psychopharmacological and Biological Psychiatry* **31(5)**:1044–1053 DOI 10.1016/j.pnpbp.2007.03.004.

Kissin EY, Lemaire R, Korn JH, Lafyatis R. 2002. Transforming growth factor β induces fibroblast fibrillin-1 matrix formation. *Arthritis and Rheumatism* **46(11)**:3000–3009 DOI 10.1002/art.10621.

Krakauer T. 2008. Nuclear factor-κB: fine-tuning a central integrator of diverse biologic stimuli. *International Reviews of Immunology* **27(5)**:286–292.

Kröger A, Köster M, Schroeder K, Hauser H, Mueller PP. 2002. Activities of IRF-1. *Journal of Interferon Cytokine Research* **22(1)**:5–14.

Kunzmann S, Mantel P-Y, Wohlfahrt JG, Akdis M, Blaser K, Schmidt-Weber CB. 2003. Histamine enhances TGF-β1-mediated suppression of Th2 responses. *FASEB Journal* **17(9)**:1089–1095 DOI 10.1096/fj.02-1008com.

Lingappa JR, Petrovski S, Kahle E, Fellay J, Shianna K, McElrath MJ, Thomas KK, Baeten JM, Celum C, Wald A, De Bruyn G, Mullins JI, Nakku-Joloba E, Farquhar C, Essex M, Donnell D, Kiarie J, Haynes B, Goldstein D, Partners in Prevention HSV/HIV Transmission Study Team. 2011. Genomewide association study for determinants of HIV-1 acquisition and viral set point in HIV-1 serodiscordant couples with quantified virus exposure. *PLOS ONE* **6(12)**:e28632 DOI 10.1371/journal.pone.0028632.

McGuffin P, Knight J, Breen G, Brewster S, Boyd PR, Craddock N, Gill M, Korszun A, Maier W, Middleton L, Mors O, Owen MJ, Perry J, Preisig M, Reich T, Rice J, Rietschel M, Jones L, Sham P, Farmer AE. 2005. Whole genome linkage scan of recurrent depressive disorder from the depression network study. *Human Molecular Genetics* **14(22)**:3337–3345 DOI 10.1093/hmg/ddi363.

Mendlewicz J, Crisafulli C, Calati R, Kocabas NA, Massat I, Linotte S, Kasper S, Fink M, Sidoti A, Scantamburlo G, Ansseau M, Antonijevic I, Forray C, Snyder L, Bollen J, Montgomery S, Zohar J, Souery D, Serretti A. 2012. Influence of *COX-2* and *OXTR* polymorphisms on treatment outcome in treatment resistant depression. *Neuroscience Letters* **516(1)**:85–88 DOI 10.1016/j.neulet.2012.03.063.

Mihailova S, Ivanova-Genova E, Lukanov T, Stoyanova V, Milanova V, Naumova E. 2016. A study of TNF-α, TGF-β, IL-10, IL-6, and IFN-γ gene polymorphisms in patients with depression. *Journal of Neuroimmunology* **293**:123–128 DOI 10.1016/j.jneuroim.2016.03.005.

Minghetti L. 2004. Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases. *Journal of Neuropathology & Experimental Neurology* **63(9)**:901–910 DOI 10.1093/jnen/63.9.901.

Morkūnienė A, Steponaviciūt D, Utkus A, Kucinskas V. 2007. Few associations of candidate genes with nonsyndromic orofacial clefts in the population of Lithuania. *Journal of Applied Genetics* **48(1)**:89–91 DOI 10.1007/BF03194663.

Musil R, Schwarz MJ, Riedel M, Dehning S, Cerovecki A, Spellmann I, Arolt V, Müller N. 2011. Elevated macrophage migration inhibitory factor and decreased transforming growth factor-beta levels in major depression—No influence of celecoxib treatment. *Journal of Affective Disorders* **134(1–3)**:217–225 DOI 10.1016/j.jad.2011.05.047.

Nam J-S, Terabe M, Kang M-J, Chae H, Voong N, Yang Y-A, Laurence A, Michalowska A, Mamura M, Lonning S, Berzofsky JA, Wakefield LM. 2008. Transforming growth factor beta subverts the immune system into directly promoting tumor growth through interleukin-17. *Cancer Research* **68(10)**:3915–3923 DOI 10.1158/0008-5472.CAN-08-0206.

Napetschnig J, Wu H. 2013. Molecular basis of NF-κB signaling. *Annual Review Biophysics* **42**:443–468.

Pace TW, Mletzko TC, Alagbe O, Musselman DL, Nemeroff CB, Miller AH, Heim CM. 2006. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. *American Journal of Psychiatry* **163(9)**:1630–1633 DOI 10.1176/ajp.2006.163.9.1630.

Passos ST, Silver JS, O'Hara AC, Sehy D, Stumhofer JS, Hunter CA. 2010. IL-6 promotes NK cell production of IL-17 during toxoplasmosis. *Journal of Immunology* **184(4)**:1776–1783 DOI 10.4049/jimmunol.0901843.

Pinto EF, Andrade C. 2016. Interferon-related depression: a primer on mechanisms, treatment, and prevention of a common clinical problem. *Current Neuropharmacology* **14(7)**:743–748 DOI 10.2174/1570159X14666160106155129.

Reyes-Gibby CC, Spitz MR, Yennurajalingam S, Swartz M, Gu J, Wu X, Bruera E, Shete S. 2009. Role of inflammation gene polymorphisms on pain severity in lung cancer patients. *Cancer Epidemiology Biomarkers & Prevention* **18(10)**:2636–2642 DOI 10.1158/1055-9965.EPI-09-0426.

Reyes-Gibby CC, Swartz MD, Yu X, Wu X, Yennurajalingam S, Anderson KO, Spitz MR, Shete S. 2013. Symptom clusters of pain, depressed mood, and fatigue in lung cancer assessing the role of cytokine genes. *Supportive Care in Cancer* **21(11)**:3117–3125 DOI 10.1007/s00520-013-1885-5.

Schiepers OJG, Wichers MC, Maes M. 2005. Cytokines and major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **29(2)**:201–217 DOI 10.1016/j.pnpbp.2004.11.003.

Seufert BL, Poole EM, Whitton J, Xiao L, Makar KW, Campbell PT, Kulmacz RJ, Baron JA, Newcomb PA, Slattery ML, Potter JD, Ulrich CM. 2013. IκBKβ and NFκB1, NSAID use and risk of colorectal cancer in the colon cancer family registry. *Carcinogenesis* **34(1)**:79–85 DOI 10.1093/carcin/bgs296.

Shah R, Rahaman B, Hurley CK, Posch PE. 2006. Allelic diversity in the TGFB1 regulatory region: characterization of novel functional single nucleotide polymorphisms. *Human Genetics* **119(1–2)**:61–74 DOI 10.1007/s00439-005-0112-y.

Shi J, Johansson J, Woodling NS, Wang Q, Montine TJ, Andreasson K. 2010. The prostaglandin E2 E-prostanoid 4 receptor exerts anti-inflammatory effects in brain innate immunity. *Journal of Immunology* **184(12)**:7207–7218 DOI 10.4049/jimmunol.0903487.

Shyn SI, Shi J, Kraft JB, Potash JB, Knowles JA, Weissman MM, Garriock HA, Yokoyama JS, McGrath PJ, Peters EJ, Scheftner WA, Coryell W, Lawson WB, Jancic D, Gejman PV, Sanders AR, Holmans P, Slager SL, Levinson DF, Hamilton SP. 2011. Novel loci for major

depression identified by genome-wide association study of sequenced treatment alternatives to relieve depression and meta-analysis of three studies. *Molecular Psychiatry* **16(2)**:202–215 DOI 10.1038/mp.2009.125.

Siegenthaler JA, Miller MW. 2004. Transforming growth factor β1 modulates cell migration in rat cortex: effects of ethanol. *Cerebral Cortex* **14(7)**:791–802 DOI 10.1093/cercor/bhh039.

Sousa Vde O, Romao L, Neto VM, Gomes FC. 2004. Glial fibrillary acidic protein gene promoter is differently modulated by transforming growth factor-beta 1 in astrocytes from distinct brain regions. *European Journal of Neuroscience* **19(7)**:1721–1730 DOI 10.1111/j.1460-9568.2004.03249.x.

Su K-P, Huang S-Y, Peng C-Y, Lai H-C, Huang C-L, Chen Y-C, Aitchison K-J, Pariante C-M. 2010. Phospholipase A2 and cyclooxygenase 2 genes influence the risk of interferon-α–induced depression by regulating polyunsaturated fatty acids levels. *Biological Psychiatry* **67(6)**:550–557 DOI 10.1016/j.biopsych.2009.11.005.

Sullivan PF, Ripke S, Wray NR, Lewis CM, Hamilton SP, Weissman MM, Breen G, Byrne EM, Blackwood P, Boomsma DI, Cichon S, Heath AC, Holsboer F, Lucae S, Madden PA, Martin NG, McGuffin P, Muglia P, Noethen MM, Penninx BP, Pergadia ML, Potash JB, Rietschel M, Lin D, Müller-Myhsok B, Shi J, Steinberg S, Grabe HJ, Lichtenstein P, Magnusson P, Perlis RH, Preisig M, Smoller JW, Stefansson K, Uher R, Kutalik Z, Tansey KE, Teumer A, Viktorin A, Barnes MR, Bettecken T, Binder EB, Breuer R, Castro VM, Churchill SE, Coryell WH, Craddock N, Craig IW, Czamara D, De Geus EJ, Degenhardt F, Farmer AE, Fava M, Frank J, Gainer VS, Gallagher PJ, Gordon SD, Goryachev S, Gross M, Guipponi M, Henders AK, Herms S, Hickie IB, Hoefels S, Hoogendijk W, Hottenga JJ, Iosifescu DV, Ising M, Jones I, Jones L, Jung-Ying T, Knowles JA, Kohane IS, Kohli MA, Korszun A, Landen M, Lawson WB, Lewis G, Macintyre D, Maier W, Mattheisen M, McGrath PJ, McIntosh A, McLean A, Middeldorp CM, Middleton L, Montgomery GM, Murphy SN, Nauck M, Nolen WA, Nyholt DR, O'Donovan M, Oskarsson H, Pedersen N, Scheftner WA, Schulz A, Schulze TG, Shyn SI, Sigurdsson E, Slager SL, Smit JH, Stefansson H, Steffens M, Thorgeirsson T, Tozzi F, Treutlein J, Uhr M, Van Den Oord EJ, Van Grootheest G, Völzke H, Weilburg JB, Willemsen G, Zitman FG, Neale B, Daly M, Levinson DF, Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium. 2013. A mega-analysis of genome-wide association studies for major depressive disorder. *Molecular Psychiatry* **18(4)**:497–511 DOI 10.1038/mp.2012.182.

Sutcigil L, Oktenli C, Musabak U, Bozkurt A, Cansever A, Uzun O, Sanisoglu SY, Yesilova Z, Ozmenler N, Ozsahin A, Sengul A. 2007. Pro- and anti-inflammatory cytokine balance in major depression: effect of sertraline therapy. *Clinical & Developmental Immunology* **2007**:76396.

Takeda K, Akira S. 2007. Toll-like receptors. *Current Protocol Immunology* **14(14)**:14.12 DOI 10.1002/0471142735.im1412s77.

Tamura T, Yanai H, Savitsky D, Taniguchi T. 2008. The IRF family transcription factors in immunity and oncogenesis. *Annual Review of Immunology* **26(1)**:535–584 DOI 10.1146/annurev.immunol.26.021607.090400.

Ten Dijke P, Hill CS. 2004. New insights into TGF-β-Smad signalling. *Trends in Biochemical Sciences* **29(5)**:265–273 DOI 10.1016/j.tibs.2004.03.008.

Vivien D, Ali C. 2006. Transforming growth factor-β signalling in braindisorders. *Cytokine & Growth Factor Reviews* **17(1–2)**:121–128 DOI 10.1016/j.cytogfr.2005.09.011.

Wang H, Zhao Y-P, Gao C-F, Ji Q, Gressner AM, Yang Z-X, Weiskirchen R. 2008. Transforming growth factor β1 gene variants increase transcription and are associated with liver cirrhosis in Chinese. *Cytokine* **43(1)**:20–25 DOI 10.1016/j.cyto.2008.04.013.

WHO. 2018. Depression. *Available at* http://www.who.int/news-room/fact-sheets/detail/depression.

