Schizophrenia Bulletin Open
doi:10.1093/schizbullopen/sgab012

Immune Signatures of Treatment-Resistant Schizophrenia: A FondaMental Academic Centers of Expertise for Schizophrenia (FACE-SZ) Study

Marion Leboyer[1–3], Ophélia Godin[1,3], Emilie Terro[3], Wahid Boukouaci[2,3], Ching-lieng Lu[2,3], Myrtille Andre[3,4], Bruno Aouizerate[3,5,6], Fabrice Berna[3,7], Caroline Barau[8], Delphine Capdevielle[3,4], Julie Clauss-Kobayashi[3,7], Isabelle Chereau[3,9], Thierry D'Amato[3,10], Caroline Dubertret[3,11], Julien Dubreucq[3,12], Guillaume Fond[3,13], Hakim Laouamri[3], Sylvain Leignier[3,12], Christophe Lancon[3,13], Pierre-Michel Llorca[3,9], Jasmina Mallet[3,11], Philippe Le Corvoisier[8], David Misdrahi[3,14], Christine Passerieux[3,15], Romain Rey[3,10], Baptiste Pignon[1–3], Mathieu Urbach[3,15], Andrei Szoke[1–3], the FACE-SZ (FondaMental Academic Centers of Expertise for Schizophrenia) Groups[†], Franck Schürhoff[1–3], and Ryad Tamouza[*,1–3]

[1]Univ Paris Est Créteil, INSERM, IMRB, Translational Neuropsychiatry, DMU IMPACT, Fondation FondaMental, F-94010, Créteil, France; [2]AP-HP, Hôpitaux Universitaires Henri Mondor, Département Médico-Universitaire de Psychiatrie et d'Addictologie (DMU IMPACT), Fédération Hospitalo-Universitaire de Médecine de Précision en Psychiatrie (FHU ADAPT) F-94010, France; [3]Fondation FondaMental, Créteil, France; [4]Service Universitaire de Psychiatrie Adulte, Hôpital la Colombière, CHRU Montpellier, Université Montpellier 1, INSERM 1061, Montpellier, France; [5]Centre Hospitalier Charles Perrens, Université de Bordeaux, Bordeaux F-33076, France; [6]INRAE, NutriNeuro, University of Bordeaux, U1286, Bordeaux F-33076, France; [7]Hôpitaux Universitaires de Strasbourg, Université de Strasbourg, INSERM U1114, Fédération de Médecine Translationnelle de Strasbourg, Strasbourg, France; [8]INSERM, Centre Investigation Clinique 1430, AP-HP, Hôpitaux Universitaires Henri Mondor, Université Paris Est Créteil, F94010 Créteil, France; [9]CHU Clermont-Ferrand, Department of Psychiatry, University of Clermont Auvergne, EA 7280, Clermont-Ferrand, France; [10]INSERM U1028, CNRS UMR5292, Centre de Recherche en Neurosciences de Lyon, Université Claude Bernard Lyon 1, Equipe PSYR2, Centre Hospitalier Le Vinatier, Pole Est, Bron, France; [11]Université de Paris, INSERM UMR1266, AP-HP, Groupe Hospitalo-Universitaire AP-HP Nord, Service de Psychiatrie et Addictologie. Hôpital Louis Mourier, Colombes, France; [12]Centre Référent de Réhabilitation Psychosociale et de Remédiation Cognitive (C3R), CH Alpes Isère, France; [13]AP-HM, Aix-Marseille Univ, School of medicine – La Timone Medical Campus, EA 3279: CEReSS – Health Service Research and Quality of Life Center, 13005 Marseille, France; [14]Department of Adult Psychiatry, Charles Perrens Hospital, University of Bordeaux, CNRS UMR 5287-INCIA "Neuroimagerie et cognition humaine," Bordeaux, France; [15]Service Universitaire de psychiatrie et d'addictologie du Centre Hospitalier de Versailles, INSERM UMR1018, CESP, Team "DevPsy," Université de Versailles Saint-Quentin-en-Yvelines, Paris – Saclay, France

*To whom correspondence should be addressed; Département Hospitalo-Universitaire de Psychiatrie, Hôpital Albert Chenevier, 40 rue de Mesly, Créteil 94000, France; tel: +331-49-81-30-51, fax: +33 1 49 81 30 59, e-mail: tamouza.ryad@gmail.com

[†]The list of FondaMental Advanced Center of Expertise (FACE-SZ) collaborators has been mentioned in the Acknowledgments section.

Treatment-resistant schizophrenia (TRS) affects around 30% of patients with schizophrenia (SZ) resulting in poor functioning, relapses, and reduced quality of life. Convergent findings show that inflammation could contribute to resistance. We thus search for immune signatures of patients with TRS/ultra TRS (UTRS) in a sample of community-dwelling outpatients with SZ. In total, 195 stabilized SZ patients (mean age = 31.2 years, 73% male gender) were consecutively included in the network of the FondaMental Expert Centers for Schizophrenia in France and received a thorough clinical assessment. At inclusion, psychotic symptomatology was evaluated by the Positive and Negative Syndrome Scale (PANSS) for schizophrenia. Circulating serum/plasma levels of a large panel of markers reflecting the main inflammatory pathways were evaluated. TRS was defined by current treatment by clozapine (CLZ) and UTRS by current CLZ treatment + PANSS total score ≥ 70. The frequency of TRS and UTRS patients was, respectively, 20% and 7.7% and was defined using multivariable analysis elevated by high levels of interleukin (IL)-12/IL-23p40, IL-17A, IL-10, and beta 2 microglobulin (B2M) and IL-12/IL-23p40, IL-17A, IL-6, IL-10, IFNγ, and B2M, respectively. These observations suggest that resistance and ultra resistance to CLZ treatment are underpinned by pro-inflammatory molecules mainly belonging to the T helper 17 pathway, a finding making sense given the interplay between inflammation and antipsychotic treatment responses. If confirmed, our findings may allow us to consider IL-23/IL-17 pathway as a therapeutic target for patients with resistance to antipsychotics.

