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DOI
10.1016/j.jpsychires.2019.11.002

Publication date
2020

Document Version
Final published version

Published in
Journal of Psychiatric Research

Journal of Psychiatric Research 121 (2020) 31–38

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

Prefrontal cortex alterations in glia gene expression in schizophrenia with and without suicide

Lin Zhang[a], Ronald W.H. Verwer[a], Paul J. Lucassen[b], Inge Huitinga[b,c], Dick F. Swaab[a,*]

[a] Neuropsychiatric Disorders Group, Netherlands Institute for Neuroscience, An institute of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, the Netherlands
[b] Brain Plasticity Group, Faculty of Science, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, the Netherlands
[c] Neuroimmunology Group, Netherlands Institute for Neuroscience, An institute of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, the Netherlands

A B S T R A C T

Background: Patients with schizophrenia (SCZ) run a lifelong risk of suicide. Alterations in glia activities in the prefrontal cortex (PFC) have been reported in relation to suicide in patients with SCZ. While immune processes in the CNS have been related to the susceptibility and course of SCZ, there are hardly any direct comparisons between individuals with SCZ, both those who died of natural causes and those that committed suicide, and healthy controls.

Materials and methods: We compared mRNA expression using real time qPCR of 16 glia-related genes in the dorsal lateral prefrontal cortex (DLPFC) and the anterior cingulate cortex (ACC) between 35 patients with SCZ (7 suicide completers and 28 patients who died of natural causes) and 34 well-matched controls without psychiatric or neurological diseases.

Results: We found an increased expression of the astrocytic gene aldehyde dehydrogenase-1 family member L1 (ALDH1L1) mRNA, a marker involved in dopaminergic activity, in SCZ versus controls. Excluding individuals with SCZ that committed suicide resulted in an elevated expression in the DLPFC of both ALDH1L1 and glutamine synthetase (GS) genes in patients with SCZ, compared to suicide completers and non-psychiatric controls. Regarding microglia genes: in the ACC, homeostatic markers such as chemokine (C-X3-C motif) ligand 1 (CX3CR1) mRNA expression was increased in SCZ without suicide as compared to suicide completers, while no change was found when compared to controls. Another, purinergic receptor 12 (P2RY12) mRNA was exclusively elevated in the ACC of suicide completers, compared to either other group. Triggering receptor expressed on myeloid cells 2 (TREM2) expression, which maintains microglial metabolism, was reduced in non-suicide patients with SCZ, compared to suicide victims and control subjects.

Conclusions: Differential changes are found in astrocyte and microglia genes in the PFC subregions in relation to SCZ and suicide, indicating possible disturbances of glia homeostasis in these conditions.

1. Introduction

Patients with schizophrenia (SCZ) run a high risk of committing suicide. Although the 5–15% incidence of completed suicides in individuals with SCZ is lower than that of major depressive disorder (MDD) and bipolar disorder (BD) patients (respectively 15% and 25%), 20–40% of the individuals with SCZ had suicidal ideations and did one or more attempts at suicide (American Psychiatric Association, 2013; Bachmann, 2018; Ko et al., 2018). In terms of suicide risk; sex differences are well documented in the population with SCZ: males commit suicide 3–9 times more frequently than females (Johns et al., 1986; Karvonen et al., 2007). This is in line with the observation that a longer duration of the disorder (related to poor prognosis) is more prevalent in males (American Psychiatric Association, 2013). On the other hand, females were more likely to go through with a suicide attempt during a sudden aggravation of the disease (Heila et al., 1997).

Various neurobiological observations and neuroimaging studies that point to the possible involvement in suicide of the dorsolateral prefrontal cortex (DLPFC, Brodmann area 46) and anterior cingulate cortex (ACC, Brodmann area 24) are independent of the underlying psychiatric causes. In MDD patients who committed suicide, specific changes were observed in the expression of neuronal and glia-related genes as compared to MDD patients that did not commit suicide (Zhao et al., 2016, 2018, 2019). In contrast, an exclusive reduction in serotonin receptor 2A (HTR2A) expression was observed in the DLPFC of suicide victims with SCZ as compared to non-suicidal cases with SCZ and controls (Garbett et al., 2008). This observation is in accordance with the lower serotonin transmitter binding reported in the DLPFC in patients with SCZ who completed suicide (Underwood et al., 2018). Moreover, a frontal cortex-targeted magnetic resonance imaging (MRI) study in patients with SCZ showed that ACC-based cognitive control disturbance in the ACC was related to long-term suicidal ideations and behaviors,

* Corresponding author. University of Amsterdam, Netherlands Institute for Neuroscience, an institute of the Royal Netherlands Academy of Arts and Sciences, Meibergdreef 47, 1105, BA Amsterdam, the Netherlands.
E-mail address: d.f.swaab@nin.knaw.nl (D.F. Swaab).