Wray NR, Ripke S, Mattheisen M, Trzaskowski M, Byrne EM, Abdellaoui A, Adams MJ, Agerbo E, Air TM, Andlauer TMF, Bacanu S-A, Bækvad-Hansen M, Beekman AFT, Bigdeli TB, Binder EB, Blackwood DRH, Bryois J, Buttenschøn HN, Bybjerg-Grauholm J, Cai N, Castelao E, Christensen JH, Clarke T-K, Coleman JIR, Colodro-Conde L, Couvy-Duchesne B, Craddock N, Crawford GE, Crowley CA, Dashti HS, Davies G, Deary IJ, Degenhardt F, Derks EM, Direk N, Dolan CV, Dunn EC, Eley TC, Eriksson N, Escott-Price V, Kiadeh FHF, Finucane HK, Forstner AJ, Frank J, Gaspar Héléna A, Gill M, Giusti-Rodríguez P, Goes FS, Gordon SD, Grove J, Hall LS, Hannon E, Hansen CS, Hansen TF, Herms S, Hickie IB, Hoffmann P, Homuth G, Horn C, Hottenga J-J, Hougaard DM, Hu M, Hyde CL, Ising M, Jansen R, Jin F, Jorgenson E, Knowles JA, Kohane IS, Kraft J, Kretzschmar WW, Krogh J, Kutalik Zán, Lane JM, Li Y, Li Y, Lind PA, Liu X, Lu L, MacIntyre DJ, MacKinnon DF, Maier RM, Maier W, Marchini J, Mbarek H, McGrath P, McGuffin P, Medland SE, Mehta D, Middeldorp CM, Mihailov E, Milaneschi Y, Milani L, Mill J, Mondimore FM, Montgomery GW, Mostafavi S, Mullins N, Nauck M, Ng B, Nivard MG, Nyholt DR, O'Reilly PF, Oskarsson H, Owen MJ, Painter JN, Pedersen CB, Pedersen MG, Peterson RE, Pettersson E, Peyrot WJ, Pistis G, Posthuma D, Purcell SM, Quiroz JA, Qvist P, Rice JP, Riley BP, Rivera M, Saeed Mirza S, Saxena R, Schoevers R, Schulte EC, Shen L, Shi J, Shyn SI, Sigurdsson E, Sinnamon GBC, Smit JH, Smith DJ, Stefansson H, Steinberg S, Stockmeier CA, Streit F, Strohmaier J, Tansey KE, Teismann H, Teumer A, Thompson W, Thomson PA, Thorgeirsson TE, Tian C, Traylor M, Treutlein J, Trubetskoy V, Uitterlinden Aé G, Umbricht D, Van Der Auwera S, Van Hemert AM, Viktorin A, Visscher PM, Wang Y, Webb BT, Weinsheimer SM, Wellmann J, Willemsen G, Witt SH, Wu Y, Xi HS, Yang J, Zhang F, Arolt V, Baune BT, Berger K, Boomsma DI, Cichon S, Dannlowski U, De Geus ECJ, DePaulo JR, Domenici E, Domschke K, Esko T, Grabe HJ, Hamilton SP, Hayward C, Heath AC, Hinds DA, Kendler KS, Kloiber S, Lewis G, Li QS, Lucae S, Madden PFA, Magnusson PK, Martin NG, McIntosh AM, Metspalu A, Mors O, Mortensen PB, Müller-Myhsok B, Nordentoft M, Nöthen MM, O'Donovan MC, Paciga SA, Pedersen NL, Penninx BWJH, Perlis RH, Porteous DJ, Potash JB, Preisig M, Rietschel M, Schaefer C, Schulze TG, Smoller JW, Stefansson K, Tiemeier H, Uher R, Völzke H, Weissman MM, Werge T, Winslow AR, Lewis CM, Levinson DF, Breen G, Børglum AD, Sullivan PF, The Major Depressive Disorder Working Group of the Psychiatric Genomics Consortium. 2018. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nature Genetics* **50(5)**:668–681 DOI 10.1038/s41588-018-0090-3.

Yamagiwa S, Gray JD, Hashimoto S, Horwitz DA. 2001. A role for TGF beta in the generation and expansion of CD4$^+$CD25$^+$ regulatory T cells from human peripheral blood. *Journal of Immunology* **166(12)**:7282–7289 DOI 10.4049/jimmunol.166.12.7282.

Zahiu C, Mihai R. 2014. Neuropsychiatric side-effects of interferonalpha treatment: pathophysiology and therapeutic options. *MAEDICA - A Journal of Clinical Medicine* **9(2)**:121–126.

Zhang Q, Lenardo MJ, Baltimore D. 2017. 30 years of NF-κB: a blossoming of relevance to human pathobiology. *Cell* **168(1–2)**:37–57 DOI 10.1016/j.cell.2016.12.012.

Chronic Mild Stress and Venlafaxine Treatment Were Associated with Altered Expression Level and Methylation Status of New Candidate Inflammatory Genes in PBMCs and Brain Structures of Wistar Rats

Katarzyna Bialek [1], Piotr Czarny [2], Paulina Wigner [3], Ewelina Synowiec [1], Gabriela Barszczewska [1], Michal Bijak [4], Janusz Szemraj [2], Monika Niemczyk [5], Katarzyna Tota-Glowczyk [5], Mariusz Papp [5] and Tomasz Sliwinski [1,*]

[1] Laboratory of Medical Genetics, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland; biaalek.k@gmail.com (K.B.); ewelina.synowiec@biol.uni.lodz.pl (E.S.); gabriela.barszczewska@edu.uni.lodz.pl (G.B.)
[2] Department of Medical Biochemistry, Medical University of Lodz, 92-216 Lodz, Poland; piotr.czarny@umed.lodz.pl (P.C.); janusz.szemraj@umed.lodz.pl (J.S.)
[3] Department of General Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland; paulina.wigner@biol.uni.lodz.pl
[4] Biohazard Prevention Centre, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland; michal.bijak@biol.uni.lodz.pl
[5] Institute of Pharmacology, Polish Academy of Sciences, 31-343 Krakow, Poland; niemczyk@if-pan.krakow.pl (M.N.); ktech@o2.pl (K.T.-G.); nfpapp@cyfronet.pl (M.P.)
* Correspondence: tomsliw@biol.uni.lodz.pl; Tel.: +48-42-635-44-86; Fax: +48-42-635-44-84

Abstract: Preclinical studies conducted to date suggest that depression could be elicited by the elevated expression of proinflammatory molecules: these play a key role in the mediation of neurochemical, neuroendocrine and behavioral changes. Thus, this study investigates the effect of chronic mild stress (CMS) and administration of venlafaxine (SSRI) on the expression and methylation status of new target inflammatory genes: TGFA, TGFB, IRF1, PTGS2 and IKBKB, in peripheral blood mononuclear cells (PMBCs) and in selected brain structures of rats. Adult male Wistar rats were subjected to the CMS and further divided into matched subgroups to receive vehicle or venlafaxine. TaqMan gene expression assay and methylation-sensitive high-resolution melting (MS-HRM) were used to evaluate the expression of the genes and the methylation status of their promoters, respectively. Our results indicate that both CMS and chronic treatment with venlafaxine were associated with changes in expression of the studied genes and their promoter methylation status in PMBCs and the brain. Moreover, the effect of antidepressant administration clearly differed between brain structures. Summarizing, our results confirm at least a partial association between TGFA, TGFB, IRF1, PTGS2 and IKBKB and depressive disorders.

Keywords: depression; chronic mild stress; venlafaxine; inflammation; expression; methylation

1. Introduction

Being one of the most frequently diagnosed mental diseases, depression (Major depressive disorder, MDD) affects more than 260 million people worldwide and is a significant contributor to the global burden of disease. Due to the constantly increasing number of patients, MDD is estimated to be the second leading cause of social disability. Depression reduces people's functioning by inducing persistent sadness, lack of interest and anxiety. These and other symptoms often become chronic or recurrent and may lead to suicide [1]. Furthermore, above one-third of patients do not respond to antidepressant treatment [2,3].

Despite its importance, the pathogenesis of depression is not fully understood. Nevertheless, there is a growing body of evidence suggesting that it may be influenced by

the activation of the immune system. One mechanism that has been proposed for its development is given in the "cytokine hypothesis" [4]; briefly, MDD could be elicited by the elevated expression and activity of proinflammatory molecules: these act as neuromodulators and thus play a key role in the mediation of neurochemical, neuroendocrine and behavioral changes [5]. Indeed, patients affected with medical conditions associated with chronic inflammation, i.e., rheumatoid arthritis, cardiovascular diseases and autoimmune disorders, are at higher risk of depression [6]. Moreover, a great amount of evidence confirms a link between inflammation and depression in patients without other medical conditions. A rich body of research indicates that MDD patients exhibit increased concentrations of cytokines and other proinflammatory markers, such as acute phase reactants, chemokines and adhesion molecules [7–10].

Patients with depression have also demonstrated activation of microglia, i.e., immune cells resident within the central nervous system (CNS) [11]. This may also contribute to neuroinflammation, and neurotrophic system disruptions since activated microglia express proinflammatory cytokines [12]. Additionally, their mobilization is connected with the activation of nuclear factor-kB (NF-kB), which is often responsible for cytokine production [13]. However, sometimes microglia exert a neuroprotective effect by releasing anti-inflammatory molecules, including transforming growth factor β (TGFB), to antagonize inflammation-promoted CNS damage [12].

TGFB is a class of polypeptide growth factors, which together with transforming growth factor α (TGFA), constitute the TGF family. Their main functions are embryonic development and regulation of immune system reactions [14,15]. TGFB is known to play a role in brain inflammation, as well as in the peripheral immune response [16,17]. In addition, TGFB can exert neuroprotective effects in many neurodegenerative disorders [18]. However, reports about its role in MDD are inconsistent. Its level has been found to be increased in animal studies, with this increase being associated with an imbalance between Treg and Th17 cells [19], while other studies have identified lower TGFB expression in depressed patients than in healthy subjects [20,21]. In addition, TGFB stimulates not only cytokines but also prostaglandin-endoperoxide synthase 2 (PTGS2; cyclooxygenase-2—COX-2) encoded by the PTGS2 gene, which has been implicated in the pathogenesis of MDD [22,23]. Besides its role in inflammation, PTGS2 also catalyzes the conversion of arachidonic acid (AA) to prostaglandins (PGs), which further escalate inflammatory and neurodegenerative processes in CNS [24,25]. Importantly, research on an animal model of depression confirmed that PTGS2 levels are significantly elevated in various brain regions [26].

Another molecule strongly associated with inflammation is interferon regulatory factor 1 (IRF1). IRF1 was the first transcription factor identified in the interferon (IFN) system and plays a pivotal role in controlling the expression of many genes associated with the immune system [27]. IRF1 regulates IFN and other IFN-inducible genes involved in inflammation by influencing transcription [28]. Interferons, clusters of cytokines acting as signaling proteins in the immune response, play key roles in psychiatric conditions. For instance, IFN-α is an efficient stimulator of the proinflammatory cytokine network, including interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α), in both peripheral the CNS [9,29]; it is implicated in the adjustment of mood, sleep–wake cycle and behavior [29]. IRF1 also promotes the release of inflammatory cytokines and regulates the expression of interleukin 12 (IL-12) and interleukin 15 (IL-15), which are involved in MDD [28]. Furthermore, IRF1 interacts with several transcription factors, such as NF-kB [27].

Besides cytokines, various inflammatory pathways are thought to be dysregulated in MDD, including NF-kB, leading to increased levels of proinflammatory cytokines [30,31]. NF-kB is a ubiquitous transcriptional factor regulating the expression of genes involved in pleiotropic functions, including proinflammatory cytokines and costimulatory molecules [32–34]. In addition, NF-kB regulates neurogenesis and synaptic plasticity in the nervous system [35–37]. Moreover, some studies indicate the presence of an interplay between NF-kB and brain-derived

neurotrophic factor (BDNF), which is a cornerstone of the neurotrophic hypothesis of depression [38,39]. More precisely, NF-kB can regulate BDNF expression and vice versa [40]. Normal NF-kB signaling is essential for neurogenesis, brain functioning, memory and neuronal plasticity [41,42]. Canonical signaling of NF-kB is activated by the IkB kinase (IKK complex), consisting of three subunits, one of which is IKK-B (inhibitor of nuclear factor kappa-B kinase subunit β) encoded by the IKBKB gene [43–45]. Therefore, alterations in IKBKB gene expression can disrupt the NF-kB system and may influence developing depression [43].

Despite the confirmed involvement of the immune system in depression, knowledge about inflammatory molecules other than cytokines is lacking. All selected genes contribute to neuroinflammation and brain functioning involved in the pathogenesis of depression. The occurrence of any variation in such genes may result in dysregulation and disruption of the other related factors. Moreover, the majority of them have not been studied in the context of psychiatric disorders yet. Therefore, all of these genes were selected to give a broader view of the inflammatory processes that may be activated in depression, not just focusing on cytokines, especially since all these factors are related to the regulation or stimulation of cytokine expression. Moreover, we have studied these genes in the context of the correlation of their single nucleotide polymorphisms (SNPs) with the risk of depression development. Therefore, the current study is a continuation of our research [46]. Stress is known to provoke inflammation in brain regions, such as the frontal cortex, hypothalamus and hippocampus, particularly sensitive to chronic stress [47,48]. Importantly, studies indicate an imbalance between pro- and anti-inflammatory cytokines in chronic mild stress (CMS)-induced depression [49]. It is hypothesized that antidepressant drug administration could effectively reduce proinflammatory cytokines in depressed subjects [50]. Selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs), such as venlafaxine, are currently used in the first-line treatment of MDD [51,52]. However, the chronic impact of antidepressants on the levels of inflammatory molecules in the peripheral and central nervous system has been barely studied. As it is only possible to directly study the brain of depressed patients post-mortem, therefore, such study requires using an animal model to understand the complex relationship between many processes, including the inflammation and etiology of MDD.

Therefore, the present study investigates whether: (1) the CMS procedure in rats, which closely mirrors depression in humans, can induce changes in TGFA, TGFB, IRF1, PTGS2 and IKBKB expression at the mRNA level in peripheral blood mononuclear cells (PBMCs) and in selected brain structures (hippocampus, amygdala, midbrain, hypothalamus, prefrontal cortex and basal ganglia); (2) chronic administration of serotonin-norepinephrine reuptake inhibitor, venlafaxine, alters the expression of these genes in the peripheral and central nervous system; (3) the CMS procedure and chronic venlafaxine administration cause epigenetic changes in the investigated genes, such as methylation level in the promoters; (4) the changes in expression observed in PBMCs can reflect similar changes in the brain.

2. Materials and Methods

2.1. Animals

Male Wistar Han rats, approximately 5 weeks old, weighing 200–220 g at the start (Charles River, Germany), were used to carry out the study. The animals were brought into the laboratory one month before the start of the experiment to adapt to the housing conditions. With the exceptions described below, the rats were housed singly with a maintenance 12 h light/dark cycle (lights on at 8.00) in controlled temperature (20 ± 2 °C) and humidity ($50 \pm 5\%$). Food and water were allowed ad libitum. All procedures used in the experiment were approved by the Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland, and conform to the rules and principles of Directive 86/609/ECC.

