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Monocyte mitochondrial dysfunction, inflammaging, and inflammatory pyroptosis in major depression

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ABSTRACT

Background: The macrophage theory of depression states that macrophages play an important role in Major Depressive Disorder (MDD).

Methods: MDD patients (N = 140) and healthy controls (N = 120) participated in a cross-sectional study investigating the expression of apoptosis/growth and lipid/cholesterol pathway genes (BAX, BCL10, EGR1, EGR2, HB-EGF, NR1H3, ABCA1, ABCG1, MVK, CD163, HMOX1) in monocytes (macrophage/microglia precursors). Gene expressions were correlated to a set of previously determined and reported inflammation-regulating genes and analyzed with respect to various clinical parameters.

Results: MDD monocytes showed an overexpression of the apoptosis/growth/cholesterol and the TNF genes forming an inter-correlating gene cluster (cluster 3) separate from the previously described inflammation-related gene clusters (containing IL1 and IL6). While upregulation of monocyte gene cluster 3 was a hallmark of monocytes of all MDD patients, upregulation of the inflammation-related clusters was confirmed to be found only in the monocytes of patients with childhood adversity. The latter group also showed a downregulation of the cholesterol metabolism gene MVK, which is known to play an important role in trained immunity and proneness to inflammation.

Conclusions: The upregulation of cluster 3 genes in monocytes of all MDD patients suggests a premature aging of the cells, i.e. mitochondrial apoptotic dysfunction and TNF "inflammaging", as a general feature of MDD. The overexpression of the IL-1/IL-6 containing inflammation clusters and the downregulation of MVK in monocytes of patients with childhood adversity indicates a shift in this condition to a more severe inflammation form (pyroptosis) of the cells, additional to the signs of premature aging and inflammaging.

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1. Introduction

A growing body of evidence points towards the involvement of an abnormal inflammatory response system in the pathogenesis of Major Depressive Disorder (MDD). In the last decades, a large number of investigations have been carried out on inflammation-regulating cytokines, since cytokines are relatively easy to determine. Meta-analyses of these investigations revealed that cytokine levels are raised in MDD patients (Köhler et al., 2017), particularly in those with a history of childhood adversity (CA; Pedrotti Moreira et al., 2018), adiposity (Gomes et al., 2019), or cardiovascular disease (Halaris, 2016). This has strengthened the inflammation theory of depression, in which early life stress leads to low-grade inflammatory activation (with inflammatory cytokines as main indicators) which in turn leads to depressive symptoms (Slavich and Irwin, 2014; Cathomas et al., 2019). Cells of the monocyte-macrophage lineage are important producers of inflammation-regulating cytokines. Thus, the inflammation theory of mood disorders has also been referred to as the monocyte/macrophage theory of depression (Smith, 1991).

In the past decade our group has extensively studied the circulating monocytes of mood disorder patients, focusing on an abnormal expression of 30–35 genes involved in the regulation of inflammation (based on the inflammatory theory of depression). In several finding pre-studies (carried out a decade ago, see Appendix A) using whole genome screening of patient versus healthy control monocytes we had selected these 30–35 inflammation-related genes because they ranked within the top over- and underexpressed genes in patient monocytes and were known to be involved in inflammation regulation as shown in Ingenuity Pathway Analyses (IPA). In later q-PCR confirmation studies we indeed showed that this set of inflammation-regulating genes was abnormally expressed in various cohorts of MDD patients, with expression being mainly higher in patient monocytes (Carvalho et al., 2014; Grosse et al., 2015; Arteaga-Henríquez et al., 2019; Schiweck et al., 2020). The most recent report shows that the expression of inflammatory genes in monocytes is particularly high in the patient group with a history of childhood adversity (Schiweck et al., 2020).

Furthermore, the IPA of the top over- and underexpressed genes carried out a decade ago (see Appendix A) also revealed that apart from inflammatory pathways other top molecular pathways were abnormally expressed, such as those related to leukocyte apoptosis, growth, and development. Top genes in these pathways were the mitochondrial apoptosis/growth regulating genes BAX, BCL10, EGR1 and EGR2 and the growth factor HB-EGF. Although apoptosis disturbances in the context of mitochondrial dysfunction and inflammation have been studied in psychiatric patients (Allen et al., 2018), studies on the expression level of mitochondrial apoptosis and growth-related genes in monocytes of well characterized groups of MDD patients are lacking. Further, their expression in relation to the previously determined inflammation-regulating genes in patient monocytes is so far unknown.

Another impetus for the study reported here is that the cholesterol metabolism in monocytes/macrophages plays an important role in the inflammation-regulation of the cells (Tall and Yvan-Charvet, 2015). An important enzyme in the lipid metabolism of monocytes is mevalonate kinase (MVK), facilitating the transition of mevalonic acid to mevalonate. Down-stream products such as the isoprenoids are anti-inflammatory (Marcuzzi et al., 2010), and a genetic deficiency of MVK leads to an inflammatory syndrome, the hyper IgD syndrome (HIDS) with strongly pro-inflammatory activated macrophages (Tricarico et al., 2013). Cholesterol pathway genes also play a role in the removal of lipids accumulated in the vessel wall of atherosclerotic plaques, and in doing so determine the inflammatory state of vessel wall infiltrating monocytes/macrophages (Westerterp et al., 2014). The gene expression state of such vessel wall infiltrating macrophages has been studied by others previously. Vessel wall infiltrating monocytes/macrophages can either be pro-inflammatory pro-atherogenic Mox macrophages (i.e. M1-like lipid-loaden foam cells) or anti-inflammatory atheroprotective M (hb)/H-mac cells (i.e. M2-like macrophages capable of pumping out cholesterol to be bound to apo-lipoproteins; Chistiakov et al., 2015). M (hb)/H-Mac cells are characterized by an active cholesterol pump machinery, such as the ABCA1 and ABCG1 pumps (Levy and Moreno, 2006), the gene expression of which is activated by the transcription factor LXR-α (coded by NR1H3) (Schulman, 2017). M(hb)/H-Mac cells are additionally characterized by the expression of the mannose receptor (MRC1), and the haptoglobin/hemoglobin receptor (CD163), while H-Mac cells additionally express HMOX (Chistiakov et al., 2015), an important anti-oxidant mechanism to counteract the pro-atherogenic effects of taken-up oxidized lipids. Despite the importance of the cholesterol pathway genes in inflammation regulation and atherosclerosis, and despite the knowledge that there is a higher risk for atherosclerotic disease in mood disorder patients (Penninx et al., 2001), studies on the expression of important cholesterol pathway genes and genes characteristic of atherosclerotic plaque macrophages are lacking in monocytes of MDD patients.