Key words: schizophrenia/immuno-inflammatory/signatures/profile/treatment-resistant/clozapine

Introduction

Treatment-resistant schizophrenia (TRS) is usually defined as nonresponse to at least two first line of antipsychotic treatments of adequate duration and dose with documented compliance.[1] TRS affects approximately 30% of all individuals with schizophrenia (SZ) and results in poorer functioning, reduced quality of life, and higher rates of unemployment, as compared with responders to treatment,[2] altogether leading to a 3- to 11-fold increase in healthcare costs in comparison to the overall patients with SZ.[3] The only approved evidence-based treatment for TRS is clozapine (CLZ). However, due to its adverse effects, CLZ is only licensed as a third-line treatment (NICE, 2014).[2] Howes et al[4] showed that CLZ is offered years after the onset of the disorder, with an average delay of 3.9 years. Nevertheless, as it has been shown that patients with a short delay before CLZ initiation experience a better prognosis,[5] it would be of the utmost importance to be able to identify early predictors of TRS.[6] Because of the specific role of CLZ in the treatment of TRS, it constitutes an often-used proxy of resistance. A step beyond, ultra TRS (UTRS) designs cases of failure to respond to CLZ, a subgroup representing up to 60% of the patients with TRS who do not respond.[7]

Identifying biomarkers of resistance would not only help in their early prediction but also add to our understanding of underlying causes of TRS and inform the development of future treatment. Current hypotheses for the biological basis of TRS focus on the differences in dopamine functioning, changes in glutamate, or of other pathways.[8] In particular, elevated levels of inflammation at baseline have clearly been suggested to contribute to resistance. Convergent findings show that inflammation plays a role in the pathogenesis of psychotic disorders[9] with increased cytokine levels in peripheral blood and cerebrospinal fluid, both at illness onset and in later stages, particularly in TRS. Neuroinflammation early in life followed by chronic overactivation has been hypothesized to contribute to the etiology of TRS.[1,10,11] We have previously shown that c-reactive protein was associated with ultra-high resistance to SZ, defined by CLZ resistance.[12] However, this finding was not replicated in a sample of first-episode SZ patients who later developed TRS.[13] Strawbridge et al[14] thus suggested that a composite measure of inflammatory markers would better contribute to predicting resistance in depression than single marker of inflammation.

The objective of our study is thus to search for immune signatures of patients with TRS/UTRS in a population of 195 patients with SZ assessed clinically and biologically tested for a large number of immuno-inflammatory markers.

Materials and Methods

Study Population

This is a cross-sectional study based on a national cohort issued from FondaMental Academic Centers of Expertise (FACE), the FACE-SZ cohort.[15] The FACE-SZ cohort is based on a French national network of 10 Expert Centers, which is coordinated by the Fondation FondaMental (www.fondation-fondamental.org) and funded by the French Ministries of Research and Health in order to develop, establish, and provide precision medicine and adaptive care for patients suffering from SZ spectrum disorders. All individuals included were outpatients receiving stable medication for more than 4 weeks. A subgroup of 195 patients with a thorough description of their current and past psychotropic treatments along with clinical assessment and measures of serum inflammatory markers were included in this analysis. The assessment protocol was approved by the relevant ethical review board (CPP-Ile-de-France IX, January 18, 2010). All data were collected anonymously.

Data Collection

Diagnostic interviews were carried out by psychiatrists according to the Structured Clinical Interview for Mental Disorders (SCID 1.0).[16] Information about sociodemographic data, onset and course of illness, duration of untreated psychosis (DUP), comorbidities, in particular history of suicide attempt, lifetime alcohol, and/or cannabis abuse, and body mass index (BMI) were recorded. Psychotic and general psychopathology was assessed using the Positive and Negative Syndrome Scale (PANSS).[17] Current depressive symptoms were evaluated using the Calgary Depression Rating Scale (CDRS) for SZ.[18] Daily global functioning was evaluated with the Global Assessment of Functioning (GAF) Scale.[19] Ongoing and past psychotropic treatment were recorded and self-reported adherence to pharmacological treatment was evaluated using the Medication Adherence Rating Scale (MARS).[20]

Biological Data

Circulating serum/plasma levels of a large panel of markers reflecting the main inflammatory pathways, including pro- and anti-inflammatory cytokines, chemokines, growth factors, adhesion molecules, and nonspecific inflammatory molecules, were evaluated. More precisely, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12-IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-21, IL-22, IL-23, IL-27, IL-31, interferon gamma (IFN γ), TNF α, tumor necrosis factor (TNF) β, vascular endothelial growth factor (VEGF), macrophage inflammatory factor (MIP) 1, monocyte chemoattractant protein (MCP) 1, Granulocyte-macrophage

colony-stimulating (GM-CSF), B cell activating factor (BAFF), brain-derived neurotrophic factor (BDNF), eotaxin, eotaxin 3, intercellular adhesion molecule 1 (ICAM1), interferon gamma-induced protein 10 (IP10), M1, MCP4, macrophage-derived chemokine (MDC), MIP1α, MIP1β, MIP3α, programmed death (PD) 1, programmed death-ligand 2 (PDL2), serum amyloid A (SAA), thymus- and activation-regulated chemokine (TARC), and vascular cell adhesion molecule 1 (VCAM1) were measured using a MesoScale Discovery (MSD) human V-Plex electrochemiluminescence assay (MSD), while soluble isoforms of beta 2 microglobulin (B2M) and human leukocyte antigen E (HLA-E) by commercially available ELISA kits (BioVendor and Cloud-Clone Corp, respectively). Both methods were performed according to the manufacturer's recommendations. Biological markers with more than 15% of missing data and cytokines with more than 50% of concentrations outside the limit of detection (LLOD) were excluded from the analysis. For the remaining cytokines, concentrations below or upper limit of detection were, respectively, replaced by LLOD divided by 2 and by maximum value +1.

Definition of Response to Treatment Status

Treatment-Resistant Schizophrenia: TRS was defined by current treatment by CLZ.

Ultra Treatment-Resistant Schizophrenia: Among TRS individuals, UTRS was defined by failure to respond to CLZ as reflected by a mean PANSS score ≥ 70, which corresponds to "moderately to severely ill" patients.