2.2. Chronic Mild Stress Procedure

Male Wistar Han rats were brought into the laboratory one month before the start of the experiment to adapt to the housing conditions. First, after acclimatization, the animals were trained to consume a 1% sucrose solution in baseline tests conducted once a week in the home cage. Sucrose solution consumption is the most common, adequate way to quantify the behavioral effect of CMS procedure by measuring the ability to respond to reward stimuli. It reflects the key symptom investigated in a depressed subject, which is anhedonia—inability to feel pleasure [53]. Sucrose solution was presented for one hour after 14 h water and food deprivation. Consumption of the sucrose was verified once a week, under controlled conditions, until the experiment was ended. Subsequently, based on their sucrose intakes in the final baseline test, the animals were divided into two matched groups. The control group (of nonstressed animals) was housed in separate rooms to exclude contact with the stressed animals. In this group, food and water were freely available, except for 14 h deprivation before each weekly sucrose test. The stressed group was exposed to the CMS procedure for a period of two or seven weeks. Each week of the stress regimen consisted of two periods of food and water deprivation, two periods of 45-degree cage tilt, two periods of intermittent illumination (light on and off every two hours), two periods of a soiled cage (250 mL water in sawdust bedding), one period of paired housing, two periods of low-intensity stroboscopic illumination (150 flashes/min), and three periods without stress. All stressors were applied for 10–14 h and were used individually and continuously, day and night. The rats subjected to the CMS procedure demonstrated a gradual decrease in sucrose solution consumption to approximately 40% of pre-stress values. After stabilization of this effect, named after two weeks of initial stress, the animals were either decapitated or further divided into matched subgroups and daily administrated with vehicle (1 mL/kg, IP) or venlafaxine (10 mg/kg, IP) for the subsequent five weeks. The drug was administrated to both control and stressed animals. The weekly sucrose tests were carried out 24 h after the last dose. After the final sucrose test, i.e., after seven weeks of stress, or rather, the completion of five-week administration of vehicle or drug, the animals were decapitated, and blood and brain samples were collected. Before decapitation, no anesthesia was used to avoid possible changes in the expression of genes in the brain caused by the anesthetic. The detailed description of stressors and CMS schedule are presented in Table 1.

2.3. Specimen Collection

Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples collected into 5 mL vacutainers with EDTA. Isolation was based on differential migration of cells during centrifugation. Precisely, blood was mixed with equal volumes of PBS, layered on top of Gradisol L (Aqua-Med, Lodz, Poland) and centrifuged. The interfacial layer (lymphocyte coat) was transferred to a new tube and centrifuged. The supernatant was removed, and PBMCs stored as pellets at −20 °C until used.

2.4. RNA and DNA Isolation from Peripheral Blood Mononuclear Cells

RNA and DNA isolation was performed using the commercial spin column methods with elution in RNAse-Free water (GenElute mammalian total RNA miniprep kit, Sigma-Aldrich, St. Louis, MO, USA; QIAamp DNA mini kit, Qiagen, Hilden, Germany, respectively), following the manufacturer's instructions. Total DNA and RNA concentrations were determined spectrophotometrically. The purity of samples was measured as 260/280 nm OD ratio with expected values of 1.8–2.0. RNA and DNA samples were stored at −20 °C until further analysis.

Table 1. Schedule of CMS procedure and detailed description of all applied stressors.

Experiment Start		
5 weeks adaptation to 1% sucrose consumption test		
2 weeks without stress	2 weeks of initial stress	
5 weeks without stress and with venlafaxine administration	5 weeks of stress with saline administration	5 weeks of stress with venlafaxine administration

Stress Procedure		
Stressor	Duration	Number of periods
Food and water deprivation	10–14 h	2 periods
45-degree cage tilt	10–14 h	2 periods
Soiled cage (250 mL water in sawdust bedding)	10–14 h	2 periods
Paired housing	10–14 h	1 period
Low-intensity stroboscopic illumination (150 flashes/min)	10–14 h	2 periods
Intermittent illumination	10–14 h (light on and off every two hours)	2 periods
No stress	10–14 h	3 periods
Final sucrose consumption test and decapitation		

2.5. Specimen Collection; RNA and DNA Isolation from Brain Tissues

Brain regions, i.e., hippocampus, amygdala, midbrain, hypothalamus, prefrontal cortex and basal ganglia, were separated and immediately frozen in liquid nitrogen and stored at $-80\ ^{\circ}$C. In the isolation procedure, a sufficient volume of PBS was added to each sample and then homogenized using FastGene® tissue grinder (Nippon Genetics Europe, Düren, Germany). The homogenized samples were then sonicated, centrifuged and rinsed with PBS by a commercial kit (ISOLATE II RNA/DNA/protein kit; Bioline), according to the manufacturer's protocol. The purity of the RNA and DNA and their concentrations were measured spectrophotometrically by calculating the ratio between absorbance at 260 nm and 280 nm. Samples were stored at $-20\ ^{\circ}$C until further analysis.

2.6. Reverse Transcription and Gene Expression

The reverse transcription reaction was performed with the use of a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). The total reaction volume was 20 µL. The mixture contained nuclease-free water, 10xRT buffer, 10xRT random primers, 25xdNTP Mix (100 mM), total RNA (0.5 ng/µL) and MultiScribe® reverse transcriptase. The reaction tubes were incubated for 10 min at 25 $^{\circ}$C, 120 min at 37 $^{\circ}$C, and then for 5 min at 85 $^{\circ}$C to inactivate the reverse transcriptase. PCR was performed in a C1000™ programmed thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). After the reaction, the cDNA samples were stored at $-20\ ^{\circ}$C. TaqMan gene expression assay (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used to examine the expression of the following genes: *IKBKB* (assay ID: Rn00584379_m1), *TGFA* (assay ID: Rn00446234_m1), *TGFB* (assay ID: Rn00572010_m1), *IRF1* (assay ID: Rn01483828_m1), *PTGS2* (assay ID: Rn01483828_m1). The reaction was performed using CFX96™ real-time PCR detection system thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). The housekeeping gene 18S ribosomal RNA gene (18S) (assay ID: Hs99999901_s1) was applied

as an internal control (reference gene) for all reverse transcription–quantitative polymerase chain reactions (RT–qPCR). The reaction mixture contained the following: cDNA samples, a TaqMan Universal master mix, no UNG (Applied Biosystems, Foster City, CA, USA), TaqMan probe (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and RNAse-free water. The PCR protocol was as follows: 10 min at 95 °C (enzyme activation), followed by 60 cycles of 30 s at 95 °C (denaturation), and one minute at 60 °C (for annealing/extension). The cycle threshold (Ct) values were calculated automatically by a CFX96 real-time PCR detection system software System (Bio-Rad Laboratories Inc., Hercules, CA, USA). For each sample, the gene expression of the target mRNA was calculated relative to a reference gene (ΔCt sample = Ct target gene − Ct reference gene). The levels of gene expression are given as a normalization ratio calculated as fold = 2 − ΔCt sample.

2.7. Methylation and HRM Analysis

The methylation status of investigated gene promoters was obtained by methylation-sensitive high-resolution melting [54,55]. Genes sequences were checked for the numbers of promoters and the presence of CpG islands. The promoter sequence was obtained from the Eukaryotic promoter database EPD (http://epd.vital-it.ch (accessed on 1 December 2018)) [56]. For all investigated genes, the region from −499 to 100 bp relative to the transcription start site (TSS) was used to design primers. The selected region contains all core promoter motifs, required CpG island as well, as is characterized by the presence of curved DNA elements relevant to the transcription process. Primers were designed for promoters containing CpG islands using Methyl Primer Express™ Software v 1.0 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to recommendations provided by Wojdacz et al. (2009) [57]. It was not possible to design suitable MS-HRM primers for TGFB (Table 2). The bisulfite conversion reaction was performed using 200 ng of DNA with a CiTi converter DNA methylation kit (A&A Biotechnology, Gdynia, Poland), according to the manufacturer's instruction. Methylated DNA (CpGenome™ rat methylated genomic DNA standard; Merck Millipore, Burlington, MA, USA) and unmethylated DNA (CpGenome™ rat unmethylated genomic DNA standard; Merck Millipore, Burlington, MA, USA) were used as controls for the MS-HRM experiments. To maintain accuracy and control the sensitivity of methylation detection, a series of dilutions were prepared, namely: nonmethylated, 10% methylated, 25% methylated, 50% methylated, 75% methylated, and 100% methylated DNA. These reactions were performed using the Bio-Rad CFX96 real-time PCR detection system and analyzed in HRM Powered by Precision Melt Analysis™ software (Bio-Rad Laboratories Inc., Hercules, CA, USA). Each reaction mixture contained 5× HOT FIREPol® EvaGreen® HRM Mix (no ROX) (Solis BioDyne, Tartu, Estonia), 500 nM of each primer and 10 ng of bisulfite converted DNA (theoretical calculation). The parameters for amplification and HRM analyses included initial activation for 12 min at 95 °C, 45 cycles of 95 °C for 15 s; annealing at optimal primer temperatures (tested experimentally) for 20 s and elongation at 72 °C for 20 s. The HRM analysis consisted of denaturation at 95 °C for 15 s, reannealing at 60 °C for one minute and melting from 60 to 95 °C at a ramp rate of 0.2 °C.

Table 2. The specification of primers used for the analysis of methylation levels in the promoter regions of the studied genes.

Gene	Starter Sequence (5′->3′)	Tm (°C)	Product Size (bp)	Number of CpG Islands	Product %CGs	CpGs in Product
IKBKB	F:AGGGTGGTTTTTTATTTTTATTTT R:AACCCCCACTAAAACTAACTTAA	55	117	1	36.75	5
IRF1	F:TTGGAGATTTAGGGAGTTAGGT R:CCCCTTACCTATCTTAAAAAACC	55	123	1	43.90	4
PTGS2	F:GTAATAGTAGGGAGGAAAAATTTTAA R:ATCCTAACAAACCCCAAA	55	111	1	37.84	10
TGFA	F:GTTTTTTTAGGTGGTTGGTTAAG R:CTTCAAACACCTCCCTACAATA	55	188	1	42.55	11

2.8. Drugs

Venlafaxine HCl (Carbosynth Ltd., Compton, Berkshire, UK) was dissolved in 0.9% sterile saline, which was used for vehicle administration. The drug was then administered IP at a volume of 1 mL/kg of body weight, i.e., a dose of 10 mg/kg, as used previously [58,59].

2.9. Statistical Analysis

The effect of initial two-week stress on sucrose consumption was analyzed by *t*-test for normally distributed data or the Mann–Whitney rank-sum test for non-normally distributed data. In addition, when the data were normally distributed, the sucrose intake, gene expression and methylation data were analyzed using one-way analysis of variance (one-way ANOVA), with Tukey's test as a post hoc test; F ratios were significant for the groups' control/vehicle, stressed/vehicle and stressed/venlafaxine. If the data were not normally distributed, these relationships were tested using the Kruskal–Wallis one-way ANOVA on ranks, followed by post hoc Student–Newman–Keuls test. The student's t-test was used to analyze differences between blood and brain samples. *p* values < 0.05 were considered significant. Analyses were performed using Statistica 12 (StatSoft, Tulsa, OK, USA), SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA) and GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Sucrose Intakes and Body Weights of Animals Exposed to CMS and Venlafaxine Administration

The 1% sucrose solution intake was comparable in all groups before CMS procedure initiation (week 0). Following the initial two-week stress, the consumption decreased to approximately 60% of initial values (week 2; stressed). Intakes remained at low levels in stressed animals administered with the vehicle until the end of the experiment (week 7; stressed/saline). Although chronic (five-week) venlafaxine treatment yielded no effect in control animals, it normalized sucrose consumption in stressed rats (Table 3). Both stress and venlafaxine had no significant effect on the body weights of the control or CMS animals (Table S1, Supplementary Materials).

Table 3. Sucrose intakes in animals exposed to chronic mild stress (CMS) for two weeks and in animals exposed to CMS or venlafaxine.

| Weeks of CMS	Control	Stressed	Stressed/Saline	Stressed/Venlafaxine	Control/Venlafaxine
Week 0	12.6 ± 1.6	11.0 ± 0.7	11.7 ± 0.7	11.4 ± 0.5	11.9 ± 0.7
Week 2	15.6 ± 1.9	6.8 ± 1.0 **	4.9 ± 0.6 ****	5.8 ± 0.5 *	13.9 ± 0.9
Week 7	-	-	6.1 ± 0.7	12.6 ± 1.0 ***	13.3 ± 1.3

Data represent means ± SEM. N = 6. ** $p < 0.01$; relative to week 0 in the stressed group. * $p < 0.05$; relative to week 0 in the stressed/venlafaxine group. *** $p < 0.01$; relative to week 2 in the stressed/venlafaxine group. **** $p < 0.001$; relative to week 0 in the stressed/saline group.