The aim of the study reported here to investigate the expression of the mitochondrial apoptosis/growth regulating genes BAX, BCL10, EGR1, and EGR2, the growth factor HB-EGF, and the cholesterol metabolism pathway genes NR1H3, ABCA1, ABCG1, MVK, CD163, HMOX1 in the monocytes of the MDD patients and healthy controls, thereby relating those to the previously studied inflammation-related genes by hierarchical clustering. Further, various sociodemographic and clinical parameters, such as sex, age, BMI, duration of illness, medication state, and childhood adversity will be taken into account analyzing gene expression data. Since childhood adversity was found to be an important determinant for a higher expression of the inflammation-regulating genes in the previous study of Schiweck et al. (2020), it will be of particular interest.

2. Methods

2.1. Participants

Using a cross-sectional study design, data were collected at three different sites using uniform procedures: the university psychiatric hospitals of Münster (Germany), München (Germany), and Leuven (Belgium). Samples of 140 MDD patients and 120 age- and sex-matched healthy controls (HC) were analyzed for gene expressions. Adult men and women patients (ages 18–65 years) were included if they were diagnosed with MDD and were free of the following diseases: clinical inflammation-related symptoms (including fever), current or recent inflammatory or infectious disease, uncontrolled systemic disease, uncontrolled metabolic disease, other uncontrolled somatic disorder affecting mood. Participants were excluded if they used somatic medication that affects mood or the immune system, e.g. statins, corticosteroids, non-steroidal anti-inflammatory drugs. Healthy controls were excluded if they were not in self-declared health (specifically lacking any form of auto-immune disease and/or atopic disease) and/or used somatic medication that affects mood or the immune system. For both the patient and control group, pregnant women or women who had delivered within the previous 6 months were excluded. The study was conducted in accordance with the declaration of Helsinki and its subsequent revisions and approved by ethical committees of the participating universities (reference numbers: Leuven: S51723; Munich: 291-09, Münster: 2009-019-f-S). Written informed consent was obtained from all participants.

2.2. Clinical assessment

Patients were diagnosed with major depression according to DSM-IV using the Mini- International Neuropsychiatric Interview (MINI; Sheehan et al., 1998). Healthy controls were screened for the presence of psychiatric symptoms using the MINI Screening version. In patients, depression severity was assessed by the Inventory of Depression

Symptoms (IDS-C30; Rush et al., 1996) in a face-to-face interview, and in healthy controls by the self-report version (IDS-SR30). Presence of adverse events during childhood was measured by the Childhood Adversity Questionnaire (CTQ; Bernstein et al., 2003). Positivity for childhood adversity (CA) was defined by presence of trauma on at least one subscale of the CTQ. Presence of CA on a single subscale was defined according to Walker et al. (1999). Body Mass Index was calculated after assessing self-reported height and body weight. Duration of illness was calculated by subtracting the date of disease onset from age at the time of study assessments. Disease onset and medication were obtained from clinical records.

2.3. Laboratory assessment

The assessment procedure of monocyte gene expression applied in this study has been described in detail before (Grosse et al., 2015). In brief, blood samples were drawn and PBMC suspensions were prepared and stored at −80 °C (Knijff et al., 2006). CD14+ monocytes were isolated by magnetic cell sorting system (Miltenyi Biotec, B.V., Bergisch Gladbach, Germany) from thawed PBMC, then RNA was isolated (RNAeasy minikit; Qiagen, Hilden, Germany). Of RNA, 1 μg was reverse transcribed (high capacity cDNA kit) to produce cDNA for quantitative-polymerase chain reaction (q-PCR) to determine monocyte gene expression by comparative threshold cycle method (CT values; Applied Biosystems, Carlsbad, CA, USA). cDNA was stored at −80 °C. Using the cDNA, the genes were determined using the probe and primer sets of Applied Biosystems (see Appendix B., Supplementary Table 1). All gene values were normalized by the value of the housekeeping gene ABL1 (ΔCT values), which is a superior housekeeping gene for leukocytes (Beillard et al., 2003). Controlling for site, for each gene the average ΔCT value of healthy controls without CA of each site was subtracted from the ΔCT values of patients of the same site resulting in corrected ΔΔCT values before data was pooled and used for analyses (Livak and Schmittgen, 2001). Negative ΔΔCT indicate upregulation, positive ΔΔCT values indicate downregulation of gene expression in reference to healthy controls without CA.

2.4. Statistical analyses

Statistical analyses were performed with R software version 3.5.2. Comparison of demographical data between MDD and HC were computed using the Chi-square test for categorical data and Wilcoxon rank sum test for continuous data. Missing gene values (5.9% of healthy control genes and 5.1% of MDD genes) were imputed using the median of patient values for missing patient values and of HC values for missing HC values for visual data presentation and initial single gene analyses. Hierarchical cluster analysis of MDD monocyte gene expression intercorrelations was conducted using Spearman rank correlation matrix and ward.d2 method. For initial analysis and graphical presentation, *p*-values of single gene expressions of 44 genes were obtained using one-sample Wilcoxon signed rank test and were adjusted for multiple testing using the Benjamini-Hochberg-method (false discovery rate). *P*-values of single gene group comparisons were calculated using Wilcoxon rank sum test (Benjamini-Hochberg-corrected). Visual data presentation follows the order resulting from hierarchical cluster analysis. For multivariate analyses, missing data were imputed by multiple imputation using chained equations (mice package). Five imputed datasets were created and analyses were run on each dataset. *P*-values were calculated based on pooled F-values from all datasets. A clusterwise multivariate analysis of co-variance (Mancova) was performed to obtain and verify pooled differences of gene expression profiles as were observed after clusterwise depiction of single gene expressions. BMI and age were included as covariates. In a second step, the effect of sex, duration of illness, and medication (binary variable anti-depressants yes/no) were also studied and added as covariates, respectively. Since we suspected differential gene expression in women and men, both groups are depicted, separately. In the Mancova analyses, we were interested in the main effects due to missing significant interaction terms and therefore used a type II sum square calculation (jmv package).

3. Results

3.1. Demographics

Table 1A shows that the distributions of age, sex, and BMI were not significantly different between patients and controls. Significant differences emerged, not surprisingly, for depression severity and CA (prevalence patients 57.0%, prevalence healthy controls 31.4%). The vast majority of patients were receiving drug treatment. Table 1B shows the varieties of medication: 11.5% were not receiving any drug treatment at the time of testing, 11.5% were receiving a benzodiazepine only, while the remaining patients often used a variety of anti-depressants.

3.2. A monocyte gene expression signature

We first conducted a hierarchical cluster analysis using the expression levels of the apoptosis/growth genes, the cholesterol pathway genes and the previously determined inflammation-regulating genes which resulted in the clusters shown in Fig. 1: The two inflammation-related gene clusters found in previously published analyses (Schiweck et al., 2020) were confirmed, and the additionally determined apoptosis/growth/cholesterol pathway genes by and large formed a separate cluster (cluster 3). Cluster 1 was composed of various cytokine production-associated genes (with important cytokine genes such as IL1 and IL6), while cluster 2 consisted of chemotaxis, adhesion, and coagulation-regulating genes. Fig. 1 futhermore shows that cluster 1 and cluster 2 inflammatory genes are in general strongly positively intercorrelated, while cluster 3 genes correlated weaker amongst themselves and to cluster 1 and 2 genes (positively and negatively). It is also of note that the newly determined M(hb) gene HMOX was not part of cluster 3 but positioned as a cluster 2 gene and correlated negatively to many of the cluster 1 inflammation-related genes in accordance with its strong anti-oxidant function (see Fig. 1).