https://doi.org/10.1016/j.jpsychires.2019.11.002
Received 31 July 2019; Received in revised form 1 October 2019; Accepted 8 November 2019

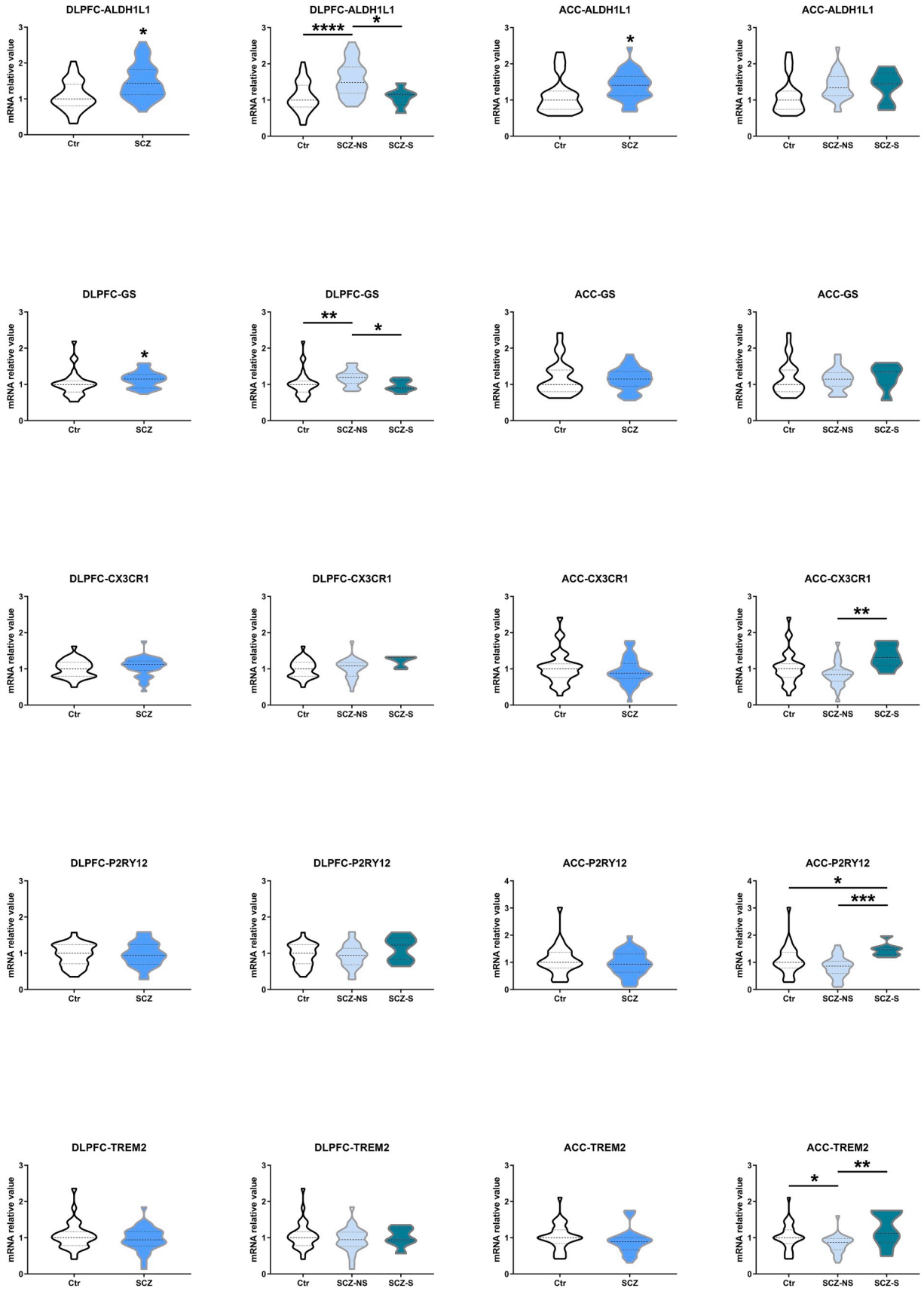

Fig. 1. Results were plotted with violin plots. Transcript levels of astrocyte (ALDH1L1 and GS) and micrcoglia related genes (CX3CR1, P2RY12 and TREM2) in the DLPFC and ACC in controls (Ctr) and patients with schizophrenia (SCZ) that died of suicide (SCZ-S) or other causes than suicide (SCZ-NS). Abbreviations: ACC, anterior cingulate cortex; DLPFC, dorsal lateral prefrontal cortex; * indicates $0.01 < P \leq 0.05$, ** indicates $0.001 < P \leq 0.01$, *** indicates $0.001 < P \leq 0.0001$, **** indicates $0.001 < P \leq 0.0001$.

whereas such a connection was not present in the DLPFC (Minzenberg et al., 2014). In addition, it was shown that noninvasive prefrontal stimulation can improve clinical symptoms and may have anti-suicidal effects in patients with SCZ (George et al., 2014; Linsambarth et al., 2019; Mehta et al., 2019). This indicates that these brain areas may play a causal role in these signs and symptoms of SCZ.