Statistical Analysis

Categorical variables are presented using frequency distribution and continuous variables by measure of means (SD) or median (interquartile range [IQR]) depending on the variable's distribution. Normal distribution was examined using the Shapiro-Wilk test. Comparison between TRS and non-TRS individuals, ie, good responders as well as between UTRS and non-UTRS individuals regarding demographic and clinical characteristics, was performed using the Chi-square or Fisher exact test for categorical variables and Student *t*-tests or Mann-Whitney Wilcoxon test for continuous variables. Immuno-inflammatory makers were log(1 + *x*) transformed, and separate multivariable models for each immuno-inflammatory marker were performed. Variables significantly associated with TRS/UTRS in univariate analysis were included in the multivariable analysis of covariance model. As more traditional levels (such as .05) can fail to identify variables known to be important, sensitivity analysis including variables with *P*-value < .20 in univariate analysis was performed. Multivariable analyses were then adjusted for (1) age, gender, age of SZ onset, DUP, psychotic symptomatology, global functioning, history of suicide attempt, current smoker, lifetime cannabis abuse, first-generation antipsychotic, antidepressant medication, anticholinergic treatment, and the number of baseline psychotropic medication for TRS and (2) age, gender, age of SZ onset, DUP, duration of illness, psychotic symptomatology, global functioning, depressive symptoms, history of suicide attempt, current smoker, lifetime cannabis abuse, body mass index, anticholinergic treatment, and the number of baseline psychotropic medication for UTRS. All statistical tests were 2-tailed, with α level set at 0.05. Data were conducted using R software.

Results

We included a sample of 195 SZ patients with a mean age of 31.2 (SD = 8.3) years, mean illness duration 10 years (SD = 7.9), and a majority of men (73%). The mean PANSS total score was 62.9 +/− 19.6. The frequency of TRS and UTRS patients was, respectively, 20% (n = 39) and 7.7% (n = 15). The mean duration of CLZ treatment was 34 months (SD = 45) for TRS individuals and 45 months (SD = 42) for UTRS patients.

Individuals with TRS had a significantly younger age at onset (18.6 ± 3.3 vs 22.4 ± 6.5 years; P = .0004) and a shorter DUP (1.7 ± 4.5 vs 2.0 ± 4.7 years, P = .0450) as compared with good responders. They also were less likely to be smokers (33.3% vs 53.8%, P = .0254) and to have lifetime cannabis abuse (20.5% vs 34%, P = .0354). No association with other clinical variables was observed (table 1). In comparison to good responders, UTRS individuals had a significantly earlier age at onset (18.7 ± 3.4 vs 22.4 ± 6.5 years; P = .0236), shorter DUP (0.35 ± 1.3 vs 2.0 ± 4.7 years, P = .0052), lower global functioning, higher depressive symptoms, and were more likely to have a history of suicide attempt (40.0% vs 15.4% in UTRS and good responders, respectively, P = .0340). As expected, they had persistent psychotic symptomatology for total and all sub-scores of the PANSS scoring scales. Similarly to TRS, they were less likely to be smokers, to have lifetime cannabis abuse, and more likely to be treated with anticholinergic medication.

Table 2 presents the results of univariable analysis of potential associations between immuno-inflammatory markers and TRS and UTRS subtypes of patients as compared with the good responders' subset. We found that both TRS and UTRS individuals had significantly higher levels of IL-12/IL-23p40, IL-17A, IL-6, IFNγ, and B2M as compared with good responders alongside a trend for increased expression of TNFα, which, however, failed to reach statistical significance. Moreover, TRS individuals exhibited a higher level of IL-10. The multivariable analysis confirmed the associations of high levels of IL-12/IL-23p40, IL-17A, IL-10, and B2M with TRS (P = .0036, .0138, .1935, .0347, .0715, and .0041, respectively) and of IL-12/IL-23p40, IL-17A, IL-6, IL-10, IFNγ, and B2M

Table 1. Sociodemographic and Clinical Factors Associated With Treatment-Resistance Schizophrenia (TRS) and Ultra TRS (UTRS)

	TRS				
	No	Yes	UTRS		
	N = 156 (80%)	*N* = 39 (20%)	*N* = 15 (7.7%)	*P*-value*	*P*-value**
Gender, (%)					
Women	41 (26.3)	12 (30.8)	5 (33.3)	0.5732	0.5513
Men	115 (73.7)	27 (69.2)	10 (66.7)		
Mean age (SD)	31.5 (8.1)	29.8 (9.2)	30.9 (10.4)	0.1200	0.5440
Illness characteristics					
Age of SZ onset, mean (SD)	22.4 (6.5)	18.6 (3.3)	18.7 (3.4)	**0.0004**	**0.0236**
Duration of illness, mean (SD)	10.3 (7.8)	11.7 (8.2)	13.4 (9.4)	0.2614	0.1777
DUP (y) mean (SD)	2.04 (3.7)	1.71 (4.5)	0.35 (1.3)	**0.0450**	**0.0052**
Psychotic symptomatology, mean (SD)	61.9 (19.3)	66.9 (20.3)	88.8 (9.7)	0.1899	**3.50E-07**
Positive symptoms, mean (SD)	13.1 (5.8)	14.7 (5.6)	18.7 (6.0)	0.1360	**0.0006**
Negative symptoms, mean (SD)	16.8 (7.3)	17.5 (7.6)	24.6 (4.8)	0.6711	**7.56E-05**
General psychopathology, mean (SD)	32.2 (9.8)	34.8 (10.7)	45.5 (6.8)	0.1515	**8.14E-07**
Depressive symptoms (CDRS), mean (SD)	3.5 (4.1)	4.0 (4.1)	6.1 (5.0)	0.4921	**0.0226**
Global functioning (GAF), mean (SD)	53.1 (16.6)	48.4 (14.3)	39.4 (10.4)	0.1181	**0.0030**
History of suicide attempt, *n* (%)	24 (15.4)	10 (25.6)	6 (40.0)	0.1453	**0.0340**
Comorbidities					
Current smokers, *n* (%)	84 (53.8)	13 (33.3)	4 (26.7)	**0.0254**	0.0522
Lifetime alcohol abuse, *n* (%)	68 (43.6)	15 (38.5)	5 (33.3)	0.2175	0.2302
Lifetime cannabis abuse, *n* (%)	53 (34.0)	8 (20.5)	1 (6.7)	**0.0354**	**0.0083**
Body mass index, mean (SD)	27.3 (5.7)	28.5 (6.1)	29.4 (5.0)	0.2471	0.1804
Treatment					
Adherence to medication (MARS), mean (SD)	6.5 (2.3)	6.5 (2.1)	5.8 (2.2)	0.9907	0.2919
First antipsychotic generation, *n* (%)	47 (30.1)	7 (17.9)	4 (26.7)	0.1284	1.0000
Antidepressant, *n* (%)	56 (35.9)	9 (23.1)	4 (26.7)	0.1287	0.4743
Anticholinergic treatment, *n* (%)	22 (14.1)	11 (28.2)	6 (40.0)	**0.0357**	**0.0197**
Mood stabilizers, *n* (%)	20 (12.8)	8 (20.5)	3 (20.0)	0.2205	0.4297
Benzo & apparentés *N* (%)	47 (30.1)	13 (33.3)	6 (40.0)	0.6981	0.5593
Number of psychotropic treatment, mean (SD)	2.6 (1.6)	2.9 (1.6)	3.3 (1.9)	0.1872	0.1472