3.3. Overexpression of cluster 3 genes is a hallmark of MDD patients, irrespective of childhood adversity. Dependency on age and BMI

Since we previously described that CA is a major determinant for the higher expression of the inflammation-related genes in this set of MDD patients (Schiweck et al., 2020), we analyzed data not only in the entire group of MDD patients, but also split into those with and without a history of CA. Fig. 2 shows the expression levels of the various genes per

Table 1A
Demographic data of patients and healthy controls.

	MDD (N)	HC (N)	*test*	*df*	*p*-value
Sex (women, %)	64.29 (140)	65.83 (120)	$\chi^2 = 0.02$	1	0.90
Age (*Md/ IQR*)	41.88/ 20.52 (140)	37.50/ 22.29 (120)	*W* = 7551.00	–	0.16
BMI (*Md/ IQR*)	23.96/ 5.09 (140)	23.59/ 4.82 (120)	*W* = 7292.50	–	0.08
IDS score (*Md/ IQR*)	31.00/ 15.00 (140)	5.50/ 6.25 (120)	*W* = 287.00	–	<0.001
CA yes (%)	57.04 (135)	31.36 (118)	$\chi^2 = 15.75$	1	<0.001
DD (*Md/ IQR*)	4.00/ 11.25 (118)	–	–	–	–
Suicide risk yes (%)	74.05 (131)	–	–	–	–

Legend. MDD Major Depressive Disorder; HC healthy control; Age in years; BMI body mass index; IDS Inventory of Depression Symptoms; CA childhood adversity; DD disease duration in years; χ2 Chi-Square statistic; Md median; IQR interquartile range; W Wilcoxon statistic; $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Table 1B
Absolute and relative frequencies of medication taken by MDD patients.

Medication regimen	N (%)
No psychotropics	13 (11.50)
Benzodazepines only	13 (11.50)
Anti-depressants only	87 (76.99)
TCA/ TeCA	37 (32.74)
SSRI	37 (32.74)
SNRI	23 (20.35)
NARISARI	4 (3.54)
Melatonin	2 (1.77)
Neuroleptics	42 (37.17)
Lithium	13 (11.50)

Medication status was unavailable for 27 patients; cumulative frequency of anti-depressant drug regimen exceeds 100% due to multiple prescriptions; in total, 40 patients (35,40%) were taking benzodiazepines; anti-depressants only = anti-depressant drug regimen; TCA tricyclic anti-depressant; TeCA tetracyclic anti-depressant; SSRI selective serotonin reuptake inhibitor; N absolute number of patients; % percent in reference to the total patient group number.

(sub-)cluster in the monocytes of all MDD patients and those who had or had not experienced CA, respectively.

3.3.1. Cluster 1 genes

With regard to cluster 1 genes, we again showed, as in the study of Schiweck et al. (Schiweck et al., 2020), that MDD patients with a history of CA show many monocyte genes significantly upregulated (e.g. IL1B, IL1A, IL6) versus healthy controls without CA. MDD patients without a history of CA showed hardly any upregulation of cluster 1 genes. From the Mancova analysis including age and BMI, a considerably significant effect emerged for the MDD CA group, showing that the upregulation of cluster 1 is significantly linked to this mood condition, independently of the effects of age and BMI. Noteworthy, age had an important additional effect for cluster 1 genes, while BMI did not.

3.3.2. Cluster 2 genes

The Mancova analysis further showed that there existed a nearly significant ($p = 0.06$) upregulation of cluster 2 genes specific to major depression and independent from the effects of BMI and age, but only in the MDD CA group. Cluster 2 upregulation was not significantly associated with MDD without CA. It also appeared from the Mancova analysis that cluster 2 upregulation was particularly dependent on BMI and age, both in MDD patients with and without CA.

3.3.3. Cluster 3 genes

Cluster 3 was significantly upregulated in monocytes of both the MDD patient group with and without CA, independent of the effects of age and BMI. Mancova analysis showed that BMI hardly had an effect on cluster 3 expression; however, a clear age effect was present. Increasing age correlated in general to increasing expression of the cluster 3 genes.

Three subclusters of cluster 3 were observed:

3.3.4. The mitochondrial apoptosis and growth genes

Cluster 3 consisted in part of the mitochondrial apoptosis and growth regulating genes BAX, BCL10, HB-EGF, EGR1 and EGR2, of which BAX and BCL10 were significantly upregulated in MDD patients, in