Glia cells, implicated in SCZ pathophysiology, have been widely investigated in different cortical regions and glial subtypes, and an elevation of astrocyte-related glutamate transporter-related transcripts was found in, e.g., the DLPFC in SCZ (Toker et al., 2018). Glial fibrillary acidic protein (GFAP) expression was also increased in SCZ, at both the messenger and protein level, although this appeared to occur mainly when there was mention in their clinical files of neuroinflammation, old age or dementia (Arnold et al., 1996; Catts et al., 2014; Martins-de-Souza et al., 2009). In the ACC, no differences were reported in astrocytic genes in SCZ, except for some marker reductions in the deep cortical layers (Katsel et al., 2011). Microglial density based on immunocytochemical detection of human leukocyte antigen-DR (HLA-DR) was significantly increased in the DLPFC and ACC in individuals with SCZ who committed suicide, independent of their underlying psychiatric condition (Steiner et al., 2006, 2008). This observation suggests that also microglial alterations may be related to suicide in SCZ. Another aspect of altered glia responses, i.e. myelin basic protein (MBP), did not change in the DLPFC in SCZ according to one study (Baruch et al., 2009), whereas a reduction of this marker was observed by proteomic analysis in another study in elderly individuals with SCZ (Martins-de-Souza et al., 2009). In these postmortem studies, research was predominately carried out in samples with neurodegenerative or neuroinflammatory changes, and it is thus not clear whether the alterations occur specifically in patients with SCZ or in patients who committed suicide.

So far, studies referring to suicide-specific alterations in SCZ are limited to the few mentioned above. Therefore, we investigate here whether suicidal and non-suicidal patients with SCZ differ from each other in terms of glial gene expression in the PFC. Quantitative real-time PCR (qPCR) was performed on isolated grey matter of the DLPFC and ACC, using a panel of common markers for astrocytes, microglia and oligodendrocytes in patients with SCZ who had died from either suicide or from natural causes and who were compared to well-matched controls.

2. Materials and methods

2.1. Brain samples

One hundred and thirty-eight brain samples, from 25 male and 9 female controls and 26 male and 9 female patients with SCZ, were provided by the Stanley Medical Research Institute (SMRI, Bethesda, MD, USA. Director: Dr. Maree J. Webster. For demographic and clinical information see Tables 1 and SI Tables 2A and 2B). The next of kin provided permission for the use of brain material for scientific research. Diagnoses were made by senior psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorder IV (DSM-IV). Diagnoses of unaffected controls were based on structured interviews with family member(s) by a senior psychiatrist to rule out Axis I diagnoses. Non-psychiatric controls had not displayed any suicidal behaviors, nor had they suffered from any psychiatric or neurological disorder.

The brain regions included were microscopically examined to exclude subjects with pathological signs of neurodegeneration or other obvious lesions. The SMRI formulated exclusion criteria for all specimens, which comprised; a) significant structural brain pathology on postmortem examination by a qualified neuropathologist or by antemortem imaging; b) history of significant focal neurological signs antemortem; c) history of a central nervous system disease that could be expected to permanently alter gene expression; d) documented IQ < 70; e) poor RNA quality as indicated by an RNA integrity value (RIN) of lower than 7; f) additional exclusion criteria for unaffected controls, including age under 30 (thus, still in the period of maximum risk for SCZ) and substance abuse within 1 year before death or significant alcohol-related changes in the liver. The cause of death for 7 patients with SCZ was suicide (SCZ-S); the other cases (SCZ-NS) and all control subjects (Ctr) died from non-suicidal medical conditions or accidents (for clinico-pathological details and matching for confounding factors see Table SI 2A and 2B).

Table 1A
Demographic information for the SMRI array collection (Ctr-SCZ).

	Ctr	SCZ	*P*
Age (year, range)	45 (31–60)	43 (19–59)	0.558
Gender (M/F)	25/9	26/9	0.943[1]
PMD (hours, range)	28.5 (9–58)	31.3 (9–80)	0.631
Brain pH	6.69 (6.00–7.03)	6.50 (5.90–6.90)	0.015
Brain weight (gram, range)	1413 (1120–1900)	1420 (1170–1670)	0.972
Hemisphere	16 L; 18 R	17 L; 18 R	0.900[1]
Age of onset	–	20 (9–34)	–
Duration of illness	–	24 (1–45)	–
Suicide	–	7	–
Psychotic features	–	35	–
Fluphenazine	–	35	–

Abbreviation: Ctr, control; F, female; L, left; M, male; PMD, postmortem delay; R, right; SCZ, schizophrenia.
[1] Chi-square test.