Note: SZ, schizophrenia; DUP, duration of untreated psychosis; CDRS, Calgary Depression Rating Scale; GAF, Global Assessment of Functioning Scale; MARS, Medication Adherence Rating Scale. Fisher exact test or Chi-square for categorical variables. Student *t*-test or Wilcoxon Mann-Whitney test for continuous variables. Significant *P*-value are in bold.
*Comparison between TRS vs non-TRS.
**Comparison between UTRS vs non-UTRS.

with UTRS (*P* = .0144, .0026, .0498, .0435, .0012, and .0019, respectively), in comparison to good respondents (table 3), both after adjustment for potential confounders, ie, age, gender, age of SZ onset, DUP, psychotic symptomatology, global functioning, history of suicide attempt, current smoker, lifetime cannabis disorders, first-generation antipsychotic, antidepressant medication, anticholinergic treatment, and the number of psychotropic medication. Additional adjustment for depressive symptoms and body mass index was performed for UTRS. Furthermore, cross analysis of potential interrelationships between the herein associated immuno-inflammatory markers and TRS/UTRS showed that except IL-6, which is only associated with IL-10, all other inflammatory parameters are significantly related to each other, representing a network deserving to be dissected in future studies (supplementary table 1). Finally, concerning the other studied inflammatory markers, we did not observe any significant difference between the three groups.

Overall, it is important to mention that the increase of the majority of pro-inflammatory molecules (IL-17A, IL-6, IL-10, and B2M) along with the severity status of resistance, ie, TRS vs UTRS, strongly suggests the major implication of inflammatory processes in the mechanism of resistance to antipsychotics in SZ (figure 1).

Discussion

Resistance to treatment (TRS) is a major topic in SZ as it often contributes to clinical deterioration, increased number of hospitalizations, chronicity, and poor quality of life.[21] TRS may be driven, at least in part, by deregulated immune processes reflected by fluctuating levels of inflammatory molecules.[10,12] We found that resistance and ultra resistance to CLZ treatment are associated with high level of cytokines mainly belonging to the T helper 17 (Th17) pathway, a finding suggesting that inflammatory processes might be at work especially given the interplay between

Table 2. Immuno-Inflammatory Markers Associated With Treatment-Resistant Schizophrenia (TRS) and Ultra TRS (UTRS)

	TRS				
	No	Yes	UTRS		
	N = 156 (80%)	*N* = 39 (20%)	*N* = 15 (7.7%)	*P*-value*	*P*-value**
Immuno-inflammatory markers. Median (IQR)					
IL-12/IL-23p40 pg/ml	95.6 (76.56)	123.9 (91.25)	119.2 (97.0)	**0.0032**	**0.0099**
IL-15 pg/ml	2.5 (0.87)	2.5 (0.77)	2.6 (0.66)	0.7295	0.5832
IL-16 pg/ml	266.3 (203.28)	236.9 (177.52)	230.5 (265.51)	0.9987	0.7206
IL-17A pg/ml	2.9 (4.72)	4.1 (3.81)	5.0 (3.32)	**0.0212**	**0.0107**
IL-7 pg/ml	11.2 (6.83)	12.3 (4.10)	10.9 (5.53)	0.0930	0.5609
IL-6 pg/ml	0.6 (0.53)	0.8 (0.76)	1.0 (0.55)	**0.0050**	**0.0068**
IL-8 pg/ml	9.7 (14.62)	9.8 (43.70)	16.8 (51.90)	0.1335	0.1828
IL-10 pg/ml	0.3 (0.49)	0.4 (0.51)	0.5 (0.68)	**0.0271**	0.1116
TNFβ pg/ml	0.4 (0.59)	0.4 (0.39)	0.3 (0.41)	0.5071	0.3546
TNFα pg/ml	2.5 (1.75)	2.9 (2.56)	3.3 (2.49)	0.0971	0.0560
IFNγ pg/ml	1.9 (3.73)	3.3 (6.13)	4.5 (5.13)	**0.0546**	**0.0036**
VEGF pg/ml	99.8 (93.79)	112.6 (95.50)	105.3 (177.9)	0.7740	0.1781
BAFF pg/ml	1288.9 (404.31)	1367.0 (322.10)	1323.0 (267.46)	0.1537	0.7090
BAFFR pg/ml	366.8 (1532.38)	351.0 (708.53)	519.8 (925.14)	0.6117	0.6803
B2M mg/l	1.7 (0.44)	1.9 (0.57)	2.0 (0.47)	**0.0243**	**0.0050**
HLA-E pg/ml	141.3 (158.94)	96.6 (183.87)	96.6 (315.36)	0.4534	0.7346

Note: Student *t*-test or Wilcoxon Mann-Whitney test for continuous variables.
*Comparison between TRS vs non-TRS.
**Comparison between UTRS vs non-UTRS.