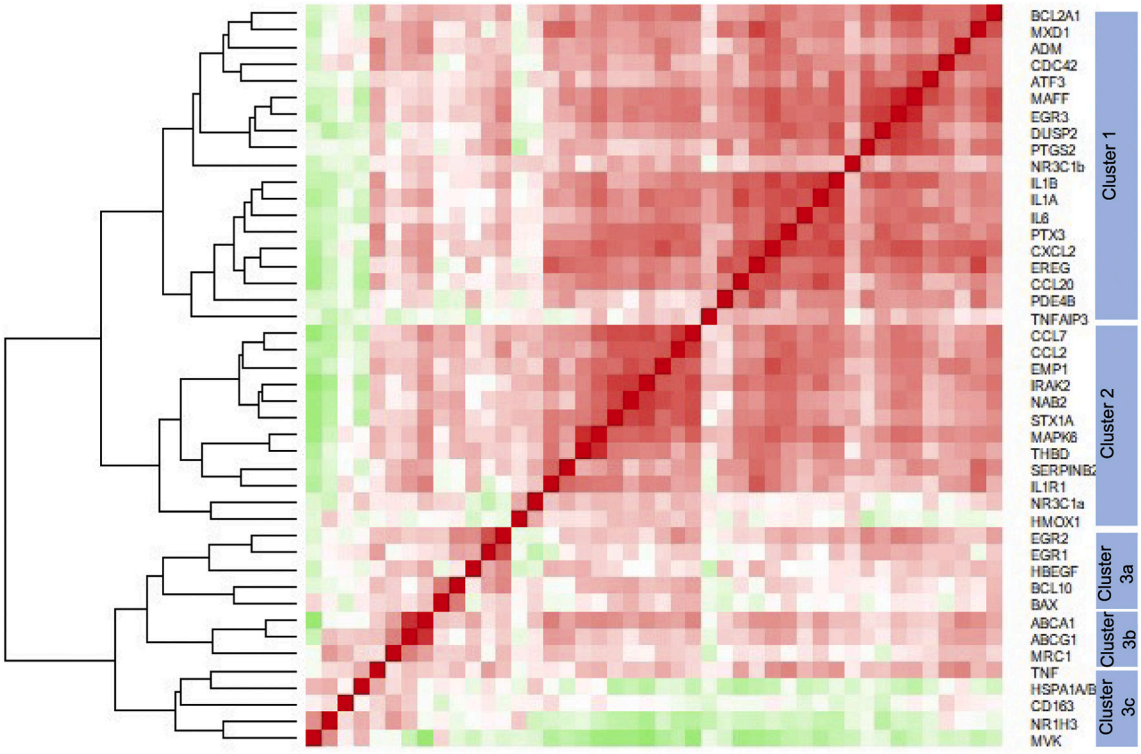
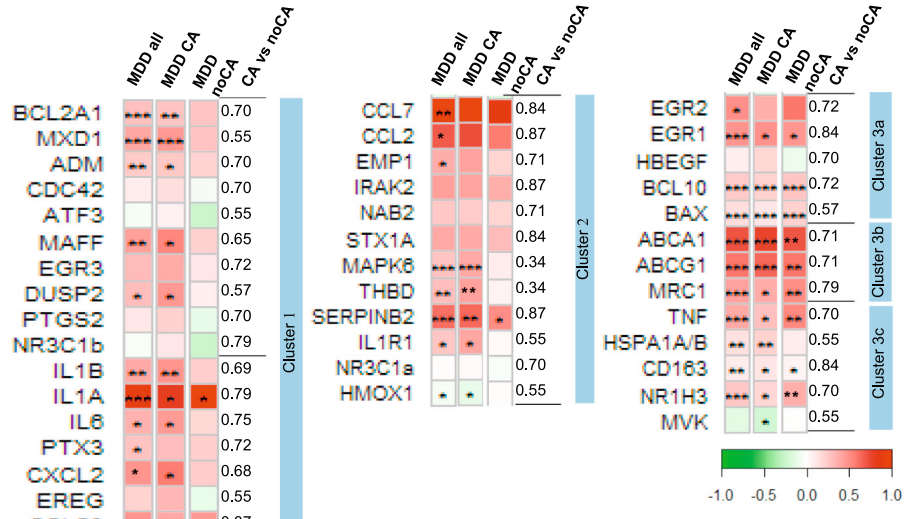


Fig. 1. Inter correlating gene clusters as expressed in monocytes of MDD patients ($N = 140$).
Legend. In this study BAX, BCL10, EGR1, EGR2, HBEGF, ABCA1, ABCG1, NR1H3, CD163, HMOX1, MRC1, and MVK were determined. In previous studies [5,6,8] ADM, ATF3, BCL2A1, CCL2, CCL20, CCL7, CDC42, CXCL2, DUSP2, EGR3, EMP1, EREG, HSPA1A_HSPA1B, IL1A, IL1B, IL1R1, IL6, IRAK2, MAFF, MAPK6, MXD1, NAB2, NR3C1a, NR3C1b, PDE4B, PTGS2, PTX3, SERPINB2, STX1A, THBD, TNF, and TNFAIP3 had been determined from the same cDNA preparations. Three main gene clusters could be identified (see dendrogram): Cluster 1 containing most of the inflammation-regulating genes (including the interleukins IL1A, IL1B and IL6). Cluster 2 comprising predominantly genes related to adhesion, coagulation, shape change and the chemotactic ability of the cells (EMP1, STX1A, THBD, CCL2, CCL7). Cluster 3 with mainly the newly determined apoptosis/growth regulating genes and cholesterol pathway genes, but also TNF and HSP1A/B. This cluster can be sub-divided in cluster 3a, 3b, and 3c.



	group				BMI				age				interpretation
	F	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*	
MDD-HCnoCA													
Cluster1	2.715	19	199	<0.001***	1.029	19	199	0.429	2.651	19	199	<0.001***	independent group effect
Cluster2	1.415	12	206	0.161	3.384	12	206	<0.001***	3.258	12	206	<0.001***	age and BMI only determinants
Cluster3	2.044	13	205	0.015*	1.755	13	205	0.035*	4.023	13	205	<0.001***	independent group effect
MDD CA-HCnoCA													
Cluster1	2.472	19	136	0.001**	1.333	19	136	0.173	3.010	19	136	<0.001***	independent group effect
Cluster2	1.832	12	143	0.048*	4.430	12	143	<0.001***	2.670	12	143	0.003**	independent group effect
Cluster3	2.091	13	142	0.018*	2.086	13	142	0.018*	4.405	13	142	<0.001***	independent group effect
MDD noCA-HCnoCA													
Cluster1	1.607	19	117	0.059^{+}	0.966	19	117	0.528	2.359	19	117	0.002**	marginal independent group effect
Cluster2	0.891	12	124	0.498	2.315	12	124	0.015*	2.516	12	124	0.010*	age and BMI only determinants
Cluster3	2.052	13	123	0.044*	1.487	13	123	0.228	1.970	13	123	0.045*	independent group effect

Fig. 2. Monocyte gene expression signature of the entire group of MDD patients ($N = 140$), and in the subgroups of patients with childhood adversity (MDD CA; $N = 77$) and without childhood adversity (MDD noCA; $N = 58$).
Legend. *MDD* Major Depressive Disorder; *CA* childhood adversity; *noCA* no childhood adversity; *HCnoCA* healthy controls without experience of childhood adversity; *BMI* body mass index. Mean values are expressed relative to the expression level of HCnoCA; the intensity of red reflects higher expression, intensity of green lower expression; stars indicate level of significance; *p*-values of group comparison are given. Pooled F-values and estimated *p*-values of the clusterwise gene expression analysis (Mancova) with group as factor and BMI and age as covariates are displayed. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{+}p \sim 0.05$.

particular, irrespective of the presence of CA (see Fig. 2).

3.3.5. *M(hb) characteristic genes*

Cluster 3 also consisted of the genes characteristic of anti-inflammatory M(hb) cells, such as the cholesterol pump genes ABCA1/ABCG1, NR1H3 (coding LXRα, the transcription factor for the cholesterol pumps), CD163 and MRC1. Of these, ABCA1 and ABCG1 were the strongest upregulated genes in MDD patients, irrespective of the presence of CA (see Fig. 2).