3.2. Gene Expression

3.2.1. Gene Expression in PBMCs after CMS Procedure and Venlafaxine Administration

The mRNA expression level of TGFA, TGFB, PTGS2, IRF1 and IKBKB in PBMCs did not differ between the control and stressed groups for the initial two weeks. However, animals stressed for seven weeks and administered saline demonstrated significantly greater expression of all studied genes compared to the control group, i.e., TGFA (F = 22.027, df = 4, $p < 0.001$, Tukey's test $p < 0.001$), TGFB (F = 11.383, df = 4, $p < 0.001$, Tukey's test $p < 0.001$), PTGS2 (F = 20.803, df = 4, $p < 0.001$, Tukey's test $p < 0.001$), IRF1 (F = 11.239, df = 4, $p < 0.001$, Tukey's test $p < 0.001$), IKBKB (F = 13.817, df = 4, $p < 0.001$, Tukey's test $p < 0.001$). Chronic treatment with venlafaxine (five weeks) yielded no effect in control animals, but caused a significant decrease in the expression of all studied genes in stressed rats, i.e., TGFA (F = 22.027, df = 4, $p < 0.001$, Tukey's test $p < 0.001$), TGFB (F = 11.383, df = 4, $p < 0.001$, Tukey's test $p < 0.001$), PTGS2 (F = 20.803, df = 4, $p < 0.001$, Tukey's test

$p < 0.001$), IRF1 (F = 11.239, df = 4, $p < 0.001$, Tukey's test $p < 0.001$), IKBKB (F = 13.817, df = 4, $p < 0.001$, Tukey's test $p < 0.001$) (Figure 1).
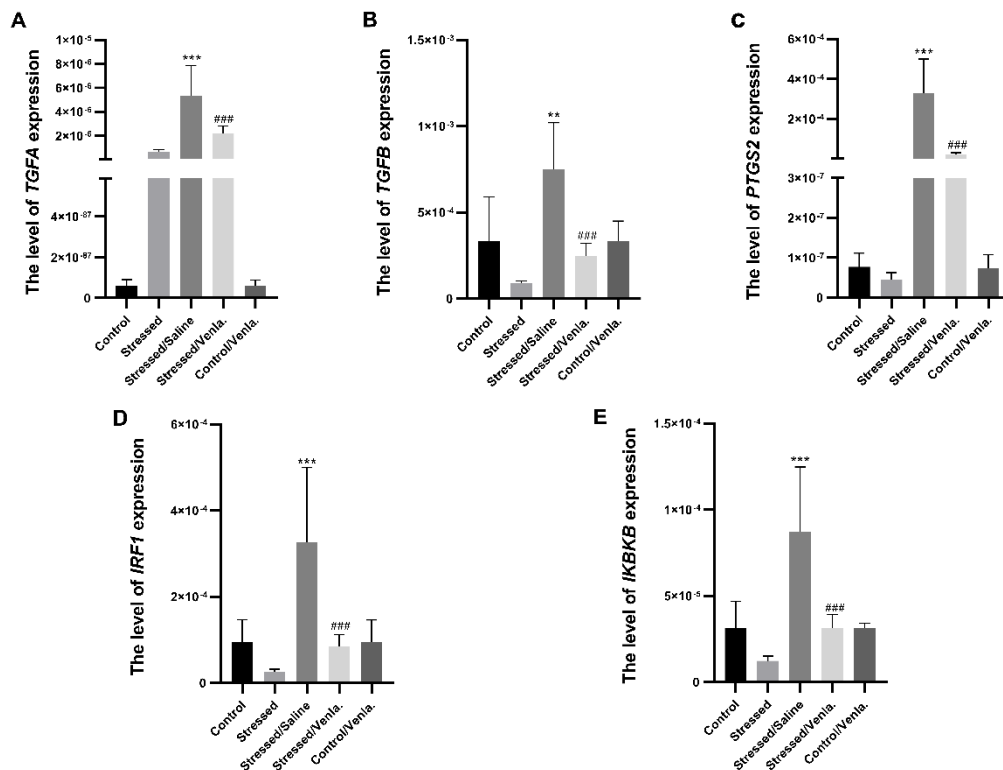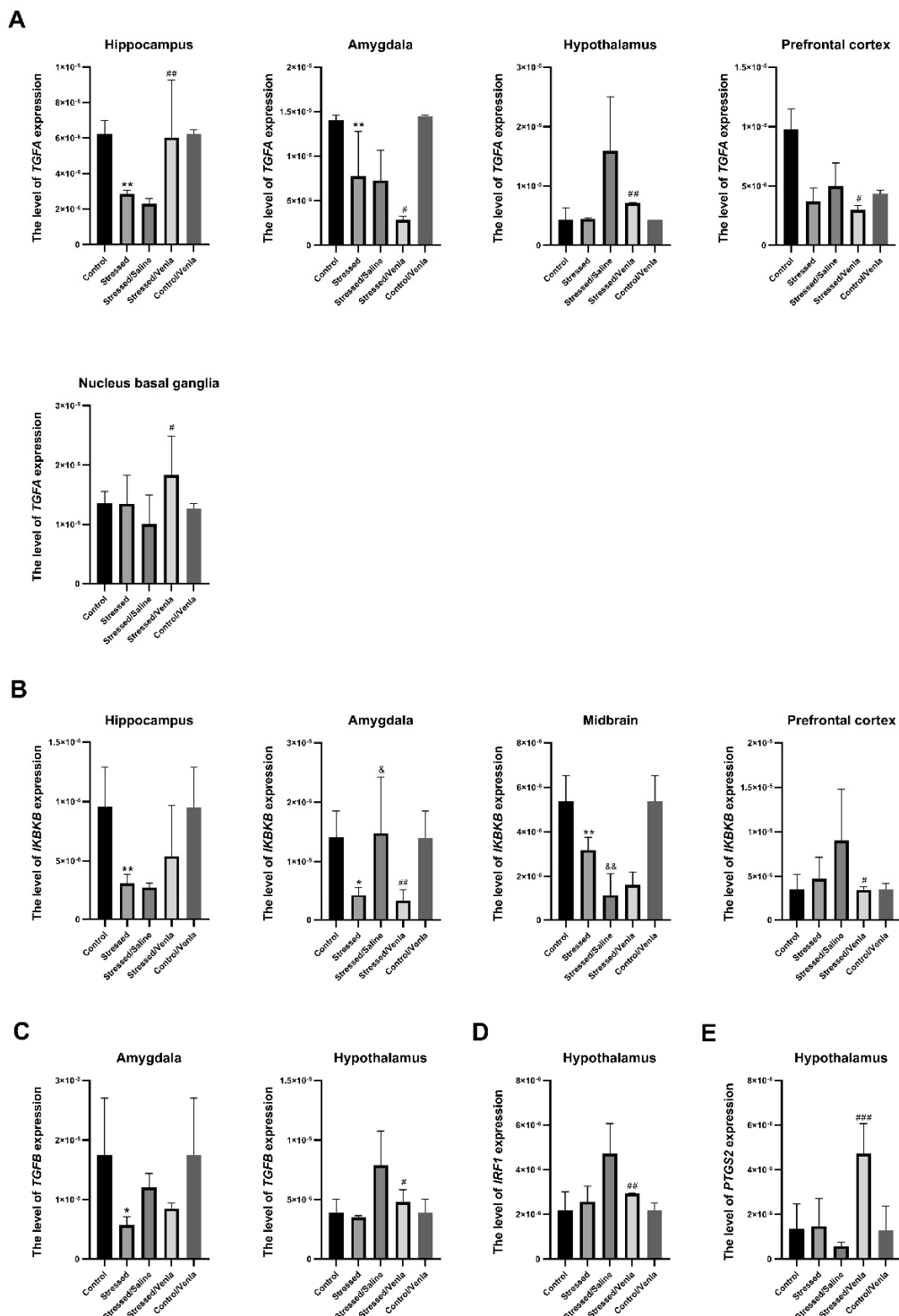

Figure 1. mRNA expression of TGFA (**A**), TGFB (**B**), PTGS2 (**C**), IRF1 (**D**) and IKBKB (**E**) in PBMCs of animals exposed to chronic mild stress (CMS) for two weeks (control, stressed) and in animals exposed to CMS for seven weeks and administered vehicle (1 mL/kg) or venlafaxine (10 mg/kg) for five weeks (stressed/saline, stressed/venlafaxine, control/venlafaxine). Relative gene expression levels were estimated using the 2−ΔCt (Ct gene–Ct 18S) method. Data represent means ± SD. N = 6; ** $p < 0.01$; *** $p < 0.001$ relative to control group; ### $p < 0.001$ relative to stressed/saline group.

3.2.2. Gene Expression in Brain Structures after CMS Procedure and Venlafaxine Administration

The effect of CMS and antidepressant administration on the mRNA expression of the studied genes clearly differed between brain structures. All statistically significant results are shown in Figure 2. The two-week CMS caused a significant decrease of TGFA (F = 10.364, df = 4, $p < 0.001$, Tukey's test $p = 0.006$), and IKBKB (F = 7.985, df = 4, $p < 0.001$, Tukey's test $p = 0.006$) expression in the hippocampus. Furthermore, stress induced lower expression of TGFA (F = 19.543, df = 4, $p < 0.001$, Tukey's test $p = 0.004$), TGFB (F = 4.408, df = 4, $p = 0.008$, Tukey's test $p = 0.022$) and IKBKB (F = 7.311, df = 4, $p < 0.001$, Tukey's test $p = 0.024$) in the amygdala, and in the midbrain in the case of IKBKB (F = 27.746, df = 4, $p < 0.001$, Tukey's test $p = 0.004$). Interestingly, this effect was intensified in animals after the seven-week CMS procedure. After venlafaxine administration, the stressed animals demonstrated downregulation of TGFA (F = 8.635, df = 4, $p < 0.001$, Tukey's test $p < 0.001$), TGFB (F = 8.058, df = 4, $p < 0.001$, Tukey's test $p < 0.001$) and IRF1 (F = 10.804, df = 4, $p < 0.001$, Tukey's test $p < 0.001$) in the hypothalamus, IKBKB (F = 4.029, df = 4, $p = 0.012$, Tukey's test $p = 0.024$) levels in the prefrontal cortex and IKBKB (F = 7.311, df = 4, $p < 0.001$, Tukey's test $p < 0.015$) in the amygdala. On the other hand, venlafaxine treatment also increased the expression of TGFA in the hippocampus (F = 10.364, df = 4, $p < 0.001$, Tukey's test $p = 0.002$) and nucleus basal ganglia (F = 2.815, df = 4, $p = 0.047$, Tukey's test $p = 0.024$), as well as PTGS2 level in the hypothalamus (F = 13.733, df = 4, $p < 0.001$, Tukey's test $p < 0.001$). Furthermore, no differences in mRNA expression level

were found after venlafaxine administration in the nonstressed control group (Figure S1, Supplementary Materials).

Figure 2. mRNA expression of TGFA (**A**), IKBKB (**B**), TGFB (**C**), IRF1 (**D**) and PTGS2 (**E**) in the brain structures of animals exposed to chronic mild stress (CMS) for two weeks (control, stressed) and in animals exposed to CMS for seven weeks and administered vehicle (1 mL/kg) or venlafaxine (10 mg/kg) for five weeks (control/venlafaxine, stressed/saline, stressed/venlafaxine). Relative gene expression levels were estimated using a $2-\Delta Ct$ (Ctgene–Ct18S) method. Data represent means $\pm$ SD. N = 6. *$p < 0.05$; **$p < 0.01$ relative to control group. # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ relative to stressed/saline group. & $p < 0.05$; && $p < 0.01$ relative to stressed group.

3.3. Methylation of Studied Genes Promoters

#### 3.3.1. Methylation Status in PBMCs after CMS Procedure and Venlafaxine Administration

The only significant change in methylation status was found in the case of the IKBKB promoter (Figure 3), where two-week exposure to CMS caused increased methylation compared with nonstressed controls (F = 5.777, df = 4, $p = 0.002$, Tukey's test $p = 0.011$). No significant differences were observed for promoters of other investigated genes in PMBCs.
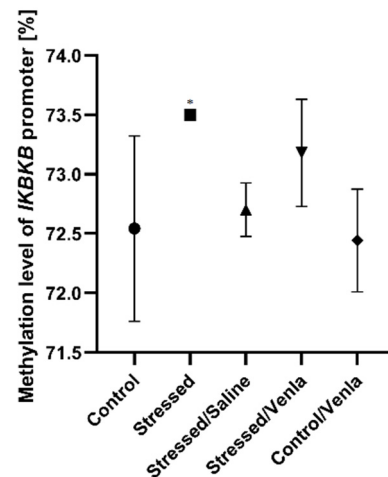
Figure 3. Methylation level of IKBKB promoter in PBMCs of animals exposed to chronic mild stress (CMS) for two weeks (control, stressed) and in animals exposed to CMS for seven weeks, including five-week administration of vehicle (1 mL/kg) or venlafaxine (10 mg/kg) (control/venlafaxine, stressed/saline, stressed/venlafaxine). Data represent means ± SD. N = 6. * $p < 0.05$ relative to control group.