Table 3. Multivariable Association Between Immune-Inflammatory Markers and Treatment-Resistant Schizophrenia (TRS)/Ultra TRS (UTRS)

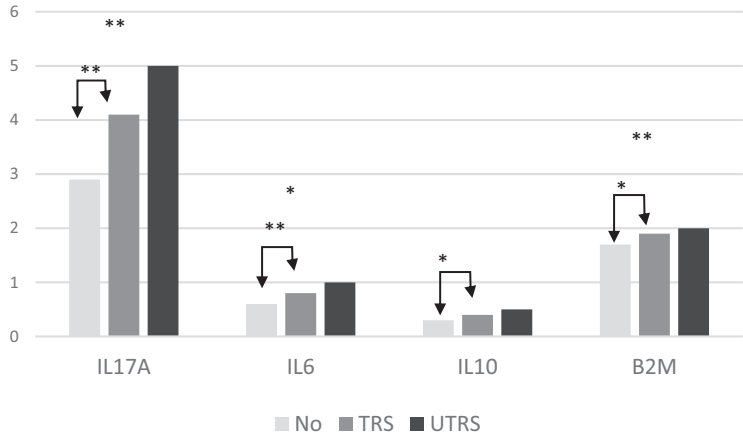
	TRS vs non-TRS			UTRS vs non-UTRS		
	F	df	*P*-value*	*F*	df	*P*-value *
IL-12/IL-23p40	8.7838	1	**0.0036**	6.1758	1	**0.0144**
IL-17A	6.2218	1	**0.0138**	9.4431	1	**0.0026**
IL-6	1.7077	1	0.1935	3.9303	1	**0.0498**
IL-10	4.5546	1	**0.0347**	4.1651	1	**0.0435**
IFNγ	3.3000	1	0.0715	11.0103	1	**0.0012**
B2M	8.5417	1	**0.0041**	10.0651	1	**0.0019**

Note: df, degree of freedom.
*Multivariable analysis of covariance.

inflammation and antipsychotic treatment responses.[22,23] In terms of immune signatures, it seems that TRS and UTRS are associated with elevated circulating levels of IL-12/IL-23p40, IL-17A, IL-10, and B2M and IL-12/IL-23p40, IL-17A, IL-6, IL-10, IFNγ, and B2M, respectively. In the two situations, the concomitant increased levels of IL-17A, IL-12/IL-23p4, and IL-6 in the UTRS group evoke the implication of the Th17 immune pathway, which makes sense both for inflammation and SZ.

Indeed, the Th17 subset produces pro-inflammatory cytokines, a property explaining their well-known link with both inflammatory/autoimmune disorders[24] and SZ.[22,23] Th17 cells predominantly produce IL-17 along with IL-22 and TNF-α. Their development and differentiation require coordinated actions of multiple factors in particular of IL-6 and IL-23 as differentiation and maintenance elements, respectively.[25] Due to its potent role in the maintenance of pathogenic Th17 cell populations, IL-23 is also recognized as one of the critical cytokines involved in autoimmune/inflammatory processes. IL-23-activated Th17 cells are known to be essential to sustain chronic inflammation in the brain during infection and auto-immunity.[26] IL-17 molecules are expressed on endothelial cells of the blood-brain barrier and can disrupt tight junction, thus impairing its integrity.[27]

Dysregulations of the Th17 pathway in psychosis have been reported by several studies although the measurement of circulating of IL-17 levels yielded discrepant results. Indeed, while high levels of IL-17 and high proportion of TH17 cells were found in drug-naïve SZ



TRS : Treatment-Resistant Schizophrenia ; UTRS : Ultra Treatment-Resistant Schizophrenia

*p value<0.05, ** p value <0.005

Fig. 1. Association between pro-inflammatory molecules (IL-17A, IL-6, IL-10, and B2M) along with the severity status of resistance. Abbreviations: TRS, treatment-resistant schizophrenia; UTRS, ultra treatment-resistant schizophrenia. **P*-value < .05, ***P*-value < .005.

patients[28,29] and in subjects at ultra-high risk of SZ,[30] diminished levels have also been observed in first-episode SZ patients[31] and in stable SZ patients.[32] Associations between elevated[33] or low IL-17 levels have been found with disease severity.[34] Finally, a recent meta-analysis of IL-17 levels in drug-naïve first-episode psychosis found no implications of IL-17 in the pathophysiology of SZ.[35] Altogether, such controversial findings might be attributable to differences in study designs, methods of cytokine measurement, and/or to patient's heterogeneity in terms of the time of analysis, disease progression, treatment, and cognitive decline.

Beside the increased level of IL-17, we also observed an upregulation of the common IL-12 and IL-23 cytokines' p40 subunit in the two resistant subgroups of individuals. IL-12 and IL-23 are two immunoregulatory cytokines mainly produced by dendritic cells and are both pivotal for anti-infectious immune responses. Both cytokines exist as heterodimers with a common p40 subunit. The p40 subunit mainly functions in conjunction with either p35 (to form IL-12) or p19 (to form IL-23) to support differentiation and maintenance of Th1 or Th17 cells, respectively.[36] While IL-12 promotes Th1 differentiation and IFN-γ production, IL-23 contributes to Th17 development and function. Increased levels of these two cytokines were repeatedly reported in various immune settings including autoimmunity. Given the high expression level of IL-17a observed here, it seems plausible that the p40 subunit likely belongs to the IL-23 cytokine. It is worthy to mention that increased levels of IL-23 have been found both in first-episode patients, in SZ relapsing patients whatever antipsychotic treatment,[37] and in patients under CLZ. However, the overexpression of INF-γ observed only in the UTRS subgroup cannot exclude a Th1 induction and hence an additional implication of IL-12 cytokine in the resistance to treatment processes. Altogether, these data suggest that the activation of IL-17 pathways is possibly present from the onset of the disorder and seems to increase with the progression of the disorder, up to resistance, and ultra resistance. This hypothesis is totally in line with our observation of an increase of the level of pro-inflammatory markers along with resistance worsening status, ie, responders, TRS, and UTRS. One may thus hypothesize that when inflammatory processes exceed interindividual homeostatic capacities, resistance to treatment appears and gradually worsens possibly explaining TRS and UTRS. One indirect example in favor of such hypothesis is the in vitro demonstrated capacity of CLZ to mediate protective effects against inflammation-induced damage of Dopaminergic neurons by preventing microglial overactivation in experimental models.[38]

In addition, we found elevated levels of IL-6 in TRS and in UTRS, which confirms converging evidence of implications of IL-6 in SZ.[39] As mentioned above, IL-6 is one of the major effector cytokines of Th17 cells. However, it is also possible that the high levels of IL-6 observed in the UTRS subgroup may indicate a skewing toward a chronicity-related Th2 pathway.