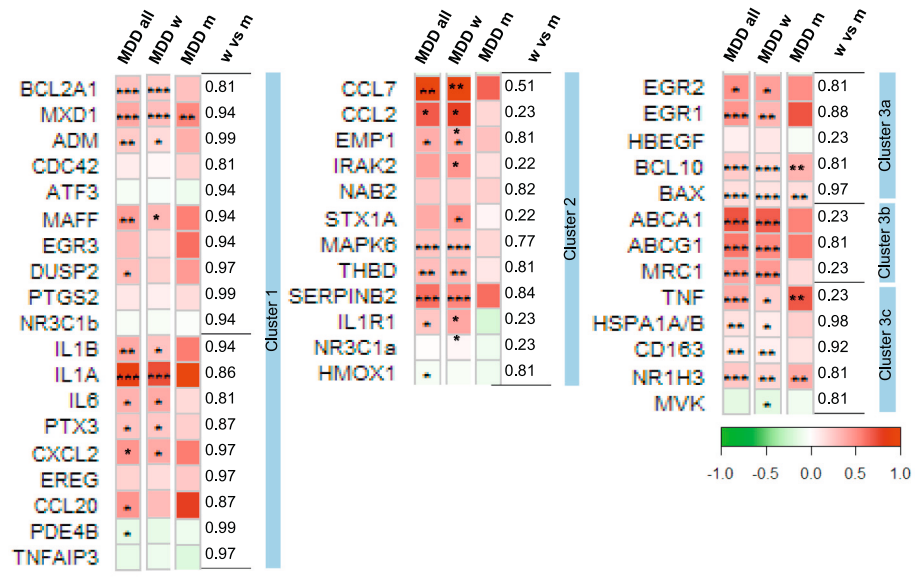
3.3.6. *Other cluster 3 genes*

Cluster 3 also contained the pro-inflammatory cytokine TNF (see Fig. 2), which was overexpressed in both the MDD group with and without CA. The overexpression of TNF in cluster 3 is peculiar regarding the M(hb) profile of cluster 3 (which is in general regarded as an anti-inflammatory profile). Interestingly, MDD patients with a history of CA showed a significant downregulation of the MVK gene (see Fig. 2), which correlated strongly but negatively to cluster 1 and 2 inflammation-regulating genes (see Fig. 1). The strongest negative correlation emerged between MVK and cluster 2 genes (Spearman-Rho ranged from −0.48 to −0.27 for 10 of the 12 cluster 2 genes; *p*-values ≤0.001).

With regard to the different subtypes of childhood adversity, Appendix C (Supplementary Fig. 1) shows that emotional abuse and emotional neglect contributed especially to the higher expression of cluster 3 genes. Schiweck et al. (2020) previously reported that emotional neglect and emotional abuse also contributed strongest to the higher expression of the inflammation-related gene clusters 1 and 2.

3.4. *Effects of sex, duration of disease, and medication on monocyte gene expression in MDD patients*

Statistically, monocytes of women and men with MDD had an equally higher expression of the three clusters of genes with reference to the monocytes of healthy controls (Fig. 3). However, men had a somewhat weaker expression level than females, as is evident from the expression levels in single gene analysis (see Fig. 3). Mancova analysis showed that age and BMI effects on monocyte gene expression were particularly evident in women. In addition, we studied the effect of disease duration and anti-depressant medication on monocyte gene



	group				BMI				age				disease duration			
	F	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*
MDD women-HcnoCA																
Cluster1	2.074	19	134	0.009**	1.040	19	134	0.420	3.050	19	134	<0.001***	0.727	19	134	0.786
Cluster2	2.151	12	141	0.017*	3.416	12	141	<0.001***	2.590	12	141	0.004**	1.244	12	141	0.259
Cluster3	2.003	13	140	0.025*	2.440	13	140	0.005**	3.840	13	140	<0.001***	1.622	13	140	0.086
MDD men-HcnoCA																
Cluster1	2.196	19	100	0.006**	1.264	19	100	0.225	1.789	19	100	0.034	0.851	19	100	0.642
Cluster2	1.959	12	107	0.035*	1.762	12	107	0.064	1.646	12	107	0.090	1.835	12	107	0.051^{+}
Cluster3	2.104	13	106	0.020*	1.543	13	106	0.114	1.939	13	106	0.033*	1.218	13	106	0.277
	group				**BMI**				**age**				**medication**			
	F	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*
MDD women-HCnoCA Cluster1	2.110	19	129	0.008**	1.115	19	129	0.344	3.439	19	129	<0.001***	1.465	19	129	0.109
Cluster2	1.859	12	136	0.045*	3.680	12	136	<0.001***	3.213	12	136	<0.001***	1.916	12	136	0.037*
Cluster3	1.647	13	135	0.080	3.735	13	135	<0.001***	4.136	13	135	<0.001***	2.782	13	135	0.002*
MDD men-HCnoCA																
Cluster1	2.057	19	100	0.012*	1.125	19	100	0.339	1.792	19	100	0.034*	1.730	19	100	0.043*
Cluster2	1.974	12	107	0.034	1.389	12	107	0.182	2.044	12	107	0.027*	1.199	12	107	0.293
Cluster3	1.989	13	106	0.028*	1.305	13	106	0.221	1.921	13	106	0.035*	1.331	13	106	0.206

Fig. 3. Monocyte gene expression signature of MDD patients (N = 140), of women (MDD w; *N* = 90) and men (MDD m; *N* = 50), and after control for the additional covariates duration of disease and medication.
Legend. *MDD* Major Depressive Disorder; *w* women; *m* men; *HCnoCA* healthy controls without experience of childhood adversity; *BMI* body mass index. Mean values are expressed relative to the expression level of HCnoCA; the intensity of red reflects higher expression, intensity of green lower expression; stars indicate level of significance; p-values of group comparison are given. Pooled F-values and estimated p-values of the clusterwise gene expression analysis (Mancova) with group as factor and BMI and age as covariates are displayed. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{+}p \sim 0.05$.

expression in this analysis. The Mancova analyses given in Fig. 3 show that an effect of disease duration on monocyte gene expression could not be detected. Medication, dichotomized as non-medicated and benzodazepine-only treated patients versus the patients with bona-fide anti-depressants, showed a significant effect for the expression of cluster 2 and cluster 3 genes in women (not in men).

4. Discussion

The results reported here are new in showing an aberrant expression of a cluster of genes in the monocytes of MDD patients composed of a subcluster of mitochondrial apoptosis/growth regulating genes (BAX, BCL10, EGR1, and EGR2), various genes previously described in M(hb) macrophages (ABCA1, ABCG1, NR1H3, MRC1, CD163), the gene for pro-apoptotic/pro-inflammatory TNF, the gene for the immune regulating/protein chaperone molecule HSP70, and the cholesterol pathway gene MVK. This gene cluster was abnormally expressed in all MDD patients irrespective of a history of CA. Here we show once more that the monocytes of the studied group of MDD patients also have a higher expression level of the inflammation-related gene clusters 1 and 2, but virtually only in the MDD cases with CA (see also Schiweck et al., 2020). In this report, we take this observation further in showing that the higher expression of cluster 1 and 2 genes correlates to the down-regulation of the cholesterol pathway gene MVK in MDD patients with CA. Fig. 4 shows an illustration of the interaction of all here tested genes and their role in monocyte/macrophage cellular functions such as