3.3.2. Methylation Status in Brain after CMS Procedure and Venlafaxine Administration

All statistically significant results are shown in Figure 4. CMS procedure significantly increased the methylation level of the TGFA promoter in the amygdala (F = 45.000, df = 4, $p < 0.001$, Tukey's test $p = 0.006$). Stressed animals also demonstrated a higher methylation status in the case of the IRF1 promoter in the amygdala (F = 14.765, df = 4, $p < 0.001$, Tukey's test $p < 0.001$) and prefrontal cortex (F = 29.138, df = 4, $p < 0.001$, Tukey's test $p < 0.001$), as well as in the case of the PTGS2 promoter in the hippocampus (F = 9.749, df = 4, $p < 0.001$, Tukey's test $p < 0.001$) and amygdala (F = 44.933, df = 4, $p < 0.001$, Tukey's test $p < 0.001$). However, CMS also caused a decrease in PTGS2 (F = 9.777, df = 4, $p < 0.001$, Tukey's test $p < 0.001$) as well as TGFA (F = 12.000, df = 4, $p < 0.001$, Tukey's test $p = 0.003$) promoter methylation in the prefrontal cortex Interestingly, chronic five-week administration of venlafaxine resulted in increased IKBKB promoter methylation in the amygdala (F = 24.000, df = 4, $p < 0.001$, Tukey's test $p < 0.001$) and nucleus basal ganglia (F = 5.803, df = 4, $p = 0.002$, Tukey's test $p < 0.001$), and the IRF1 promoter in the amygdala (F = 14.765, df = 4, $p < 0.001$, Tukey's test $p = 0.006$). A similar effect was observed in the case of the TGFA promoter, where the methylation status was higher in the hippocampus (F = 13.500, df = 4, $p < 0.001$, Tukey's test $p < 0.001$) and amygdala (F = 45.000, df = 4, $p < 0.001$, Tukey's test $p < 0.001$). No other differences in mRNA expression level were found (Figure S2, Supplementary Materials).
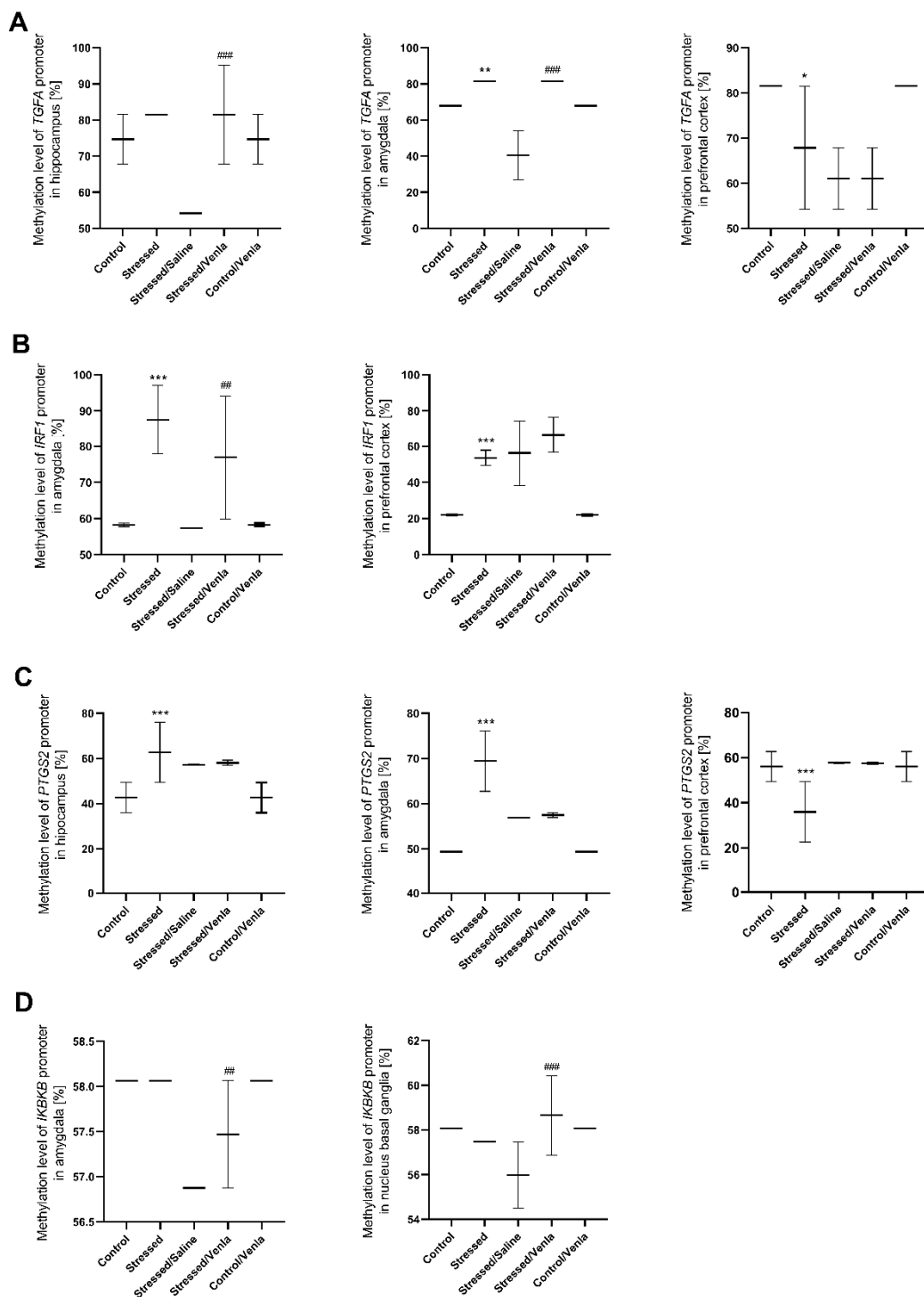
Figure 4. Methylation levels of the TGFA (**A**), IRF1 (**B**), PTGS2 (**C**) and IKBKB (**D**) promoter in brain regions of animals exposed to chronic mild stress (CMS) for two weeks (control, stressed) and in animals exposed to CMS for seven weeks, including five-week administration of vehicle (1 mL/kg) or venlafaxine (10 mg/kg) (control/venlafaxine, stressed/saline, stressed/venlafaxine). Data represent means ± SD. N = 6. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ relative to control group. ## $p < 0.01$ ###; $p < 0.001$ relative to stressed/saline group.

4. Discussion

The present study is the first to investigate the levels of TGFA, IRF1 and IKBKB mRNAs in an animal model of depression. The study also briefly examined the influence of other genes, such as PTGS2 and TGFB, and the effect of prior venlafaxine treatment on depression

as knowledge about their role in the etiopathomechanism of depressive disorders is lacking; this is particularly important as all these factors play roles in neuroinflammatory processes and brain functioning. Moreover, any variation in one of the results in dysregulation and disruption in the others. Therefore, all of these genes were investigated to give a broader view of the inflammatory processes that may be activated in depression, not just focusing on cytokines.

Our study is the first to describe the effect of CMS [53] on the expression of selected genes in the PBMCs and six brain regions (hippocampus, amygdala, hypothalamus, midbrain, prefrontal cortex and basal ganglia). They also present the impact of chronic administration of venlafaxine on the mRNA level in this context. The results are also enriched with a study of whether these factors can induce epigenetic changes, i.e., the methylation status of the gene promoters studied, in blood and brain samples. Our results indicate that for the initial two weeks, the mRNA expression of TGFA, TGFB, PTGS2, IRF1 and IKBKB in PBMCs did not differ between the control and stressed groups. However, after longer exposure to CMS, i.e., for a subsequent five weeks, the expression of all studied genes was significantly upregulated. It might suggest that activation of inflammatory pathways in the periphery could only be triggered after longer exposure to chronic stress conditions.

Our results regarding TGFB are consistent with those of other animal studies demonstrating its increased expression in mice subjected to depression induced by unpredictable mild stress [19]. On the other hand, other studies suggest a significant blood TGFB level is significantly lower in depressed patients compared to healthy controls [20,21,60,61]. Interestingly, we observed that expression of TGFB was significantly diminished in the amygdala after CMS procedure and that chronic (five-week) treatment with venlafaxine caused a decrease of TGFB expression in the PBMCs and the hypothalamus of stressed rats. This observation contradicts previous findings concerning antidepressant drugs; more precisely, it has been reported that treatment with antidepressants caused up-regulation of TGFB in plasma [61,62]. It has been proposed that its protein product plays a role in maintaining the stability of immunologically privileged sites, such as the central nervous system. In addition, as TFGB plays a complex role in stimulating the production of various cytokines [63], it is possible that using antidepressants, including venlafaxine, may change the balance between pro- and anti-inflammatory cytokines by changing the levels of TGFB in depression. It is, therefore, also possible that increased expression of TGFB in chronic stress conditions may occur in response to the elevated levels of proinflammatory agents commonly found in depression. Another investigated gene in the TGF family, TGFA, encodes polypeptide growth factor. Both genes regulate embryonic development and immune response [14,15]. In the present study, low expression of TGFA was observed in the PBMCs of control animals; however, this was significantly upregulated after CMS; interestingly, the TGFA mRNA levels were higher in the hippocampus and amygdala of the nonstressed group than the CMS rats. In the case of the hippocampus, this effect was normalized after venlafaxine administration. Similarly, in PMBCs, antidepressant therapy led to downregulation of TGFA expression, reversing the effects of the CMS procedure. In addition, lower TGFA expression was observed after venlafaxine administration in the amygdala, hypothalamus and prefrontal cortex. Interestingly, CMS caused increased TGFA promoter methylation in the amygdala, which could be associated with lower levels of TGFA expression. In addition, higher levels of methylation were observed after antidepressant therapy, which could be connected with the downregulation of TGFA expression observed in the amygdala of rats treated with venlafaxine. This is the first set of such results concerning the role of TGFA in depression or other psychiatric disorders. However, it has been found to play a role in the induction of proliferation and differentiation of neural cells in the adult mammalian brain: exogenous TGFA administration was observed to trigger repair mechanisms after nervous system injury and to have neuroprotective properties against cytotoxic and apoptotic signals [64]. It is hypothesized that TGFA could improve or the state of neurodegenerative disorders, such as Parkinson's disease, as well as post-traumatic and stroke brain injury, and even reverse some of their characteristic

features. Therefore, future studies should consider the possible role of TGFA in psychiatric disorders. Another gene believed to be associated with mechanisms of depression is PTGS2. It is widely accepted that PTGS2 participates in inflammatory processes partly involved in neurodegeneration in the CNS. PTGS2 and its downstream product PGs play important roles in triggering an inflammatory cascade in depression [24,25]. Our results indicate that its expression was significantly upregulated in PBMCs after seven-week chronic stress, and this effect was, at least partially, reversed by chronic venlafaxine administration. In the case of brain tissues, we only observed one significant change after the antidepressant treatment: a higher level of PTGS2 mRNA in the hypothalamus. We also observed that the CMS procedure increased PTGS2 promoter methylation in the hippocampus and amygdala and reduced it in the prefrontal cortex. However, this methylation pattern seemed to be unrelated to PTGS2 expression. Our findings regarding the PTGS2 mRNA expression gene are consistent with previous reports indicating significantly increased levels in the peripheral blood cells of depressed patients versus healthy controls [65]. Furthermore, in a model depression in adult rats caused by neonatal treatment with the antidepressant drug clomipramine, PTGS2 mRNA expression was increased in the hippocampus. At the same time, the protein level was elevated in the entorhinal cortex, and that the usage of NSAID PTGS2 inhibitors, i.e., COX-2 inhibitors, could reverse depressive behavior [26]. This is in line with another study proving that chronic unpredictable mild stress caused increased PTGS2 expression accompanied with depressive symptoms, which was further neutralized by PTGS2 RNAi lentivirus inhibitor [66]. Administration of COX2 selective inhibitor in depressed rats has also been found to reduce depressive behavior and diminish the levels of cytokines in the hypothalamus of rats [67]. Moreover, for patients with severe depression, therapy with a selective inhibitor cannot only alleviate depressive behavior but also reduce the serum level of proinflammatory cytokines [68]. The upregulation of the PTGS2 gene observed in the course of depression, together with the effectiveness of its inhibitors in therapy, confirm that PTGS2 plays a role in developing depressive disorders. Furthermore, our findings also suggest that venlafaxine has anti-inflammatory activity. The present research examined whether the expression of IRF1 changes during a depression-like state since it plays a pivotal role in controlling the expression of a number of genes whose products are essential in immunity [27]. As stated, IRF1 regulates the transcription of IFN and other IFN-inducible genes, all of which play a role in inflammation [28]. In the present study, IRF1 mRNA level was found to be significantly increased in the PMBCs of rats exposed to CMS; however, this fell to around control levels after venlafaxine administration. Venlafaxine treatment also lowered IRF1 expression in the hypothalamus. Regarding epigenetics, the CMS procedure resulted in an increase of IRF1 promoter methylation in the amygdala and prefrontal cortex, while antidepressant treatment caused higher IRF1 methylation in the amygdala. However, these changes did not affect the mRNA expression of the gene. This may suggest that other processes have a greater impact on the expression of this gene than the methylation of promoter sequences. To date, there has been no research regarding the role of IRF1 in depression and other psychiatric disorders, nor the level of its expression in these conditions. However, we could hypothesize that stress causes increased IRF1 expression and thus increased activation of inflammatory pathways, which is commonly observed in the course of depression. In addition, disruptions in NF-kB signaling, commonly observed in MDD, result in increased levels of proinflammatory cytokines. NF-kB regulates the expression of various genes involved in the immune response [30,31]. One of the IkB kinase subunits, IKKB, encoded by the IKBKB gene, is known to regulate NF-kB activity [43]. Studies have suggested that chronic unpredictable mild stress (CUMS) induces an increase of IKKB protein levels in the hippocampus [69]; however, our present findings indicate that CMS reduced IKBKB mRNA levels in the hippocampus, as well as in the amygdala and midbrain. In addition, in contrast to the brain, CMS resulted in increased IKBKB mRNA expression in PMBCs, which was decreased by venlafaxine treatment. Therefore, it is possible that inhibition of IKKB- NF-kB signaling pathways may exert an antidepressant-like effect and silence the neuroinflammation [69,70]. As the

activation of NF-kB signaling promotes the release of proinflammatory cytokines, increased mRNA expression of IKBKB in PMBCs, which acts as a regulatory factor for NF-kB, could contribute to the activation of inflammatory pathways in blood cells; however, this effect is not reflected in the brain. These differences between tissue types may be associated with their response to stress stimuli. However, it is worth adding that the fact that an elevated level of IKBKB is not associated with high promoter methylation status. The change in promoter methylation status is low enough (approximately 1%) that despite its statistical significance, it may not be biologically relevant. Moreover, it could also suggest that other forms of expression regulation may have a greater influence.

Our findings are mostly in line with those of previous reports and support the concept that depressive disorders accompany alterations of multiple aspects of the immune response, both in the peripheral nervous system and in the central nervous system. Our work has some limitations, particularly a lack of protein level analysis. However, such an examination could not be performed in this study due to material limitations. Moreover, obtained results were characterized by a wide variability between different parts of the brain and between blood and brain samples. It is worth mentioning that in most cases, the investigated genes demonstrated significantly higher expression in blood than brain tissues; however, it can only be speculated whether this is due to a distinct tissue response or other factors. The promoter methylation changes, despite being statistically significant, are not always reflected by altered expression patterns. This suggests that these changes have not been biologically relevant or/and other factors may have a greater influence on expression regulation. Mainly, expression changes are controlled by methylation status. However, other epigenetic modifications, such as modification of histones and microRNAs, could be implicated [71]. Additionally, discordant changes in methylation and expression patterns may be dependent on methylation changes in other cytosines, either outside investigated regions or associated with non-CpG sites. Moreover, other variables that must be taken into consideration are sequences recognized by methylation-sensitive transcriptional factors. In this case, even single methylated or unmethylated cytosine influence the affinity of the TF, and therefore, impact the gene expression. However, this phenomenon is still not well-known, as well as the list of the potentially methylation-sensitive TF is continuously changing [72]. Therefore, it is an interesting perspective research area. MS-HRM analysis could have some potential limitations, such as primer competition, finding suitable primer binding sites as well as issue of the PCR bias. However, all the imperfections of the method can be minimized or even eliminated following the rules carefully [57,73]. As mentioned, MS-HRM analysis has some limitations. In our study, it was not possible to find suitable primer-binding sites in sequences with high CpG content and thus to design primers for the TGFB gene. Additionally, it is worth adding that non-CpG sites, which have not been analyzed in our study, might be differentially methylated and thus affect gene expression [74]. Therefore, observed results should be extrapolated with caution. It is also difficult to develop a single stable and faultless animal model of depression, particularly since many human symptoms cannot be modeled in laboratory animals. We used a validated CMS animal model, which closely mirrors depression in humans. However, it should be remembered that it is based only on anhedonia, reflected by reduced sucrose intake [53]. Moreover, daily injections of drugs or vehicles may act as an additional stress factor of the CMS. However, we believe that such research moves one step closer to the possibility of conducting research on patients.