Two brain areas were studied: the DLPFC (Brodmann area 46) and ACC (Brodmann area 24). Both groups were matched for sex, age, postmortem delay (PMD), month of death (MOD), RIN value and brain weight (BW). RNA from isolated grey matter, the corresponding demographic information and medical data were provided by the SMRI. All analyses were performed by investigators ignorant of the patient information.

2.2. Quantitative real-time PCR

cDNA synthesis was performed as described by us before (Zhao et al., 2016, 2018). RIN values did not show any significant differences between the diagnostic groups ($P = 0.30$). The investigated genes were as follows: *astrocyte-related genes*, i.e. aldehyde dehydrogenase-1 family, member L1 (ALDH1L1), GFAP, glutamate transporter 1 (GLT1), glutamine synthetase (GS) and S100 calcium binding protein b (S100b); *microglia-related genes*, i.e. cluster of differentiation 68 (CD68), chemokine (C-X3-C motif) receptor 1 (CX3CR1), human leukocyte antigen-DRA (HLA-DRA), ionized calcium-binding adapter molecule-1 (IBA1), purinergic receptor 12 (P2RY12), triggering receptor expressed on myeloid cells 2 (TREM2) and translocator protein (TSPO); *oligodendrocyte-related genes,* i.e. MBP, myelin oligodendrocyte glycoprotein (MOG), oligodendrocyte transcription factor 2 (OLIG2) and myelin proteolipid protein 1 (PLP1). Additional information on all tested genes and the sequences for each primer pair are shown in SI Table 1.

A cDNA template (equivalent to 5 ng of total RNA) was amplified in a final volume of 10 µl using SYBR Green PCR master mix (Applied Biosystems, CA, USA) and a mixture of forward and reverse primers (each 2 pmoL/µl). Data were acquired and processed automatically by an Applied Biosystems 7300 Real-time PCR System. The specificity of amplification was checked by melting curve analysis. Sterile water and RNA samples without the addition of reverse transcriptase during cDNA synthesis served as negative controls. The linearity of each qPCR assay was tested by preparing a series of dilutions of the same stock cDNA in multiple plates. In this way, also the efficiency of the polymerase reaction of each gene could be estimated and applied to calculate the expression values. Reference genes were selected to reduce the effect of sample variability (Vandesompele et al., 2002; Zhao et al., 2018). The initial set of reference genes was: actin beta (ACTβ), glyceraldehyde-phosphate dehydrogenase (GAPDH), hypoxanthine phosphoribosyl

Table 1B
Demographic information for the SMRI array collection (Ctr-SCZNS-SCZS).

	Ctr	SCZ-NS	SCZ-S	*P*
Age (year, range)	45 (31–60)	44 (19–59)	39 (24–45)	0.118
Gender (M/F)	25/9	22/6	4/3	0.512 [1]
PMD (hours, range)	28.5 (9–58)	30.0 (9–80)	27.0 (15–65)	0.806
Brain pH	6.69 (6.00–7.03)	6.46 (5.90–6.90)	6.52 (6.19–6.80)	0.043
Brain weight (gram, range)	1413 (1120–1900)	1440 (1170–1670)	1480 (1340–1510)	0.837
Hemisphere	16 L; 18 R	15 L; 13 R	2 L; 5 R	0.492 [1]
Age of onset	–	19 (9–31)	29 (20–34)	–
Duration of illness	–	24 (1–45)	5 (3–18)	–
Suicide	–	–	7	–
Psychotic features	–	28	7	–
Fluphenazine	–	28	7	–

Abbreviation: Ctr, control; F, female; L, left; M, male; PMD, postmortem delay; R, right; SCZ-NS, schizophrenia patients who died of other causes than suicide; SCZ-S, schizophrenia patients who committed suicide.
[1] Chi-square test.

transferase 1 (HPRT1), tubulin alpha (TUBα), tubulin beta (TUBβ) and ubiquitin C (UBC). For the comparisons in the DLPFC, the specific selection was ACTβ, TUBβ and UBC; in the ACC, the corresponding selection was ACTβ, GAPDH and TUBβ.

2.3. Statistical analysis

S+ software (version 8.2, TIBCO, Seattle, WA, USA) was used for statistical analysis. To analyze categorical data, the Mann-Whitney test (2 samples), the Kruskal-Wallis test with multiple comparisons (3 samples) or Chi-square test was used. A Mardia-Watson-Wheeler test was performed for month-of-death analyses (Batschelet, 1981; Zar, 1999). For interval data the Mann-Whitney test (2 samples) or the Kruskal-Wallis test with multiple comparisons (3 samples) was used (Conover, 1980). Before processing the gene expression data, the values were ^{10}log-transformed to enable simple reference gene correction and conventional statistical procedures. In multiple testing situations the Benjamini-Hochberg correction (Benjamini and Hochberg, 1995) of *P*-values was applied. When the Kruskal-Wallis test was used in combination with the Benjamini-Hochberg correction we used 2-step analysis. As multiple comparisons in the Kruskal-Wallis test are only allowed if the global $P < 0.05$ (Conover, 1980), we first corrected the global *P*-values and then selected for further analysis those genes for which this requirement was met. For each appropriate comparison the corresponding *P*-values were pooled and corrected according to Benjamini-Hochberg. All tests were 2-sided.