We also observed high levels of IL-10 and B2M in TRS and UTRS. The upregulation of the potent anti-inflammatory IL-10 cytokine may reflect the induction of counteractive homeostatic processes whereas that of B2M may reflect either a nonspecific hyperactivation of T cells and/or age-dependent negative regulation of cognitive and regenerative functions, processes known to be operating in murine model of SZ.[40]

Beside the discussion around the possible therapeutic strategies to be developed to target and alleviate immune-related mechanisms of resistance, it is also important to take into account the pathophysiology of SZ per se. SZ is best conceived as a

neurodevelopmental heterogeneous disorder, induced by environmental triggers, such as prenatal infections, interacting with specific immuno-genetic background.[41] Prenatal infections are known to predispose offspring to increased SZ risk[42] and might lead to the initial differentiation of Th17 cells, via the induction of IL-6 and IL-1b, inducing the upregulation of IL-23 expression.[43] In addition, genetic variations in the complement C4 cluster with consequent overexpression of the C4 molecules have been reported to be associated with SZ,[44] an important notion given the capacity of complement proteins to modulate the production of IL-17 and IL-23.

Altogether, as depicted by the abovementioned cross analysis of inflammatory markers, a global pro-inflammatory pathway seems to underlie both SZ and resistance to treatment but distinguishing which is the egg or the chicken remains to be elucidated. One may hypothesize that such global pathway induces a vicious circle of inflammation generating a progressively worsened resistance to treatment. Finally, even if the studied patients beneficiate from a yearly psychiatric and somatic examination, we cannot exclude that an underlying infra-clinic immune-related condition may sustain inflammatory processes, hence contributing to the immune profiles observed herein and consequently to resistance to antipsychotic treatment. Thus, personalized use of anti-inflammatory compounds may disrupt such self-sustaining processes.

Future longitudinal studies are warranted to follow IL-17-related changes according to treatment responses as well as to stage progression and handicap along with cognitive decline aggravation. If confirmed, our findings may allow us to consider IL-23/IL-17 pathway as a therapeutic target for patients with resistance against antipsychotics especially as many anti-inflammatory drugs have been proposed as an add-on to treat SZ symptoms such as *N*-Acetylcysteine, which seem to reduce the production of IL-17. Future clinical trials targeting this pathway are also warranted to monitor its effects on resistance processes by themselves. Some limitations in our study should be noted: due to the cross-sectional nature of the study, we were unable to draw any firm conclusions concerning the causal nature of the associations observed. Longitudinal studies would be required for this purpose. Furthermore, our study was relatively small and was probably not representative of all patients with SZ, particularly because institutionalized and hospitalized patients were not referred to the participating expert centers.

Conclusions

The IL-23/Th17 axis seems to play a prominent role in the pathophysiology of SZ, as in several chronic inflammatory disorders, including psoriasis,[45] multiple sclerosis, and rheumatoid arthritis,[46] where the upregulation of this pathway induces a pro-inflammatory status. Herein, we have found that patients with resistant or ultra-resistant SZ show an upregulation of this pathway.

Supplementary Material

Supplementary data are available at *Schizophrenia Bulletin Open* online.

Supplementary Table 1. Correlation Between Immune-Inflammatory Markers

Funding

This work was funded by Assistance Publique des Hôpitaux de Paris, Fondation FondaMental (RTRS Santé Mentale); by the "Investissements d′Avenir" programs managed by the Agence Nationale de Recherche (ANR), France, under references ANR-11-IDEX-0004-02 and ANR-10-COHO-10-01; by Era Per Med program; by Institut National de la Santé et de la Recherche Médicale; and by International Foundation.

Acknowledgments

We express all our thanks to the nurses and to the patients who were included in the present study. We would also like to thank Hakim Laouamri and his team for the development of the FACE-SZ computer interface, data management, quality control, and regulatory aspects. The authors have declared that there are no conflicts of interest in relation to the subject of this study.

List of FondaMental Advanced Center of Expertise (FACE-SZ) collaborators:

FACE-SZ Clinical Coordinating Center (Fondation FondaMental); F. Berna, E. Haffen, M. Leboyer, P. M. Llorca, and F. Schürhoff;

FACE-SZ Data Coordinating Center (Fondation FondaMental); V. Barteau, S. Bensalem, O. Godin, H. Laouamri, and K. Souryis;

FACE-SZ Clinical Sites and Principal Collaborators in France; AP-HP, INSERM U955, Translational Psychiatry Team, DHU Pe-PSY, Centre Expert Schizophrénie, Pôle de Psychiatrie et d′Addictologie des Hôpitaux Universitaires Henri Mondor, Paris Est University, 40 rue de Mesly, 94000 Créteil, France: M. Leboyer, I. Offerlin-Meyer, B. Pignon, F. Schürhoff, and A. Szöke; Department of Adult Psychiatry, Charles Perrens Hospital, F-33076 Bordeaux, France; Laboratory of Nutrition and Integrative Neurobiology (UMR INRA 1286), University of Bordeaux, France: B. Aouizerate; Department of Adult Psychiatry, Charles Perrens Hospital, F-33076 Bordeaux; University of Bordeaux, CNRS UMR 5287-INCIA, Bordeaux, France: A. Deloge, D. Misdrahi, and E. Vilà; CHU Clermont-Ferrand, Department of Psychiatry (service de psychatrie