5. Conclusions

Our main findings indicate that the TGFA, TGFB, PTGS2, IRF1 and IKBKB genes could be responsible for activating inflammatory pathways after stress stimuli. More precisely, this research confirms that CMS is associated with changes in the mRNA expression of these genes, both in PMBCs and regions of the brain, which in turn could trigger an inflammatory cascade. Another key finding is the fact that chronic administration of venlafaxine may cause anti-inflammatory effects by affecting the expression of the

investigated genes. Furthermore, both CMS and venlafaxine administration caused changes in promoter methylation status. However, contradictory results in this area suggest that other epigenetic mechanisms could play a significant role in the expression regulation of the aforementioned genes. The results also indicate that individual brain structures demonstrate different tissue responses for stress and antidepressant drugs, suggesting that reactions are region-specific. Nevertheless, our findings confirm at least a partial association between TGFA, TGFB, PTGS2, IRF1 and IKBKB genes and depression, and hence it is highly likely that inflammation plays a role in psychiatric disorders. Such observations serve as a further step towards understanding the underlying processes of depression and the mechanisms of action of antidepressants.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/genes12050667/s1, Table S1: The effect of CMS procedure and venlafaxine on the body weights of the animals; Figure S1: mRNA expression of TGFA (A), IKBKB (B), TGFB (C), IRF1 (D) and PTGS2 (E) in the brain structures of animals exposed to chronic mild stress (CMS) for two weeks (control, stressed) and in animals exposed to CMS for seven weeks and administered vehicle (1 mL/kg) or venlafaxine (10 mg/kg) for five weeks (control/venlafaxine, stressed/saline, stressed/venlafaxine). Relative gene expression levels were estimated using a $2-\Delta Ct$ (Ctgene–Ct18S) method. Data represent means $\pm$ SD. N = 6.; Figure S2: Methylation levels of the TGFA (A), IRF1 (B), PTGS2 (C) and IKBKB (D) promoter in brain regions of animals exposed to chronic mild stress (CMS) for two weeks (control, stressed) and in animals exposed to CMS for seven weeks, including five-week administration of vehicle (1 mL/kg) or venlafaxine (10 mg/kg) (control/venlafaxine, stressed/saline, stressed/venlafaxine). Data represent means $\pm$ SD. N = 6.

Author Contributions: Conceptualization, J.S., M.P. and T.S.; formal analysis, K.B., P.C. and M.P.; funding acquisition, T.S.; investigation, K.B., P.C., P.W., E.S., G.B. and M.B.; methodology, K.B., P.C., P.W., E.S., M.B., M.P. and T.S.; project administration, K.B. and T.S.; supervision, T.S.; validation, K.B., P.C., P.W., M.N. and K.T.-G.; writing—original draft, K.B.; writing—review and editing, P.C., J.S., M.P. and T.S. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: The study was conducted in accordance with the rules of the 86/609/EEC Directive and was approved by the Local Bioethics Commission of the Institute of Pharmacology, Polish Academy of Sciences in Krakow.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author (T.S.) upon responsible request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. WHO. Depression. 2020. Available online: https://www.who.int/news-room/fact-sheets/detail/d (accessed on 1 June 2020).
2. Al-Harbi, K.S. Treatment-resistant depression: Therapeutic trends, challenges, and future directions. *Patient Prefer. Adherence* **2012**, *6*, 369–388. [CrossRef]
3. Ionescu, D.F.; Rosenbaum, J.F.; Alpert, J.E. Pharmacological approaches to the challenge of treatment-resistant depression. *Dialog. Clin. Neurosci.* **2015**, *17*, 111–126.
4. Capuron, L.; Miller, A.H. Immune system to brain signaling: Neuropsychopharmacological implications. *Pharmacol. Ther.* **2011**, *130*, 226–238. [CrossRef] [PubMed]
5. Schiepers, O.J.; Wichers, M.C.; Maes, M. Cytokines and major depression. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2005**, *29*, 201–217. [CrossRef] [PubMed]
6. Evans, D.L.; Charney, D.S.; Lewis, L.; Golden, R.N.; Gorman, J.M.; Krishnan, K.R.R.; Nemeroff, C.B.; Bremner, J.D.; Carney, R.M.; Coyne, J.C.; et al. Mood Disorders in the Medically Ill: Scientific Review and Recommendations. *Biol. Psychiatry* **2005**, *58*, 175–189. [CrossRef] [PubMed]
7. Zorrilla, E.P.; Luborsky, L.; McKay, J.R.; Rosenthal, R.; Houldin, A.; Tax, A.; McCorkle, R.; Seligman, D.A.; Schmidt, K. The relationship of depression and stressors to im-munological assays: A meta-analytic review. *Brain Behav. Immun.* **2001**, *15*, 199–226. [CrossRef] [PubMed]

8. Howren, M.B.; Lamkin, D.M.; Suls, J. Associations of Depression with C-Reactive Protein, IL-1, and IL-6: A Meta-Analysis. *Psychosom. Med.* **2009**, *71*, 171–186. [CrossRef] [PubMed]

9. Miller, A.H.; Maletic, V.; Raison, C.L. Inflammation and Its Discontents: The Role of Cytokines in the Pathophysiology of Major Depression. *Biol. Psychiatry* **2009**, *65*, 732–741. [CrossRef] [PubMed]

10. Dowlati, Y.; Herrmann, N.; Swardfager, W.; Liu, H.; Sham, L.; Reim, E.K.; Lanctôt, K.L. A Meta-Analysis of Cytokines in Major Depression. *Biol. Psychiatry* **2010**, *67*, 446–457. [CrossRef] [PubMed]

11. Steiner, J.; Bielau, H.; Brisch, R.; Danos, P.; Ullrich, O.; Mawrin, C.; Bernstein, H.G.; Bogerts, B. Immunological aspects in the neurobiology of suicide: Ele-vated microglial density in schizophrenia and depression is associated with suicide. *J. Psychiatr. Res.* **2008**, *42*, 151–157. [CrossRef]

12. Michelucci, A.; Heurtaux, T.; Grandbarbe, L.; Morga, E.; Heuschling, P. Characterization of the microglial phenotype under specific pro-inflammatory and anti-inflammatory conditions: Effects of oligomeric and fibrillar amyloid-β. *J. Neuroimmunol.* **2009**, *210*, 3–12. [CrossRef] [PubMed]

13. Park, J.; Min, J.S.; Kim, B.; Chae, U.B.; Yun, J.W.; Choi, M.S.; Kong, I.K.; Chang, K.T.; Lee, D.S. Mitochondrial ROS govern the LPS-induced pro-inflammatory response in microglia cells by regulating MAPK and NF-κB pathways. *Neurosci. Lett.* **2015**, *584*, 191–196. [CrossRef]

14. Kissin, E.Y.; Lemaire, R.; Korn, J.H.; Lafyatis, R. Transforming growth factor β induces fibroblast fibrillin-1 matrix formation. *Arthritis Rheum.* **2002**, *46*, 3000–3009. [CrossRef] [PubMed]

15. Yamagiwa, S.; Gray, J.D.; Hashimoto, S.; Horwitz, D.A. A Role for TGF-β in the Generation and Expansion of CD4 + CD25 + Reg-ulatory T Cells from Human Peripheral Blood. *J. Immunol.* **2001**, *166*, 7282–7289. [CrossRef] [PubMed]

16. Nam, J.-S.; Terabe, M.; Kang, M.-J.; Chae, H.; Voong, N.; Yang, Y.-A.; Laurence, A.; Michalowska, A.M.; Mamura, M.; Lonning, S.; et al. Transforming Growth Factor β Subverts the Immune System into Directly Promoting Tumor Growth through Interleukin-17. *Cancer Res.* **2008**, *68*, 3915–3923. [CrossRef]

17. Passos, S.T.; Silver, J.S.; O'Hara, A.C.; Sehy, D.; Stumhofer, J.S.; Hunter, C.A. IL-6 Promotes NK Cell Production of IL-17 during Toxo-plasmosis. *J. Immunol.* **2010**, *184*, 1776–1783. [CrossRef]

18. Vivien, D.; Ali, C. Transforming growth factor-β signalling in brain disorders. *Cytokine Growth Factor Rev.* **2006**, *17*, 121–128. [CrossRef]

19. Hong, M.; Zheng, J.; Ding, Z.-Y.; Chen, J.-H.; Yu, L.; Niu, Y.; Hua, Y.-Q.; Wang, L.-L. Imbalance between Th17 and Treg Cells May Play an Important Role in the Development of Chronic Unpredictable Mild Stress-Induced Depression in Mice. *Neuroimmunomodulation* **2013**, *20*, 39–50. [CrossRef]

20. Musil, R.; Schwarz, M.; Riedel, M.; Dehning, S.; Cerovecki, A.; Spellmann, I.; Arolt, V.; Müller, N. Elevated macrophage migration inhibitory factor and decreased transforming growth factor-beta levels in major depression—No influence of celecoxib treatment. *J. Affect. Disord.* **2011**, *134*, 217–225. [CrossRef]

21. Sutcigil, L.; Oktenli, C.; Musabak, U.; Bozkurt, A.; Cansever, A.; Uzun, O.; Sanisoglu, S.Y.; Yesilova, Z.; Ozmenler, N.; Ozsahin, A.; et al. Pro- and anti-inflammatory cytokine balance in ma-jor depression: Effect of sertraline therapy. *Clin. Dev. Immunol.* **2007**, *2007*, 76396. [CrossRef]

22. Aktan, F. iNOS-mediated nitric oxide production and its regulation. *Life Sci.* **2004**, *25*, 639–653. [CrossRef]

23. Hansson, M.; Olsson, I.; Nauseef, W.M. Biosynthesis, processing, and sorting of human myeloperoxidase. *Arch. Biochem. Biophys.* **2006**, *445*, 214–224. [CrossRef] [PubMed]

24. Minghetti, L. Cyclooxygenase-2 (COX-2) in Inflammatory and Degenerative Brain Diseases. *J. Neuropathol. Exp. Neurol.* **2004**, *63*, 901–910. [CrossRef]

25. Minghetti, L. Role of COX-2 in Inflammatory and Degenerative Brain Diseases. *Subcell. Biochem.* **2007**, *42*, 127–141. [CrossRef]

26. Cassano, P.; Hidalgo, A.; Burgos, V.; Adris, S.; Argibay, P. Hippocampal upregulation of the cyclooxygenase-2 gene following neonatal clomipramine treatment (a model of depression). *Pharm. J.* **2006**, *6*, 381–387. [CrossRef] [PubMed]

27. Kröger, A.; Köster, M.; Schroeder, K.; Hauser, H.; Mueller, P.P. Review: Activities of IRF-1. *J. Interf. Cytokine Res.* **2002**, *22*, 5–14. [CrossRef] [PubMed]

28. Tamura, T.; Yanai, H.; Savitsky, D.; Taniguchi, T. The IRF Family Transcription Factors in Immunity and Oncogenesis. *Annu. Rev. Immunol.* **2008**, *26*, 535–584. [CrossRef] [PubMed]

29. Zahiu, C.D.M.; Rimbas, M. Neuropsychiatric side-effects of interferon-alpha treatment: Pathophysiology and therapeutic op-tions. In *Maedica*; 2014; 9, pp. 121–126. Available online: https://pubmed.ncbi.nlm.nih.gov/25705266 (accessed on 1 June 2020).