3. Results

3.1. Confounder analysis

We used the Spearman test to examine the correlations between brain pH, antipsychotics and glia genes expression. We did not find a significant correlation between CSF pH and glial gene expression in either the DLPFC or the ACC. As for the use of antipsychotics, which was recorded as fluphenazine equivalents in a lifetime dosage (see SI Tables 2A and 2B), a significant positive correlation was found between this parameter and CX3CR1 expression (rho = −0.544, $P = 0.024$) and P2RY12 expression (rho = −0.483, $P = 0.039$) in the ACC. These differences were not observed in the subgroup of individuals with SCZ who had either died naturally (CX3CR1: $P = 0.080$; P2RY12: $P = 0.091$) or from suicide (CX3CR1: $P = 0.912$; P2RY12: $P = 1.000$).

3.2. Glial alterations in the DLPFC and ACC in SCZ and suicide

An overview of mRNA expression in genes is presented in Tables 2A and 2B (Fig. 1).

In the DLPFC, the expression of the astrocytic gene ALDH1L1 was significantly higher in patients with SCZ than in controls (Fold change = 1.42, $P = 0.017$). Another two astrocytic genes, which are related to the glutamate (Glu)- glutamine (Gln) cycle, i.e. GLT1 and GS, showed an upward trend in their mRNA levels, but no significant effects were present after correction for multiple testing ($P = 0.099$). When the patients who had completed suicide were studied as a separate group, both ALDH1L1 and GS showed elevated expression only in SCZ-NS, compared to the SCZ-S and controls (ALDH1L1: SCZ-NS vs. SCZ-S, fold change = 1.27, $P = 0.010$; SCZ-NS vs. controls, fold change = 1.43, $P < 0.001$. GS: SCZ-NS vs. SCZ-S, fold change = 1.34, $P = 0.024$; SCZ-NS vs. controls, fold change = 1.19, $P = 0.001$). GLT1 increases in SCZ-NS only showed a trend compared to the controls ($P = 0.065$). No changes were found in the DLPFC between control subjects and patients with SCZ with or without suicide in terms of microglia or oligodendrocyte-related gene expression.

In the ACC, ALDH1L1 expression was also elevated in SCZ (Fold change = 1.38, $P = 0.047$), but when we included suicide as a covariate the difference decreased to trend level ($P = 0.056$). Two or three-group comparisons did not yield any changes in the expression of microglia-related genes. However, an almost 60% increased expression in CX3CR1 mRNA was found in SCZ-S patients compared to SCZ-NS patients (fold change = 1.57, $P = 0.003$), but not when compared to the controls. No difference was found between SCZ-NS and controls. P2RY12 was found to be exclusively elevated in the SCZ-S compared to

Table 2A
Results of glial target gene expression in the DLPFC and ACC between patients with SCZ and matched controls.

	DLPFC			ACC		
	Fold change	P value	BHadj-p	Fold change	P value	BHadj-p
Astrocyte genes						
ALDH1L1	**1.42**	**0.001**	**0.017**	**1.38**	**0.003**	**0.047**
GFAP	1.05	0.606		1.20	0.719	
GLT1	1.27	0.019	0.099	1.10	0.302	
GS	1.16	0.013	0.099	1.20	0.517	
S100b	1.21	0.100		1.22	0.199	
Microglia genes						
CD68	1.11	0.119		1.06	0.952	
CX3CR1	1.13	0.313		−1.10	0.394	
HLA	1.25	0.885		1.00	0.285	
IBA1	1.00	0.876		−1.06	0.749	
P2RY12	−1.03	0.838		−1.04	0.471	
TREM2	−1.05	0.581		−1.12	0.095	
TSPO	1.16	0.103		1.08	0.254	
Oligodendrocyte genes						
MBP	1.10	0.259		−1.05	0.494	
MOG	1.08	0.464		1.09	0.130	
OLIG2	1.10	0.486		1.00	0.792	
PLP	1.00	0.525		−1.03	0.548	

Note: ACC: anterior cingulate cortex; BHadj-p: *P* value of Benjamini-Hochberg's adjustment; DLPFC: dorsal lateral prefrontal cortex.

Table 2B
Results of glial target gene expression in the DLPFC and ACC between suicide and non-suicide patients with SCZ and their matched controls.