B), University of Clermont Auvergne, Clermont-Ferrand, France: O. Blanc, I. Chéreau, H. Denizot, RM. Honciuc, D. Lacelle, PM. Llorca, and S. Pires; AP-HP, Department of Psychiatry, Louis Mourier Hospital, Colombes, Inserm UMR1266, Institute of Psychiatry and Neurosciences of Paris, University Paris Descartes, Université Paris Diderot, Sorbonne Paris Cité, Faculté de médecine, France: C. Dubertret, J. Mallet, and C. Portalier; Psychosocial Rehabilitation Reference Center, Alpes Isère Hospital, Grenoble, France: J. Dubreucq, C. Fluttaz, F. Gabayet, and C. Roman; University Claude Bernard Lyon 1, Le Vinatier Hospital Pole Est BP 300 39 – 95 bd Pinel – 69678 Bron Cedex, France: G. Chesnoy-Servanin, T. D'Amato, JM. Dorey, R. Rey, and A. Vehier; Department of Psychiatry (AP-HM), Sainte-Marguerite University Hospital, Marseille, France: C. Lançon, C. Faget, E. Metairie, P. Peri, and F. Vaillant; AP-HM, la Conception Hospital, Aix-Marseille Univ, School of medicine – La Timone Medical Campus, EA 3279: CEReSS – Health Service Research: L. Boyer and G. Fond; Strasbourg University Hospital, University of Strasbourg, INSERM U1114, Federation of Translational Psychiatry, Strasbourg, France: F. Berna, P. Vidailhet, and A. Zinetti-Bertschy; University Department of Adult Psychiatry, La Colombiere Hospital, CHU Montpellier, University of Montpellier 1, Inserm 1061, Montpellier, France: D. Capdevielle and H. Yazbek; Department of Adult Psychiatry, Versailles Hospital, Le Chesnay, France; HandiRESP and Quality of Life Center, 27 Boulevard Jean Moulin, 13005 Marseille, France Laboratory, EA4047, UFR Health Sciences Simone Veil, Université de Versailles Saint-Quentin-En-Yvelines, Montigny-le-Bretonneux, France: S. Esselin, M. Jarroir, C. Passerieux, and M. Urbach.

References

1. Howes OD, McCutcheon R, Agid O, *et al.* Treatment-resistant schizophrenia: Treatment Response and Resistance in Psychosis (TRRIP) working group consensus guidelines on diagnosis and terminology. *Am J Psychiatry.* 2017;174(3):216–229.
2. Kuipers E, Yesufu-Udechuku A, Taylor C, Kendall T. Management of psychosis and schizophrenia in adults: summary of updated NICE guidance. *BMJ.* 2014;348:g1173.
3. Kennedy JL, Altar CA, Taylor DL, Degtiar I, Hornberger JC. The social and economic burden of treatment-resistant schizophrenia: a systematic literature review. *Int Clin Psychopharmacol.* 2014;29(2):63–76.
4. Howes OD, Vergunst F, Gee S, McGuire P, Kapur S, Taylor D. Adherence to treatment guidelines in clinical practice: study of antipsychotic treatment prior to clozapine initiation. *Br J Psychiatry.* 2012;201(6):481–485.
5. Yoshimura B, Yada Y, So R, Takaki M, Yamada N. The critical treatment window of clozapine in treatment-resistant schizophrenia: secondary analysis of an observational study. *Psychiatry Res.* 2017;250:65–70.
6. Lally J, MacCabe JH. Antipsychotic medication in schizophrenia: a review. *Br Med Bull.* 2015;114(1):169–179.
7. Farooq S, Agid O, Foussias G, Remington G. Using treatment response to subtype schizophrenia: proposal for a new paradigm in classification. *Schizophr Bull.* 2013;39(6):1169–1172.
8. Potkin SG, Kane JM, Correll CU, *et al.* The neurobiology of treatment-resistant schizophrenia: paths to antipsychotic resistance and a roadmap for future research. *NPJ Schizophr.* 2020;6(1):1.
9. Khandaker GM, Dantzer R, Jones PB. Immunopsychiatry: important facts. *Psychol Med.* 2017;47(13):2229–2237.
10. Noto C, Maes M, Ota VK, *et al.* High predictive value of immune-inflammatory biomarkers for schizophrenia diagnosis and association with treatment resistance. *World J Biol Psychiatry.* 2015;16(6):422–429.
11. Mondelli V, Ciufolini S, Belvederi Murri M, *et al.* Cortisol and inflammatory biomarkers predict poor treatment response in first episode psychosis. *Schizophr Bull.* 2015;41(5):1162–1170.
12. Fond G, Godin O, Boyer L, *et al.* Chronic low-grade peripheral inflammation is associated with ultra resistant schizophrenia. Results from the FACE-SZ cohort. *Eur Arch Psychiatry Clin Neurosci.* 2019;269(8):985–992.
13. Horsdal HT, Wimberley T, Benros ME, Gasse C. C-reactive protein levels and treatment resistance in schizophrenia – a Danish population-based cohort study. *Hum Psychopharmacol.* 2017;32(6).
14. Strawbridge R, Arnone D, Danese A, Papadopoulos A, Herane Vives A, Cleare AJ. Inflammation and clinical response to treatment in depression: a meta-analysis. *Eur Neuropsychopharmacol.* 2015;25(10):1532–1543.
15. Schürhoff F, Fond G, Berna F, *et al.*; FondaMental Academic Centers of Expertise for Schizophrenia (FACE-SZ) collaborators. A National network of schizophrenia expert centres: an innovative tool to bridge the research-practice gap. *Eur Psychiatry.* 2015;30(6):728–735.
16. First M. *Structured Clinical Interview for the DSM-IV Axis I Disorders.* American Psychiatric Association. Published online 1996. http://www.ncbi.nlm.nih.gov/pubmed/?term=First+AND+1996. Accessed November 21, 2013.
17. Kay SR, Fiszbein A, Opler LA. The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. *Schizophr Bull.* 1987;13(2):261–276.
18. Bernard D, Lançon C, Auquier P, Reine G, Addington D. Calgary Depression Scale for Schizophrenia: a study of the validity of a French-language version in a population of schizophrenic patients. *Acta Psychiatr Scand.* 1998;97(1):36–41.
19. Startup M, Jackson MC, Bendix S. The concurrent validity of the Global Assessment of Functioning (GAF). *Br J Clin Psychol.* 2002;41(Pt 4):417–422.
20. Misdrahi D, Verdoux H, Llorca PM, Baylé FJ. [Therapeutic adherence and schizophrenia: the interest of the validation of the French translation of Medication Adherence Rating Scale (MARS)]. *Encephale.* 2004;30(4):409–410.
21. Iasevoli F, Giordano S, Balletta R, *et al.* Treatment resistant schizophrenia is associated with the worst community functioning among severely-ill highly-disabling psychiatric conditions and is the most relevant predictor of poorer achievements in functional milestones. *Prog Neuropsychopharmacol Biol Psychiatry.* 2016;65:34–48.
22. Debnath M, Berk M. Th17 pathway-mediated immunopathogenesis of schizophrenia: mechanisms and implications. *Schizophr Bull.* 2014;40(6):1412–1421.