30. Bierhaus, A.; Wolf, J.; Andrassy, M.; Rohleder, N.; Humpert, P.M.; Petrov, D.; Ferstl, R.; Von Eynatten, M.; Wendt, T.; Rudofsky, G.; et al. A mechanism converting psychosocial stress into mononuclear cell activation. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1920–1925. [CrossRef]

31. Pace, T.W.; Mletzko, T.C.; Alagbe, O.; Musselman, D.L.; Nemeroff, C.B.; Miller, A.H.; Heim, C.M. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. *Am. J. Psychiatry* **2006**, *163*, 1630–1633. [CrossRef]

32. Akira, S.; Takeda, K. Toll-like receptor signalling. *Nat. Rev. Immunol.* **2004**, *4*, 499–511. [CrossRef] [PubMed]

33. Krakauer, T. Nuclear factor-κB: Fine-tuning a central integrator of diverse biologic stimuli. *Int. Rev. Immunol.* **2008**, *27*, 286–292. [CrossRef] [PubMed]

34. Zhang, Q.; Lenardo, M.J.; Baltimore, D. 30 Years of NF-κB: A Blossoming of Relevance to Human Pathobiology. *Cell* **2017**, *168*, 37–57. [CrossRef] [PubMed]
35. Gerondakis, S.; Fulford, T.S.; Messina, N.L.; Grumont, R.J. NF-κB control of T cell development. *Nat. Immunol.* **2014**, *15*, 15–25. [CrossRef] [PubMed]
36. Xia, Y.; Shen, S.; Verma, I.M. NF-κB, an Active Player in Human Cancers. *Cancer Immunol. Res.* **2014**, *2*, 823–830. [CrossRef] [PubMed]
37. Van Delft, M.A.M.; Huitema, L.F.A.; Tas, S.W. The contribution of NF-κB signalling to immune regulation and tolerance. *Eur. J. Clin. Investig.* **2015**, *45*, 529–539. [CrossRef] [PubMed]
38. Duman, R.S.; Li, N. A neurotrophic hypothesis of depression: Role of synaptogenesis in the actions of NMDA receptor antago-nists. *Philos. Trans. R. Soc. B Biol. Sci.* **2012**, *367*, 2475–2484. [CrossRef]
39. Lindholm, J.S.O.; Castrén, E. Mice with altered BDNF signaling as models for mood disorders and antidepressant effects. *Front. Behav. Neurosci.* **2014**, *8*. [CrossRef]
40. Caviedes, A.; Lafourcade, C.; Soto, C.; Wyneken, U. BDNF/NF-κB Signaling in the Neurobiology of Depression. *Curr. Pharm. Des.* **2017**, *23*, 3154–3163. [CrossRef] [PubMed]
41. Pizzi, M.; Spano, P.F. Distinct roles of diverse nuclear factor-κB complexes in neuropathological mechanisms. *Eur. J. Pharmacol.* **2006**, *545*, 22–28. [CrossRef]
42. Meffert, M.K.; Baltimore, D. Physiological functions for brain NF-κB. *Trends Neurosci.* **2005**, *28*, 37–43. [CrossRef] [PubMed]
43. Napetschnig, J.; Wu, H. Molecular Basis of NF-κB Signaling. *Annu. Rev. Biophys.* **2013**, *42*, 443–468. [CrossRef] [PubMed]
44. Karin, M.; Ben-Neriah, Y. Phosphorylation Meets Ubiquitination: The Control of NF-κB Activity. *Annu Rev. Immunol.* **2000**, *18*, 621–663. [CrossRef] [PubMed]
45. Cardinez, C.; Miraghazadeh, B.; Tanita, K.; Da Silva, E.; Hoshino, A.; Okada, S.; Chand, R.; Asano, T.; Tsumura, M.; Yoshida, K.; et al. Gain-of-function IKBKB mutation causes human combined immune deficiency. *J. Exp. Med.* **2018**, *215*, 2715–2724. [CrossRef] [PubMed]
46. Bialek, K.; Czarny, P.; Watala, C.; Wigner, P.; Talarowska, M.; Galecki, P.; Szemraj, J.; Sliwinski, T. Novel association between TGFA, TGFB1, IRF1, PTGS2 and IKBKB single-nucleotide polymorphisms and occurrence, severity and treatment response of major depressive disorder. *PeerJ* **2020**, *8*, e8676. [CrossRef]
47. Frank, M.G.; Hershman, S.A.; Weber, M.D.; Watkins, L.R.; Maier, S.F. Chronic exposure to exogenous glucocorticoids primes micro-glia to pro-inflammatory stimuli and induces NLRP3 mRNA in the hippocampus. *Psychoneuroendocrinology* **2014**, *40*, 191–200. [CrossRef]
48. Han, A.; Yeo, H.; Park, M.J.; Kim, S.H.; Choi, H.J.; Hong, C.W.; Kwon, M.S. IL-4/10 prevents stress vulnerability following imipramine dis-continuation. *J. Neuroinflamm.* **2015**, *12*, 1–16. [CrossRef]
49. You, Z.; Luo, C.; Zhang, W.; Chen, Y.; He, J.; Zhao, Q.; Zuo, R.; Wu, Y. Pro- and anti-inflammatory cytokines expression in rat's brain and spleen exposed to chronic mild stress: Involvement in depression. *Behav. Brain Res.* **2011**, *225*, 135–141. [CrossRef]
50. Vogelzangs, N.; Duivis, H.E.; Beekman, A.; Kluft, C.; Neuteboom, J.; Hoogendijk, W.; Smit, J.H.; De Jonge, P.; Penninx, B.W.J.H. Association of depressive disorders, depression characteristics and antidepressant medication with inflammation. *Transl. Psychiatry* **2012**, *2*, e79. [CrossRef]
51. *The 100 Most Important Chemical Compounds: A Reference Guide*; ABC-CLIO: Santa Barbara, CA, USA, 2008.
52. Westenberg, H.G.M.; Sandner, C. Tolerability and safety of fluvoxamine and other antidepressants. *Int. J. Clin. Pract.* **2006**, *60*, 482–491. [CrossRef]
53. Papp, M. Models of Affective Illness: Chronic Mild Stress in the Rat. *Curr. Protoc. Pharmacol.* **2012**, *57*, 5–9. [CrossRef] [PubMed]
54. Wojdacz, T.K.; Dobrovic, A.; Hansen, L.L. Methylation-sensitive high-resolution melting. *Nat. Protoc.* **2008**, *3*, 1903. [CrossRef] [PubMed]
55. Wojdacz, T.K.; Dobrovic, A. Methylation-sensitive high resolution melting (MS-HRM): A new approach for sensitive and high-throughput assessment of methylation. *Nucleic Acids Res.* **2007**, *35*, e41. [CrossRef] [PubMed]
56. Dreos, R.; Ambrosini, G.; Groux, R.; Périer, R.C.; Bucher, P. The eukaryotic promoter database in its 30th year: Focus on non-vertebrate organisms. *Nucleic Acids Res.* **2017**, *45*, D51–D55. [CrossRef] [PubMed]
57. Wojdacz, T.K.; Borgbo, T.; Hansen, L.L. Primer design versus PCR bias in methylation independent PCR amplifications. *Epigenetics* **2009**, *4*, 231–234. [CrossRef]
58. Papp, M.; Gruca, P.; Lason, M.; Niemczyk, M.; Willner, P. The role of prefrontal cortex dopamine D2 and D3 receptors in the mech-anism of action of venlafaxine and deep brain stimulation in animal models of treatment-responsive and treatment-resistant depression. *J. Psychopharmacol.* **2019**, *33*, 748–756. [CrossRef]
59. Papp, M.; Gruca, P.; Lason-Tyburkiewicz, M.; Litwa, E.; Niemczyk, M.; Tota-Glowczyk, K.; Willner, P. Dopaminergic mechanisms in memory consolidation and antidepressant reversal of a chronic mild stress-induced cognitive impairment. *Psychopharmacology* **2017**, *234*, 2571–2585. [CrossRef]
60. Wray, N.R.; Ripke, S.; Mattheisen, M.; Trzaskowski, M.; Byrne, E.M.; Abdellaoui, A.; Adams, M.J.; Agerbo, E.; Air, T.M.; Andlauer, T.M.F.; et al. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nat. Genet.* **2018**, *50*, 668–681. [CrossRef]
61. Myint, A.-M.; Leonard, B.E.; Steinbusch, H.W.; Kim, Y.-K. Th1, Th2, and Th3 cytokine alterations in major depression. *J. Affect. Disord.* **2005**, *88*, 167–173. [CrossRef]

62. Lee, K.-M.; Kim, Y.-K. The role of IL-12 and TGF-β1 in the pathophysiology of major depressive disorder. *Int. Immunopharmacol.* **2006**, *6*, 1298–1304. [CrossRef]

63. Kunzmann, S.; Mantel, P.-Y.; Wohlfahrt, J.G.; Akdis, M.; Blaser, K.; Schmidt-Weber, C.B. Histamine enhances TGF-β1-mediated sup-pression of Th2 responses. *FASEB J.* **2003**, *17*, 1089–1095. [CrossRef]

64. Fallon, J.; Reid, S.; Kinyamu, R.; Opole, I.; Opole, R.; Baratta, J.; Korc, M.; Endo, T.L.; Duong, A.; Nguyen, G.; et al. In vivo induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 14686–14691. [CrossRef] [PubMed]

65. Gałecki, P.; Gałecka, E.; Maes, M.; Chamielec, M.; Orzechowska, A.; Bobińska, K.; Lewiński, A.; Szemraj, J. The expression of genes encoding for COX-2, MPO, iNOS, and sPLA2-IIA in patients with recurrent depressive disorder. *J. Affect. Disord.* **2012**, *138*, 360–366. [CrossRef]

66. Chen, Q.; Luo, Y.; Kuang, S.; Yang, Y.; Tian, X.; Ma, J.; Mai, S.; Xue, L.; Yang, J. Cyclooxygenase-2 Signalling Pathway in the Cortex is Involved in the Pathophysiological Mechanisms in the Rat Model of Depression. *Sci. Rep.* **2017**, *7*, 488. [CrossRef]

67. Myint, A.M.; Steinbusch, H.W.; Goeghegan, L.; Luchtman, D.; Kim, Y.K.; Leonard, B.E. Effect of the COX-2 Inhibitor Celecoxib on Behavioural and Immune Changes in an Olfactory Bulbectomised Rat Model of Depression. *Neuroimmunomodulation* **2007**, *14*, 65–71. [CrossRef] [PubMed]

68. Abbasi, S.-H.; Hosseini, F.; Modabbernia, A.; Ashrafi, M.; Akhondzadeh, S. Effect of celecoxib add-on treatment on symptoms and serum IL-6 concentrations in patients with major depressive disorder: Randomized double-blind placebo-controlled study. *J. Affect. Disord.* **2012**, *141*, 308–314. [CrossRef]

69. Dong, S.Q.; Zhang, Q.P.; Zhu, J.X.; Chen, M.; Li, C.F.; Liu, Q.; Geng, D.; Yi, L.T. Gypenosides reverses depressive behavior via inhibiting hippo-campal neuroinflammation. *Biomed. Pharm.* **2018**, *106*, 1153–1160. [CrossRef] [PubMed]

70. Liu, Y.M.; Niu, L.; Wang, L.L.; Bai, L.; Fang, X.Y.; Li, Y.C.; Yi, L.T. Berberine attenuates depressive-like behaviors by suppressing neu-ro-inflammation in stressed mice. *Brain Res. Bull.* **2017**, *134*, 220–227. [CrossRef] [PubMed]

71. Mehler, M.F. Epigenetic principles and mechanisms underlying nervous system functions in health and disease. *Prog. Neurobiol.* **2008**, *86*, 305–341. [CrossRef] [PubMed]

72. Héberlé, É.; Bardet, A.F. Sensitivity of transcription factors to DNA methylation. *Essays Biochem.* **2019**, *63*, 727–741. [CrossRef] [PubMed]

73. Wojdacz, T.K.; Hansen, L.L. Reversal of PCR bias for improved sensitivity of the DNA methylation melting curve assay. *Biotech.* **2006**, *41*, 274–278. [CrossRef] [PubMed]

74. Nicolia, V.; Cavallaro, R.A.; López-González, I.; Maccarrone, M.; Scarpa, S.; Ferrer, I.; Fuso, A. DNA Methylation Profiles of Selected Pro-Inflammatory Cytokines in Alzheimer Disease. *J. Neuropathol. Exp. Neurol.* **2017**. [CrossRef]

Oświadczenia współautorów

Łódź, 24.05.2021

Mgr Katarzyna Białek

Katedra Genetyki Molekularnej

Uniwersytet Łódzki

Oświadczenie o udziale w publikacji

Oświadczam, że w pracy **Bialek K**, Czarny P, Strycharz J, Sliwinski T. Major depressive disorders accompanying autoimmune diseases - Response to treatment. Prog Neuropsychopharmacol Biol Psychiatry. 2019;95:109678 mój udział wynosił 70% i obejmował przygotowanie manuskryptu, rycin i tabel.

Oświadczam, że w pracy **Bialek K**, Czarny P, Watala C, Synowiec E, Wigner P, Bijak M, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Preliminary Study of the Impact of Single-Nucleotide Polymorphisms of IL-1α, IL-1β and TNF-α Genes on the Occurrence, Severity and Treatment Effectiveness of the Major Depressive Disorder. Cell Mol Neurobiol. 2020; 40(6):1049-1056 mój udział wynosił 55% i obejmował planowanie prac, realizację części eksperymentalnej, opracowanie wyników i ich interpretację, przygotowanie manuskryptu oraz rycin i tabel.

Oświadczam, że w pracy **Bialek K**, Czarny P, Watala C, Wigner P, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Novel association between TGFA, TGFB1, IRF1, PTGS2 and IKBKB single-nucleotide polymorphisms and occurrence, severity and treatment response of major depressive disorder. PeerJ. 2020; 8:8676 mój udział wynosił 65% i obejmował planowanie prac, realizację części eksperymentalnej, opracowanie wyników i ich interpretację, przygotowanie manuskryptu oraz rycin i tabel.

Oświadczam, że w pracy **Bialek K**, Czarny P, Wigner P, Synowiec E, Barszczewska G, Bijak M, Szemraj J, Niemczyk M, Tota-Glowczyk K, Papp M, Sliwinski T. Chronic Mild Stress and Venlafaxine Treatment Were Associated with Altered Expression Level and Methylation Status of New Candidate Inflammatory Genes in PBMCs and Brain Structures of Wistar Rats. Genes (Basel). 2021; 12(5):667

Katarzyna Białek

mój udział wynosił 60% i i obejmował planowanie prac, realizację części eksperymentalnej, opracowanie wyników i ich interpretację, przygotowanie manuskryptu oraz rycin i tabel.

Katarzyna Białek

Łódź, 31.05.2021

Prof. dr hab. Tomasz Śliwiński

Katedra Genetyki Molekularnej

Uniwersytet Łódzki

Oświadczenie o udziale w publikacji

Oświadczam, że w pracy Bialek K, Czarny P, Strycharz J, Sliwinski T. Major depressive disorders accompanying autoimmune diseases - Response to treatment. Prog Neuropsychopharmacol Biol Psychiatry. 2019;95:109678 mój udział wynosił 15% i obejmował tworzenie koncepcji pracy, konsultacje merytoryczne, pomoc w przygotowaniu oraz redakcji manuskryptu.