	Fold change			P value	BHadj-p	BHadj-p (2-step)		
	SCZ-S/SCZ-NS	SCZ-S/Ctr	SCZ-NS/Ctr			SCZ-S/SCZ-NS	SCZ-S/Ctr	SCZ-NS/Ctr
DLPFC-astrocyte genes								
ALDH1L1	**−1.27**	1.16	**1.47**	**0.000**	**0.004**	**0.019**	0.991	**0.000**
GFAP	−1.28	−1.18	1.08	0.191				
GLT1	−1.22	1.08	1.31	**0.012**	0.065			
GS	**−1.33**	−1.12	**1.19**	**0.004**	**0.031**	**0.024**	0.991	**0.001**
S100b	1.02	1.24	1.21	0.215				
DLPFC-microglia genes								
CD68	1.05	1.16	1.11	0.292				
CX3CR1	1.22	1.31	1.08	0.054				
HLA	1.11	1.25	1.13	0.986				
IBA1	1.18	1.15	−1.03	0.751				
P2RY12	1.30	1.21	−1.08	0.450				
TREM2	1.00	−1.05	−1.05	0.722				
TSPO	−1.15	1.04	1.20	0.202				
DLPFC-oligodendrocyte genes								
MBP	1.09	1.20	1.10	0.258				
MOG	1.59	1.59	1.00	0.243				
OLIG2	1.18	1.30	1.10	0.648				
PLP	1.45	1.38	−1.03	0.127				
ACC-astrocyte genes								
ALDH1L1	1.07	1.43	1.33	**0.014**	0.056			
GFAP	−1.45	−1.22	1.19	0.796				
GLT1	1.23	1.35	1.10	0.515				
GS	1.17	1.35	1.15	0.703				
S100b	−1.33	−1.12	1.19	0.343				
ACC-microglia genes								
CD68	1.19	1.19	1.00	0.185				
CX3CR1	**1.57**	1.31	−1.19	**0.008**	**0.040**	**0.003**	0.056	0.066
HLA	1.60	1.33	−1.20	0.121				
IBA1	1.48	1.30	−1.14	0.297				
P2RY12	**1.65**	**1.43**	−1.15	**0.002**	**0.027**	**0.001**	**0.027**	0.066
TREM2	**1.25**	1.11	**−1.12**	**0.006**	**0.040**	**0.006**	0.227	**0.024**
TSPO	1.23	1.28	1.04	0.111				
ACC-oligodendrocyte genes								
MBP	1.22	1.16	−1.05	0.243				
MOG	1.08	1.21	1.12	0.284				
OLIG2	1.22	1.22	1.00	0.908				
PLP	1.06	−1.02	−1.08	0.653				

Note: ACC: anterior cingulate cortex; BHadj-p: *P* value of Benjamini-Hochberg's adjustment; Ctr: control; DLPFC: dorsal lateral prefrontal cortex; SCZ: schizophrenia; SCZ-NS: schizophrenia – non suicide; SCZ-S: schizophrenia - suicide.

the SCZ-NS group (fold change = 1.65, $P < 0.001$; SCZ-S vs. controls, fold change = 1.43, $P = 0.027$). A difference in this gene between SCZ-NS patients and control subjects was absent. In addition, the reduction in TREM2 mRNA was significant in SCZ-NS patients compared to SCZ-S (fold change = −1.25, $P = 0.006$; SCZ-NS vs. controls, fold change = −1.12, $P = 0.024$). This significance was absent in the SCZ-S group compared to controls.

Within the two Brodmann areas, neither two nor three group comparisons revealed any significant alterations in oligodendrocyte-related gene expression levels. Moreover, in the glia markers studied, sex differences were absent in both SCZ and control subjects.

4. Discussion

The present study revealed regionally different glia-related changes in individuals with SCZ who either died as a result of suicide or of other causes. In both the DLPFC and ACC, an astrocytic gene ALDH1L1, which represents dopaminergic activity, was found to be elevated in SCZ, especially in the DLPFC of patients who died of non-suicidal causes. In the DLPFC, we noticed similar differences in GS, an enzyme involved in the Glu-Gln cycle. In the ACC of individuals with SCZ, the expression of three microglial genes (CX3CR1, P2RY12 and TREM2) was increased in suicide completers than the others. Among them, CX3CR1 expression was strongly increased in suicidal patients with SCZ compared to those who died of other causes. P2RY12 showed increased expression exclusively in suicide victims. In addition, the only significant reduction in mRNA expression was observed in TREM 2 in non-suicidal SCZ. In view of the sex differences in terms of prevalence, signs and symptoms in SCZ, it is surprising that no sex differences were found in relation to the genes investigated. Our results suggest that functional disturbances in dopamine-glutamate interaction, microglia phagocytosis and purine metabolism are involved in SCZ.