23. Debnath M, Berk M. Functional implications of the IL-23/IL-17 immune axis in schizophrenia. *Mol Neurobiol.* 2017;54(10):8170–8178.
24. Louten J, Boniface K, de Waal Malefyt R. Development and function of TH17 cells in health and disease. *J Allergy Clin Immunol.* 2009;123(5):1004–1011.
25. Iwakura Y, Ishigame H. The IL-23/IL-17 axis in inflammation. *J Clin Invest.* 2006;116(5):1218–1222.
26. Langrish CL, Chen Y, Blumenschein WM, *et al.* IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med.* 2005;201(2):233–240.
27. Huppert J, Closhen D, Croxford A, *et al.* Cellular mechanisms of IL-17-induced blood-brain barrier disruption. *FASEB J.* 2010;24(4):1023–1034.
28. Ding M, Song X, Zhao J, *et al.* Activation of Th17 cells in drug naïve, first episode schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry.* 2014;51:78–82.
29. El Kissi Y, Samoud S, Mtiraoui A, *et al.* Increased interleukin-17 and decreased BAFF serum levels in drug-free acute schizophrenia. *Psychiatry Res.* 2015;225(1-2):58–63.
30. Zeni-Graiff M, Rizzo LB, Mansur RB, *et al.* Peripheral immuno-inflammatory abnormalities in ultra-high risk of developing psychosis. *Schizophr Res.* 2016;176(2-3):191–195.
31. Borovcanin M, Jovanovic I, Radosavljevic G, *et al.* Elevated serum level of type-2 cytokine and low IL-17 in first episode psychosis and schizophrenia in relapse. *J Psychiatr Res.* 2012;46(11):1421–1426.
32. Subbanna M, Shivakumar V, Talukdar PM, *et al.* Role of IL-6/RORC/IL-22 axis in driving Th17 pathway mediated immunopathogenesis of schizophrenia. *Cytokine.* 2018;111:112–118.
33. Chenniappan R, Nandeesha H, Kattimani S, Nanjaiah ND. Interleukin-17 and interleukin-10 association with disease progression in schizophrenia. *Ann Neurosci.* 2020;27(1):24–28.
34. Dimitrov DH, Lee S, Yantis J, *et al.* Differential correlations between inflammatory cytokines and psychopathology in veterans with schizophrenia: potential role for IL-17 pathway. *Schizophr Res.* 2013;151(1-3):29–35.
35. Fang X, Zhang Y, Fan W, Tang W, Zhang C. Interleukin-17 alteration in first-episode psychosis: a meta-analysis. *Mol Neuropsychiatry.* 2018;3(3):135–140.
36. Kastelein RA, Hunter CA, Cua DJ. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu Rev Immunol.* 2007;25:221–242.
37. Borovcanin M, Jovanovic I, Dejanovic SD, Radosavljevic G, Arsenijevic N, Lukic ML. Increase systemic levels of IL-23 as a possible constitutive marker in schizophrenia. *Psychoneuroendocrinology.* 2015;56:143–147.
38. Hu X, Zhou H, Zhang D, *et al.* Clozapine protects dopaminergic neurons from inflammation-induced damage by inhibiting microglial overactivation. *J Neuroimmune Pharmacol.* 2012;7(1):187–201.
39. Frydecka D, Misiak B, Pawlak-Adamska E, *et al.* Interleukin-6: the missing element of the neurocognitive deterioration in schizophrenia? The focus on genetic underpinnings, cognitive impairment and clinical manifestation. *Eur Arch Psychiatry Clin Neurosci.* 2015;265(6):449–459.
40. Smith LK, He Y, Park JS, *et al.* β2-Microglobulin is a systemic pro-aging factor that impairs cognitive function and neurogenesis. *Nat Med.* 2015;21(8):932–937.
41. International Schizophrenia Consortium, Purcell SM, Wray NR, *et al.* Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature.* 2009;460(7256):748–752.
42. Zimmer A, Youngblood A, Adnane A, Miller BJ, Goldsmith DR. Prenatal exposure to viral infection and neuropsychiatric disorders in offspring: a review of the literature and recommendations for the COVID-19 pandemic. *Brain Behav Immun.* 2021;91:756–770.
43. McGeachy MJ, Cua DJ. The link between IL-23 and Th17 cell-mediated immune pathologies. *Semin Immunol.* 2007;19(6):372–376.
44. Sekar A, Bialas AR, de Rivera H, *et al.*; Schizophrenia Working Group of the Psychiatric Genomics Consortium. Schizophrenia risk from complex variation of complement component 4. *Nature.* 2016;530(7589):177–183.
45. Egeberg A, Gisondi P, Carrascosa JM, Warren RB, Mrowietz U. The role of the interleukin-23/Th17 pathway in cardiometabolic comorbidity associated with psoriasis. *J Eur Acad Dermatol Venereol.* 2020;34(8):1695–1706.
46. Korn T, Anderson AC, Bettelli E, Oukka M. The dynamics of effector T cells and Foxp3^{+} regulatory T cells in the promotion and regulation of autoimmune encephalomyelitis. *J Neuroimmunol.* 2007;191(1-2):51–60.