Oświadczam, że w pracy Bialek K, Czarny P, Watala C, Synowiec E, Wigner P, Bijak M, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Preliminary Study of the Impact of Single-Nucleotide Polymorphisms of IL-1α, IL-1β and TNF-α Genes on the Occurrence, Severity and Treatment Effectiveness of the Major Depressive Disorder. Cell Mol Neurobiol. 2020; 40(6):1049-1056 mój udział wynosił 10% i obejmował współudział w projektowaniu badań, konsultacje merytoryczne oraz pomoc w analizie uzyskanych wyników i redakcji manuskryptu.

Oświadczam, że w pracy Bialek K, Czarny P, Watala C, Wigner P, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Novel association between TGFA, TGFB1, IRF1, PTGS2 and IKBKB single-nucleotide polymorphisms and occurrence, severity and treatment response of major depressive disorder. PeerJ. 2020; 8:8676 wynosił 10% i obejmował współudział w projektowaniu badań, konsultacje merytoryczne oraz pomoc w analizie uzyskanych wyników i redakcji manuskryptu.

Oświadczam, że w pracy Bialek K, Czarny P, Wigner P, Synowiec E, Barszczewska G, Bijak M, Szemraj J, Niemczyk M, Tota-Glowczyk K, Papp M, Sliwinski T. Chronic Mild Stress and Venlafaxine Treatment Were Associated with Altered Expression Level and Methylation Status of New Candidate Inflammatory Genes in PBMCs and Brain Structures of Wistar Rats. Genes (Basel). 2021; 12(5):667 wynosił 10% i obejmował współudział w projektowaniu badań, konsultacje merytoryczne oraz pomoc w analizie uzyskanych wyników i redakcji manuskryptu.

KIEROWNIK
PRACOWNI GENETYKI MEDYCZNEJ
Wydział Biologii i Ochrony Środowiska UŁ

prof. dr hab. Tomasz Śliwiński

Łódź, 28.05.2021

dr Piotr Czarny

Katedra Biochemii Medycznej

Uniwersytet Medyczny w Łodzi

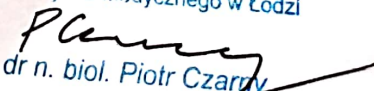## Oświadczenie o udziale w publikacji

Oświadczam, że w pracy Bialek K, **Czarny P**, Strycharz J, Sliwinski T. Major depressive disorders accompanying autoimmune diseases - Response to treatment. Prog Neuropsychopharmacol Biol Psychiatry. 2019;95:109678 mój udział wynosił 10% i obejmował tworzenie koncepcji pracy, konsultacje merytoryczne oraz współudział w przygotowaniu manuskryptu.

Oświadczam, że w pracy Bialek K, **Czarny P**, Watala C, Synowiec E, Wigner P, Bijak M, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Preliminary Study of the Impact of Single-Nucleotide Polymorphisms of IL-1α, IL-1β and TNF-α Genes on the Occurrence, Severity and Treatment Effectiveness of the Major Depressive Disorder. Cell Mol Neurobiol. 2020; 40(6):1049-1056 mój udział wynosił 7% i obejmował współudział w projektowaniu badań, przeprowadzeniu eksperymentów, konsultacje merytoryczne oraz pomoc w analizie uzyskanych wyników i redakcji manuskryptu.

Oświadczam, że w pracy Bialek K, **Czarny P**, Watala C, Wigner P, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Novel association between TGFA, TGFB1, IRF1, PTGS2 and IKBKB single-nucleotide polymorphisms and occurrence, severity and treatment response of major depressive disorder. PeerJ. 2020; 8:8676 mój udział wynosił 5% i obejmował współudział w projektowaniu badań, przeprowadzeniu eksperymentów, konsultacje merytoryczne oraz pomoc w analizie uzyskanych wyników i redakcji manuskryptu.
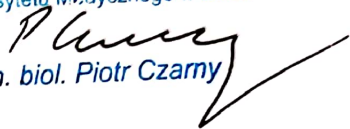
Oświadczam, że w pracy Bialek K, **Czarny P**, Wigner P, Synowiec E, Barszczewska G, Bijak M, Szemraj J, Niemczyk M, Tota-Glowczyk K, Papp M, Sliwinski T. Chronic Mild Stress and Venlafaxine Treatment Were Associated with Altered Expression Level and Methylation Status of New Candidate Inflammatory

ADIUNKT
Zakładu Biochemii Medycznej
Katedry Biochemii Medycznej
Uniwersytetu Medycznego w Łodzi

dr n. biol. Piotr Czarny

Genes in PBMCs and Brain Structures of Wistar Rats. Genes (Basel). 2021; 12(5):667 mój udział wynosił 6% i obejmował współudział w projektowaniu badań, przeprowadzeniu eksperymentów, konsultacje merytoryczne oraz pomoc w analizie uzyskanych wyników i redakcji manuskryptu.

ADIUNKT
Zakładu Biochemii Medycznej
Katedry Biochemii Medycznej
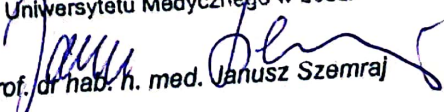Uniwersytetu Medycznego w Łodzi

dr n. biol. Piotr Czarny

Prof. dr hab. Janusz Szemraj

Katedra Biochemii Medycznej

Uniwersytet Medyczny w Łodzi

Oświadczenie o udziale w publikacji

Oświadczam, że w pracy Bialek K, Czarny P, Watala C, Synowiec E, Wigner P, Bijak M, Talarowska M, Galecki P, **Szemraj J**, Sliwinski T. Preliminary Study of the Impact of Single-Nucleotide Polymorphisms of IL-1α, IL-1β and TNF-α Genes on the Occurrence, Severity and Treatment Effectiveness of the Major Depressive Disorder. Cell Mol Neurobiol. 2020; 40(6):1049-1056 mój udział wynosił 4% i obejmował współudział w tworzeniu koncepcji, konsultacje merytoryczne oraz pomoc w redakcji manuskryptu.

Oświadczam, że w pracy Bialek K, Czarny P, Watala C, Wigner P, Talarowska M, Galecki P, **Szemraj J**, Sliwinski T. Novel association between TGFA, TGFB1, IRF1, PTGS2 and IKBKB single-nucleotide polymorphisms and occurrence, severity and treatment response of major depressive disorder. PeerJ. 2020; 8:8676 mój udział wynosił 4% i obejmował współudział w projektowaniu badań, przeprowadzeniu eksperymentów, konsultacje merytoryczne oraz pomoc w analizie uzyskanych wyników i redakcji manuskryptu.

Oświadczam, że w pracy Bialek K, Czarny P, Wigner P, Synowiec E, Barszczewska G, Bijak M, **Szemraj J**, Niemczyk M, Tota-Glowczyk K, Papp M, Sliwinski T. Chronic Mild Stress and Venlafaxine Treatment Were Associated with Altered Expression Level and Methylation Status of New Candidate Inflammatory Genes in PBMCs and Brain Structures of Wistar Rats. Genes (Basel). 2021; 12(5):667 mój udział wynosił 3% i obejmował współudział w tworzeniu koncepcji, konsultacje merytoryczne oraz pomoc w redakcji manuskryptu.

KIEROWNIK
Katedry i Zakładu Biochemii Medycznej
Uniwersytetu Medycznego w Łodzi

Prof. dr hab. n. med. Janusz Szemraj

prof. dr hab. n. med. Piotr Gałecki

Klinika Psychiatrii Dorosłych

Uniwersytet Medyczny w Łodzi

Oświadczenie o udziale w publikacji

Oświadczam, że w pracy Bialek K, Czarny P, Watala C, Synowiec E, Wigner P, Bijak M, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Preliminary Study of the Impact of Single-Nucleotide Polymorphisms of IL-1α, IL-1β and TNF-α Genes on the Occurrence, Severity and Treatment Effectiveness of the Major Depressive Disorder. Cell Mol Neurobiol. 2020; 40(6):1049-1056 mój udział wynosił 4% i obejmował współudział w tworzeniu koncepcji, diagnostyce i kwalifikacji medycznej uczestników badania, pomoc w redakcji tekstu.

Oświadczam, że w pracy Bialek K, Czarny P, Watala C, Wigner P, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Novel association between TGFA, TGFB1, IRF1, PTGS2 and IKBKB single-nucleotide polymorphisms and occurrence, severity and treatment response of major depressive disorder. PeerJ. 2020; 8:8676 mój udział wynosił 4% i obejmował współudział w tworzeniu koncepcji, diagnostyce i kwalifikacji medycznej uczestników badania, pomoc w redakcji tekstu.

Łódź, 31.05.2021

prof. dr hab. n.med. Cezary Watała
Zakład Zaburzeń Krzepnięcia Krwi
Uniwersytet Medyczny w Łodzi

Oświadczenie o udziale w publikacji

Oświadczam, że w pracy Bialek K, Czarny P, **Watala C**, Synowiec E, Wigner P, Bijak M, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Preliminary Study of the Impact of Single-Nucleotide Polymorphisms of IL-1α, IL-1β and TNF-α Genes on the Occurrence, Severity and Treatment Effectiveness of the Major Depressive Disorder. Cell Mol Neurobiol. 2020; 40(6):1049-1056 mój udział wynosił 4% i obejmował pomoc w analizie uzyskanych wyników i redakcji manuskryptu.

Oświadczam, że w pracy Bialek K, Czarny P, **Watala C**, Wigner P, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Novel association between TGFA, TGFB1, IRF1, PTGS2 and IKBKB single-nucleotide polymorphisms and occurrence, severity and treatment response of major depressive disorder. PeerJ. 2020; 8:8676 mój udział wynosił 4% i obejmował pomoc w analizie uzyskanych wyników i redakcji manuskryptu.

KIEROWNIK
Zakładu Zaburzeń Krzepnięcia Krwi
Uniwersytatu Medycznego w Łodzi

Prof. dr hab. Cezary Watała

Łódź, 25.05.2021

dr Paulina Wigner
Katedra Biochemii Ogólnej
Uniwersytet Łódzki

Oświadczenie o udziale w publikacji

Oświadczam, że w pracy Bialek K, Czarny P, Watala C, Synowiec E, Wigner P, Bijak M, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Preliminary Study of the Impact of Single-Nucleotide Polymorphisms of IL-1α, IL-1β and TNF-α Genes on the Occurrence, Severity and Treatment Effectiveness of the Major Depressive Disorder. Cell Mol Neurobiol. 2020; 40(6):1049-1056 mój udział wynosił 4% i obejmował współudział w przeprowadzeniu eksperymentów.

Oświadczam, że w pracy Bialek K, Czarny P, Watala C, Wigner P, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Novel association between TGFA, TGFB1, IRF1, PTGS2 and IKBKB single-nucleotide polymorphisms and occurrence, severity and treatment response of major depressive disorder. PeerJ. 2020; 8:8676. mój udział wynosił 4% i obejmował współudział w przeprowadzeniu eksperymentów.

Oświadczam, że w pracy Bialek K, Czarny P, Wigner P, Synowiec E, Barszczewska G, Bijak M, Szemraj J, Niemczyk M, Tota-Glowczyk K, Papp M, Sliwinski T. Chronic Mild Stress and Venlafaxine Treatment Were Associated with Altered Expression Level and Methylation Status of New Candidate Inflammatory Genes in PBMCs and Brain Structures of Wistar Rats. Genes (Basel). 2021; 12(5):667 mój udział wynosił 3% i obejmował współudział w przeprowadzeniu eksperymentów.

Paulina Wigner

Łódź, 27.05.2021

dr Ewelina Synowiec
Katedra Genetyki Molekularnej
Uniwersytet Łódzki

Oświadczenie o udziale w publikacji

Oświadczam, że w pracy Bialek K, Czarny P, Watala C, **Synowiec E**, Wigner P, Bijak M, Talarowska M, Galecki P, Szemraj J, Sliwinski T. Preliminary Study of the Impact of Single-Nucleotide Polymorphisms of IL-1α, IL-1β and TNF-α Genes on the Occurrence, Severity and Treatment Effectiveness of the Major Depressive Disorder. Cell Mol Neurobiol. 2020; 40(6):1049-1056 mój udział wynosił 4% i obejmował współudział w wykonywaniu eksperymentów.

Oświadczam, że w pracy Bialek K, Czarny P, Wigner P, **Synowiec E**, Barszczewska G, Bijak M, Szemraj J, Niemczyk M, Tota-Glowczyk K, Papp M, Sliwinski T. Chronic Mild Stress and Venlafaxine Treatment Were Associated with Altered Expression Level and Methylation Status of New Candidate Inflammatory Genes in PBMCs and Brain Structures of Wistar Rats. Genes (Basel). 2021; 12(5):667 wynosił 3% i obejmował współudział w wykonywaniu eksperymentów.

Łódź, 25.05.2021

mgr inż. Gabriela Barszczewska

Katedra Genetyki Molekularnej

Uniwersytet Łódzki

Oświadczenie o udziale w publikacji

Oświadczam, że w pracy Bialek K, Czarny P, Wigner P, Synowiec E, Barszczewska G, Bijak M, Szemraj J, Niemczyk M, Tota-Glowczyk K, Papp M, Sliwinski T. Chronic Mild Stress and Venlafaxine Treatment Were Associated with Altered Expression Level and Methylation Status of New Candidate Inflammatory Genes in PBMCs and Brain Structures of Wistar Rats. Genes (Basel). 2021; 12(5):667 mój udział wynosił 3% i obejmował współudział w przeprowadzeniu eksperymentów.

Gabriela Barszczewska

Łódź, 24.05.2021

mgr Justyna Strycharz
Katedra Biochemii Medycznej
Uniwersytet Medyczny w Łodzi

Oświadczenie o udziale w publikacji

Oświadczam, że w pracy Bialek K, Czarny P, Strycharz J, Sliwinski T. Major depressive disorders accompanying autoimmune diseases - Response to treatment. Prog Neuropsychopharmacol Biol Psychiatry. 2019;95:109678 mój udział wynosił 5% i obejmował współudział w przygotowaniu manuskryptu.