Dysfunctional astrocyte activity has been implicated in the pathogenesis and pathophysiology of schizophrenia before (Mei et al., 2018; Xia et al., 2016). This relationship is supported by glia-related molecular alterations on both the transcriptional and translational level and by rare genetic variants in different astrocytic genes associated with an increased risk for SCZ (Catts et al., 2014; González-Peñas et al., 2019). ALDH1L1 has been regarded as a dopamine-related astrocytic gene, not only because it was reported to be expressed in astrocytes, but also since it is present in, and functionally correlated with, human dopamine (DA) neurons (Galter et al., 2003). ALDH1L1 is one of the isoenzymes of ALDH1, which plays a main role in supporting acetaldehyde dehydrogenase (ALDH1) activities (Shen et al., 2016). A study based upon sub-cortical microdissection reported an overall increase of ALDH1L1 transcripts in the anteroventral, mediodorsal thalamic nucleus, internal capsule and putamen in SCZ, all components of cortico-striato-thalamic circuits, from which especially the mediodorsal thalamic nucleus projects directly to the DLPFC (Barley et al., 2009).

To our knowledge, this is the first time that ALDH1L1 is reported to be increased in the cortex of patients with SCZ, although not in suicide cases. In suicidal individuals with SCZ, ALDH1L1 transcripts may possibly be replaced by other members of the ALDH family (Fiori et al., 2011; Hishimoto et al., 2010; Monson et al., 2017) or by single

nucleotide polymorphisms (SNPs), that indeed go together with a higher incidence in suicide cases (Erlangsen et al., 2018). An RNA-sequencing study showed significant increases in the expression of specific astrocyte-related genes in the cingulate cortex of patients with SCZ that were medication independent (González-Peñas et al., 2019). The brain samples in that study were from the same collection as used in our present study and our data of increased anterior cingulate cortical ALDH1L1 mRNA expression in our data thus confirmed, with different techniques, their finding. In our study, similarly enhanced astrocytic expression in the DLPFC was observed using the markers ALDH1L1 and GS in non-suicidal subjects with SCZ, implicating the involvement of dopaminergic and glutamatergic overexposure in SCZ. The presence of such alterations has further been supported by functional MRI of the DLPFC in genetic risk variants for SCZ that were related to DA and Glu, which predicted the incidence and clinical manifestations of SCZ (Nixon et al., 2011; Tost and Meyer-Lindenberg, 2011). Our present data also supports our previous work, showing that astrocyte-related genes involved in the Glu-Gln cycle are only elevated in the DLPFC of non-suicidal MDD patients (Zhao et al., 2016).

The enhanced trend of GLT1 transcripts, another astrocytic gene involved in the Glu-Gln cycle that was observed in SCZ-NS patients, further supports this finding. Consistent with this, a microarray analysis reported a reduction of GS mRNA in the PFC of suicidal cases with SCZ (Kim et al., 2007). The observation that increased GS mRNA was also present in the thalamus of patients with SCZ in subregions that are connected to the DLPFC and show enhanced expression of ALDH1L1 (Bruneau et al., 2005) further supports our observation. One exception is the reduced GS expression that was reported in an older SCZ study (Burbaeva et al., 2003). However, as this analysis was based upon combined DLPFC and anterior prefrontal cortex (APFC, BA10) samples, regional differences in the APFC samples may have contributed to this finding.

Functional alterations of microglia in the prefrontal cortex of patients with SCZ are quite heterogeneous, as are these brain immune cells themselves (Böttcher et al., 2019), which may represent the different roles they play in SCZ (Notter and Meyer, 2017). For our study, we selected seven microglia-related genes reflecting microglia activation, phagocytosis, migration, antigen presentation, angiogenetic regulation, purine metabolism and inflammatory response. There were no significant alterations among them for the total group of individuals with SCZ as compared to controls. However, different expression levels between suicide victims and patients who died from other causes were found in the ACC. In suicide victims with SCZ, increased CX3CR1 expression was present when compared to patients with SCZ who did not commit suicide. As a member of the chemokine family, the ligand of CX3CR1, chemokine (C-X3-C motif) ligand 1 (CX3CL1, also known as fractalkine), has not been explicitly mentioned in relation to suicide or SCZ before, but it has been mentioned in relation to mood disorders (Stuart and Baune, 2014). In addition, one study reported that the expression of this gene was dramatically increased in moderate to severe MDD patients (Merendino et al., 2004), who had a high suicide risk.

Strikingly, we found more than 50% enhancement of P2RY12 mRNA only in the group of suicidal individuals with SCZ in the ACC, indicating that an abnormal metabolism in purinergic signaling may be related to suicide, probably independent of the illness. As a member of the P2RY family, P2RY12 activity is inhibited by ADP during purine metabolism (Tulapurkar et al., 2005). In body fluids, such as CSF and urine, lower levels of hypoxanthine, a spontaneous de-amination product of adenine, were present in severely affected MDD patients that had suicide ideations or had completed suicide (Agren et al., 1983; Lis et al., 1975). In addition, the serotonin augmentation index, assessed by measuring the serotonin-mediated enhancement of ADP-induced platelet aggregation, was impaired in patients with a high risk for suicide (Mann et al., 1992). This may explain the reduced ADP activity in SCZ-suicidal behaviors. We may, therefore, assume that higher P2RY12 expression in suicidality might be due to the partially lifted inhibition from ADP inactivation. In addition, our results showed upregulations of both P2RY12 and CX3CR1, two homeostatic microglial genes, that indicated a sign of imbalanced and activated homeostasis in suicidal individuals with SCZ (Landsman et al., 2009).