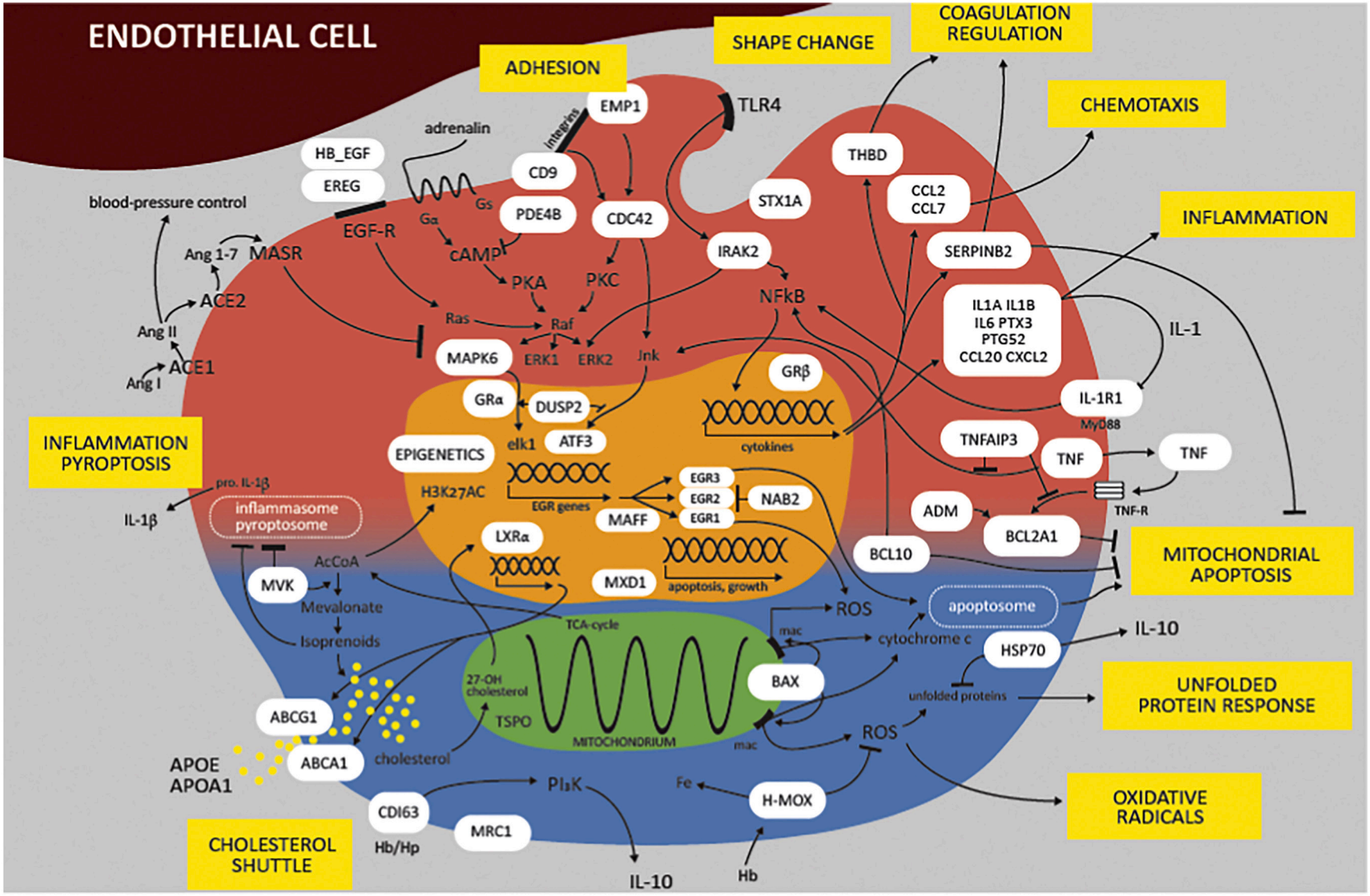


Fig. 4. Molecular gene modules activated in monocytes of MDD patients.

Legend. An illustration is shown on the interaction of the various molecules coded by the genes in the molecular modules, which are activated in monocytes of MDD patients. It is important to note that all described molecular modules contain both pro- and anti-inflammatory/apoptosis/coagulation factors and this underscores the notion that in MDD patients processes of both pro- and anti-inflammation/apoptosis/coagulation are activated. Thus, there rather exists a disequilibrium than a plain activation or inhibition of these processes in MDD monocytes. Shortly:

Mitochondrial apoptosis module, a sign of immune senescence (cluster 3a genes, lower right part of the cartoon): Includes the genes for BAX, BCL10, EGR1, EGR2 and HB-EGF (see Fig. 1).

The module of the senescence associated secretory phenotype (SASP) and unfolded protein response (cluster 3c genes, also lower right part of the cartoon): Includes the gene for TNF and HSP1A/1B. *Cholesterol metabolism module* (cluster 3b and some 3c genes, lower left part of the cartoon): includes ABCA1, ABCG1, MRC1, NR1H3 (encoding the LXR-α transcription factor) and CD163.

The module of MAP Kinases and EGRs (mainly cluster 1, upper left part of the illustration): Includes various mitogen activated protein kinases (MAPKs) such as MAPK6/ERK3, the MAPK pathway regulating genes ATF3, DUSP2, NAB2, MAFF and MXD1 and down-stream transcription factors such as EGR3. EREG feeds into this route, while PDE4B regulates the input of, e.g. adrenaline into this route.

The module of pro-inflammatory cytokines and compounds (mainly cluster 1a, upper right part of the cartoon): Activation of the MAP-kinase pathway not only leads to growth and apoptosis regulation but also to the production of pro-inflammatory cytokines and compounds. The genes IRAK-2, IL-B, IL-1A, IL-6, PTX3, PTGS2, and CXCL2 are examples.
Pyroptosis module (cluster 3c and cluster 1a, middle left part of the illustration): MVK, TNFAIP3, BCL2A1 and ADM.
Module of shape change, chemotaxis, adhesion, and coagulation (mainly top of the cartoon, all cluster 2 genes). This cluster is composed of CCL2, CCL7, EMP1, STX1A, CDC42, SERPINB2, THBD, IL-1R1, HMOX-1 and the activating GRα.
For a more detailed description of function and interaction of these separate genes (as reviewed from the literature), see Appendix D.

inflammation-regulation, chemotaxis, adhesion, shape change, coagulation regulation, mitochondrial apoptosis, growth regulation, the redox potential, the unfolded protein response, and the cholesterol shuttle (for detailed information on the separate molecules see Appendix D). The monocyte data presented here thus indicate that the immune abnormalities found in MDD are not restricted to a higher state of chronic low-grade inflammation in monocytes (which virtually only occurs in CA cases), but also involve various other disturbances of monocyte cellular functions, related to mitochondrial apoptosis, growth regulation, the redox potential, the unfolded protein response, and the cholesterol shuttle. Below we discuss the putative consequences of the abnormal expression of the cluster 3 genes in monocytes of MDD patients.