A reduction of TREM2-mRNA was found in the ACC only in non-suicidal cases with SCZ. While TREM2 was repeatedly reported to be expressed at higher mRNA levels and at lower DNA methylation rates, e.g. in peripheral leukocytes from patients with SCZ (Mori et al., 2015; Yoshino et al., 2016, 2017), our results now show opposite changes in the brain. This difference may be explained by the presence of different inflammatory responses in peripheral neutrophils and central monocytes/macrophages. Indeed, on the basis of molecular changes observed in TREM2 knockdown mice, it was proposed that a TREM2 deficiency due to genetic mutations might rapidly induce psychotic symptoms (Penberthy, 2007), which is in agreement with similar manifestations and common psychotic features in SCZ. As a marker that sustains microglia metabolism and its response towards amyloid β plague pathology in Alzheimer's disease, one can indicate that functionally deficient TREM2 expression may be in relation to the high risk of psychotic symptoms in some neurodegenerative status (Ropacki and Jeste, 2005; Ulland and Colonna, 2018). Furthermore, it was proven that TREM2 is necessary for synapse elimination, which is accompanied by modulating excitatory neurotransmission and changes in long-range functional connectivity (Filipello et al., 2018; Sellgren et al., 2019). Indeed, increased numbers of glutamatergic axons and axo-spinous synapses were found in the cingulate cortex of patients with SCZ (Harrison, 1999), supporting the idea that hyperfunctional glutamatergic synapses might be a primary genetic abnormality in SCZ etiology (Owen et al., 2005). We may further speculate that the elevated synaptic density in ACC is due to microglia deficiency, which may be mediated by the reduction in TREM2, known to disrupt adequate synapse regulation.

We did not find changes in oligodendrocyte markers in SCZ. Morphological studies of oligodendrocyte densities gave variable results and oligodendrocyte densities in the DLPFC were reported to be unchanged in the BA 9 (Hercher et al., 2014) but reduced in layer III, V and VI in the BA 10 (Kolomeets and Uranova, 2018; Vostrikov and Uranova, 2011, 2018), indicating regional and even layer-specific changes. In the ACC, previous data did not report changes in MBP expression, either on the mRNA or protein level in patients with SCZ (Dracheva et al., 2006; Haroutunian et al., 2007). Our results confirm these data, now also for the patients that committed suicide.

One of the possible confounding factors in this postmortem study is medication. A relation between fluphenazine equivalents, as a measure for the amount of dopamine receptor D1 and D2 antagonists used during life, and microglia activities has not been shown before. Our data indicate that CX3CR1 and P2RY12 may play a novel role as microglia-related genes that are targeted towards dopaminergic neurons. We also observed, for the first time, a dose-effect relationship of fluphenazine on microglia suppression in the subgroup of SCZ-NS. However, these negative correlations were disturbed in suicidal SCZs compared to the other patients, and may imply that the elevated expression of CX3CR1 and P2RY12 in suicide completers seems to counteract the microglia-suppressing effect of fluphenazine.

In conclusion, our present study highlights the heterogeneity in glia gene alterations in patients with SCZ in relation to suicide. A glutamate-related astrocytic gene, ALDH1L1, was the only one that was significantly elevated in both the DLPFC and ACC, which supports the possible presence of a hyperfunctional dopaminergic involvement in SCZ. Different, or even opposite, astrocytic and microglia alterations were found in individuals with SCZ, depending on whether they had died from natural causes or from suicide. Moreover, functional disturbances in glutamate-dopamine interaction, microglia phagocytosis and purinergic metabolism seem to participate in the pathophysiology of SCZ. Therefore, we want to emphasize that, in future research, it is of crucial importance to separately study groups of non-suicidal and

suicidal patients with SCZ. The same holds for other psychiatric disorders that go together with suicide (Zhao et al., 2019).

Role of funding source

This research was supported by the 'Stichting Vrienden van het Herseninstituut'.

Contributors

L. Zhang, I. Huitinga and D.F. Swaab designed the research protocol. L. Zhang undertook data collection. R.W.H. Verwer performed the statistical analysis. L. Zhang wrote the first draft, D.F. Swaab, I. Huitinga and P.J. Lucassen amended the manuscript. All authors have approved and contributed to the final manuscript. D.F. Swaab provided the financial support.

Declaration of competing interest

None to declare.

Acknowledgments

Postmortem brain samples were obtained from the Array Collection of the Stanley Medical Research Institute (Director: Dr. Maree J. Webster). We thank Arja Sluiter for her technical support and Wilma Verweij for secretarial assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2019.11.002.

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