4.1. Monocyte mitochondrial dysfunction in MDD: A sign of premature monocyte aging?

One of the over-expressed cluster 3 genes in monocytes of MDD patients is BAX. BAX is a key player in inducing mitochondrial apoptosis; after oligomerization it forms the mitochondrial apoptosis-induced channel (MAC) in the mitochondrial external membrane (see Fig. 4). This channel is amongst others important for the release of Reactive Oxygen Species (ROS) and cytochrome *c* from mitochondria. Cytochrome c activates the formation of the apoptosome (Tricarico et al., 2013). The clear upregulation of the mitochondrial apoptosis regulating genes BAX and BCL10 in the monocytes of MDD patients irrespective of CA supports the view that mitochondrial dysfunction is a prime hallmark of MDD. There is pertinent literature on mitochondrial dysfunction in mood disorders showing mtDNA damage, morphological changes in the mitochondria, less ATP production, electron chain changes and higher ROS production, leading to dysregulated apoptosis, abnormal cell maturation/differentiation and proinflammatory activity (Allen et al., 2018; Bansal and Kuhad, 2016; Labra Ruiz et al., 2018). Indeed, in our study pro-apoptotic/inflammatory TNF was higher expressed as part of cluster 3.

The above described pattern of mitochondrial dysfunction, altered apoptosis, and high production of TNF is compatible with the functional and molecular profile known of senescent cells. The increased inflammatory compound production in senescent cells is known as the senescence-associated secretory phenotype (SASP) and often nicknamed "inflammaging". Senescence of monocytes/macrophages has been described, though scarcely (Ong et al., 2018). It is characterized by mitochondrial dysfunction, an increased ER stress with unfolded proteins, a high expression of MRC-1 (a characteristic of anti-inflammatory M2 like macrophages), with nevertheless a higher production of pro-inflammatory cytokines and oxidative radicals (Van Beek et al., 2019). Interestingly, the monocytes of the MDD patients in this study indeed show prime features of anti-inflammatory M2 cells such as a high expression of MRC1 and CD163 together with a high expression of the chaperone molecule HSP70 (combatting ER stress), however all in combination with a high expression of the gene for the inflammatory cytokine TNF.

To our knowledge, our study is the first indicative of a premature senescent state of monocytes of MDD patients, reinforcing the earlier expressed view that MDD belongs to the group of disorders characterized by a premature cell senescence based on shortened telomere length in circulating leukocytes (Verhoeven et al., 2014) and an over-representation of terminally differentiated pro-inflammatory senescent circulating T cells (Elwenspoek et al., 2017; Ford et al., 2020). Whether such premature immune aging is genetically programmed or the result of environmental factors such as CMV infection is under investigation (Bauer, 2008; Verhoeven et al., 2019; Reed et al., 2019; Leng et al., 2011). There are indications that CMV infection might play an important role in monocyte aging (Leng et al., 2011).

4.2. Abnormal monocyte cholesterol metabolism in MDD

Cholesterol metabolism genes known from anti-inflammatory lipid-handling M(hb) macrophages (described as athero-protective cells in atherosclerotic plaques) were also upregulated in the circulating monocytes of the MDD patients irrespective of CA. Upregulated genes were NR1H3 (LXRα) and ABCA1/ABCG1, which play prominent roles in pumping out intracellular cholesterol, a molecule that has a pro-inflammatory activity when intra-cellularly accumulated (Tall and Yvan-Charvet, 2015). An increase of the pumps can therefore be considered an anti-inflammatory event, and the upregulated expression of the M2 marker MRC1 is in accordance with such a view. However, the principally anti-inflammatory M(hb) cells show a pro-inflammatory SASP-like senescent profile with a high TNF expression (see above) in our MDD patients. It is tempting to speculate that this occurrence of the peculiar "inflammation-prone senescent M(hb) cells" is a factor in the well-known higher prevalence of atherosclerotic cardiovascular disease in MDD patients. Nevertheless, our observations support the view that the interaction between fat metabolism and immunity plays a role in the pathogenesis of MDD, as is expressed in the so-called theory of immunometabolic depression (Milaneschi et al., 2020).

4.3. Mevalonate kinase and the proneness of monocytes to induce inflammation

Our study is novel in showing that MDD patients with a history of CA and a concomitant upregulation of cluster 1 and 2 inflammation-regulating genes, show a significant downregulation of MVK. Patients with mutations in the MVK gene (HIDS patients) are known for their periodic inflammatory episodes and dysregulated pro-inflammatory cytokine levels, particularly that of IL-1β. In its most severe form, brain developmental abnormalities are at the forefront in HIDS patients (Hoffmann et al., 1993). The pathological mechanisms of auto-inflammation in mevalonate kinase deficiency are not well understood; however, reduced synthesis of isoprenoid lipids downstream of MVK are thought to play a central role (Tricarico et al., 2015). These are necessary for the prenylation (the addition of hydrophobic compounds) of small GTPases (such as CDC42, a cluster 2 gene). Reduced prenylation of GTPases results in altered autophagy, mitochondrial dysfunction, and redox balance with an over-activation of the pyroptosomes and, consequently, in a dysregulated production of IL-1β and IL-18 (Tricarico et al., 2015). Thus, it is not surprising that the reduced MVK in the monocytes of MDD patients with a history of CA is linked to the upregulation of cluster 1 and 2 genes in the patient monocytes. Furthermore, MVK also plays a prominent role in "trained immunity" (Bekkering et al., 2018). Trained immunity is the capability of monocytes/macrophages to build up a long-term non-specific memory towards danger signals via an epigenetic imprinting (Netea et al., 2016). The metabolite mevalonate is the mediator of training via activation of mTOR and subsequent histone modifications in the inflammatory pathway (Bekkering et al., 2018).

Monocytes of HIDS patients, which are deficient in MVK, accumulate mevalonate and show a trained phenotype (Bekkering et al., 2018). From our studies it can be hypothesized that CA, as an early danger signal, induces a monocyte pro-inflammatory epigenetic training program with concomitant reduced MVK activity and with long-lasting effects on the inflammatory state of monocytes/macrophages.

4.4. How do our data relate to monocyte gene expression data in previous literature?

Literature from other research groups on monocyte gene expression in MDD cases is scarce and fragmented. Zhu et al. (2019) performed gene expression studies in monocytes of monozygotic twin pairs discordant for MDD. These authors described how genes related to mitochondrial energy production, the oxidative stress response, and the cytokine secretion, and how the zinc family genes were dysregulated in MDD monocytes. Of the differentially methylated regions, those involved in apoptosis, growth, and the MAPK/ERK pathway stood out. Similarities to the profiles described here are obvious. Overexpression of inflammatory genes in monocytes of MDD patients have also been found by Chiang et al. (2019) and Hasselmann et al. (2018). Hasselmann et al. (2018) in addition described steroid resistance of the MDD monocytes, i.e. reduced expression of the GR. We did not find abnormalities in GR expression in this series of MDD patients. However, in a previous study on unmedicated older melancholic patients with a higher depression score as described here, we did find a higher expression of the inflammation-related monocyte genes together with an overexpression of the blocking GRβ and a reduction in the activating GRα correlating to the depression score (Carvalho et al., 2014). Lisi et al. (2013) stimulated monocytes of drug-free MDD patients with LPS and found a reduced PTGS2 gene expression in the presence of a trend for the IL1B and IL6 genes to be over-expressed. We found similar data, i.e. in non-stimulated MDD monocytes PTGS2 normally expressed and IL1A, IL1B, and IL6 overexpressed. Hung et al. (Hung et al., 2017; Hung, 2018) showed an inflammatory state of circulating monocytes of unmedicated MDD patients, i.e. a higher expression of inflammation-inducing toll-like receptors (TLRs) in the presence of a reduced expression of their negative regulators. The investigators specifically focused on the negative regulator TNFAIP3, one of our panel genes, which they found downregulated in unmedicated patients, but upregulated after SSRI treatment (Hung et al., 2017). We also found this gene downregulated. However, the majority of our patients was medicated. Schwaiger et al. (2016) studied monocyte gene expression after an acute stressor in healthy individuals with a history of childhood adversity. The investigators found increased gene expressions for modules of cytokine and chemokine activity/cytokine-receptor interaction and alterations in gene expression modules for steroid binding and hormone activity (Schwaiger et al., 2016). The authors also suggested that childhood adversity leads to persistent alterations in transcriptional control in monocytes, as this report also shows (Schwaiger et al., 2016).

4.5. Limitations

The present study has several limitations. The vast majority of patients was medicated by a variety of drugs and only few were not medicated at all. Although we do report data adjusted for medication, we consider outcomes as preliminary due to the low number of non-medicated patients, the high variability of multiple medication usage, and the more or less arbitrary binary coding of its use. Our outcomes show an expression of cluster 2 and 3 genes only in female MDD patients connected to the treatment with anti-depressants. This seems peculiar with respect to the general idea that anti-depressants may have anti-inflammatory effects (Dionisie et al., 2021). Probably, the use of anti-depressants in our patients indicates a genuine MDD patient group, but this is speculative. As women showed a somewhat stronger upregulation, anti-depressant use may also relate to disease severity. Future studies should evaluate the effect of anti-depressant medication on monocyte gene expression more elaborately and over time, preferably in drug-naïve patients at enrollment.

Due to a paucity of cDNA material, fewer MDD cases could be tested for the new cluster 3 genes than evaluated by Schiweck et al. (2020). Moreover, some of the genes were tested in even smaller subgroups leading to reduced statistical power and probable type II error. Further, we have not recorded the prevalence of cardiovascular abnormalities and lipid profiles in our test subjects. It is well-known that such pathologies are related to MDD (Penninx et al., 2001; Wei et al., 2020). In previous studies of our group on metabolic syndrome patients, we found the expression profile of monocyte microRNAs/mRNAs compatible with an upregulation of cluster 2 genes (Baldeón et al., 2015). Here, we also show that the BMI is a prime factor in cluster 2 gene regulation. Lastly, our study used convenience sampling. However, important subject characteristics were not different between MDD patients and HC, and the multiple sites increase generalizability of study results.

4.6. Conclusion

The gene expression profile of monocytes of MDD patients supports a view that the low-grade inflammatory state (reported by many investigators for MDD) is part of a broader immune abnormality, i.e. a premature senescence of immune cells characterized by various mitochondrial dysfunctions related to an abnormal apoptosis/growth and cholesterol metabolism, and by inflammaging (high TNF). A history of CA is related to the downregulation of MVK and an upregulation of a cluster of various pro-inflammatory genes (amongst which IL1 and IL6) in the monocytes of the MDD patients, showing a shift to more severe monocyte inflammation (pyroptosis) on top of the premature monocyte inflammaging. Thus, we here report novel observations on biological underpinnings of MDD going beyond the previous concept of chronic low-grade inflammation extending the immune abnormalities in MDD patients to monocyte mitochondrial dysfunction and early/premature aging of the myeloid system in combination with immuno-metabolic abnormalities. Uncovering these molecular mechanisms in depression pathophysiology also revealed the importance of considering the differential roles of childhood adversity for monocyte gene expression signatures. Future studies should investigate the relationship of premature aging of monocytes to the well-described premature T cell aging in MDD (Elwenspoek et al., 2017; Ford et al., 2020; Bauer, 2008) and the role of genetic susceptibility and chronic (viral) infections in monocyte aging. Further, possibilities to use the monocyte expression signature for stratifying patients for immune therapy, such as anti-inflammatory and anti-microbial interventions, should be studied as well.

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Disclosures

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Part of the samples and genes of this report were used for prior publication as referenced in the main body text, and part of the data were presented as poster and abstract at the Innate Immune Memory (2019, Nijmegen) and EPA (2020, virtual) congresses, respectively. A previous draft of the manuscript was uploaded to the SSRN preprint server. The present work has neither been published previously nor is it currently under consideration for publication elsewhere.

Author contributions

MSS: data analysis and interpretation, drafting the manuscript. CS: provision of data analysis expertise, draft revision. GAH: support of data preparation, draft revision. SP: support of data preparation, draft revision. BCMH: support of conception, supervising material collection, draft revision. WAD: support of conception, laboratory analyses, draft revision. MS: support of conception, laboratory analyses, draft revision. EV: site coordinator, support of conception, supervising data acquisition, draft revision. OM: support of conception, supervision data collection, laboratory analyses, draft revision. SJ: supervising data acquisition, draft revision. RM: supervising data acquisition, draft revision. SC: site coordinator, support of conception, supervising data acquisition, draft revision. BTB: site coordinator, supervision data collection, draft revision. ML: deputy coordinator, support of conception, draft revision. FB: site coordinator, support of conception, collection of material, draft revision. RF: support of conception, supervising laboratory analyses, draft revision. RB: support of conception, laboratory analyses, draft revision. HdW: support of conception, laboratory analyses, draft revision. AW: laboratory analyses, draft revision. VA: site coordinator, support of conception, supervising data acquisition, draft revision. NM: site coordinator, support of conception, supervising data acquisition, draft revision. HAD: coordinator, drafting the manuscript.

All authors approved the manuscript and are accountable for the presented work.

Code availability

The R code is available from the first author upon request.

Ethical statement

The study was conducted in accordance with the standards for Good Clinical Practice (GCP) und the declaration of Helsinki and its subsequent revisions in order to protect the rights, safety, and well-being of all participants. The relevant European and national regulations were adhered to. The study was approved by the ethical committees of the participating universities (reference numbers: Leuven: S51723; Munich: 291–09, Münster: 2009–019-f-S). Before the performance of any study-related procedures, written informed consent was obtained from all participants.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2021.110391.

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