Published in final edited form as:

Schizophr Res. 2017 November ; 189: 217–218. doi:10.1016/j.schres.2017.02.005.

Validation of schizophrenia gene expression profile in a preclinical model of maternal infection during pregnancy

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Animal models of schizophrenia constitute at this moment fundamental experimentation tools to further explore on the etiological basis of this pathology, leading to the discovery of new biological targets and innovative therapeutic strategies for its treatment. Any improvement in the translation and validation of biological data between preclinical and clinical models will therefore represent a substantial advance in the research of this mental disease. Recent data from our group obtained by next-generation sequencing from blood

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Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.schres.2017.02.005>.

Conflict of interest

The authors declare no conflict of interest.

transcriptome samples identified, among 22,278 genes analyzed, differential expression of 200 genes in schizophrenia patients related to biological processes associated with immunological responses (Sainz et al., 2013). Further investigations conducted by the same experimental approach and focused on 6 particular genes selected from those 200 previously detected, *i.e.*, *ADAMTS2*, *CD177*, *CNTNAP3*, *ENTPD2*, *RFX2* and *UNC45B*, revealed an overexpression of these particular genes in drug-naïve patients that was reverted to the control expression levels after antipsychotic treatment (Crespo-Facorro et al., 2014). Pre-clinical models of schizophrenia present important limitations due to the complexity of emulating and/or translating a human mental disease into a particular animal phenotype. We have extensively characterized a neurochemical alteration as a possible molecular explanation for some of the symptoms associated with schizophrenia. This consists in the dysregulation of the elements that constitute the heteroreceptor complex formed by 5-HT_{2A} serotonin and mGlu2 glutamate receptors (Gonzalez-Maeso et al., 2008). Particularly, the up-regulation of 5-HT_{2A} accompanied by the down-regulation of mGlu2 receptors results in a final signaling balance at the cellular level that predispose mice to a “psychotic” phenotype according to behavioral experimental models (Fribourg et al., 2011). This behavioral phenotype resides essentially in an increase in the head-twitch response evoked by hallucinogenic serotonergic agonists accompanied by locomotor hyperactivity induced by dissociative glutamate antagonists. Additionally, we have also described in mouse models of both maternal influenza viral infection (Moreno et al., 2011) and maternal variable stress during pregnancy (Holloway et al., 2013) that descendants presented neurochemical and behavioral changes in line with the dysregulation of the expression level of 5-HT_{2A}-mGlu2 receptor heteromer causing schizophrenia-like alterations in mice only in post-pubertal ages. These models of alterations during pregnancy are useful to explore those hypotheses maintaining that there are environmental events occurring during pre- or perinatal phases of development determinant for the onset of clinical symptoms (Rapoport et al., 2005). One of the hypothesis supporting this concept proposes that exposure to adverse factors during gestation such as certain infections, maternal stress, malnutrition or maternal medical complications would predispose individuals to a latent immune vulnerability that will be manifested at puberty or early adulthood by affecting different processes in the central nervous system (Kinney et al., 2010).

In order to preclinically validate our previous data obtained from blood human samples, we proceeded to evaluate the expression of the orthologous version of these genes in adult mice born to mothers submitted to influenza viral infection during gestation. Pregnant CD1 mice were subjected to influenza viral infection according to the protocol previously described (Moreno et al., 2011) and adult offspring were assessed for behavioral and neurochemical traits corresponding to schizophrenia-like phenotype, essentially increased head-twitch response elicited by treatment with the hallucinogenic drug DOI and changes in the relative 5-HT_{2A} and mGlu2 receptor expression levels detected by *ex vivo* radioligand binding assays. A group of eight animals that statistically presented these alterations were pair matched with control mice. In both experimental groups, we tested animals originating from at least two different litters. Tissue samples from mouse frontal cortex were obtained and mRNA was extracted to conduct subsequent quantitative real-time PCR (qRT-PCR) experiments as previously described with minor modifications (Gonzalez-Maeso et al.,

2003) (primer sequences are included in Supplemental material 1). qRT-PCR results demonstrated, first, that these six genes are expressed in cortical tissue from mouse brain. Second and more importantly, statistical analysis comparing control and influenza-infected descendant mice (Fig. 1), performed by correcting a value as described before (Kurita et al., 2012), revealed a significant overexpression of at least three of the six total genes considered in this study, in particular *CD177*, *CNTNAP3* and *ENTPD2* ($t_{14} = 3.85$, $P = 0.0018$; $t_{14} = 3.31$, $P = 0.0052$; $t_{14} = 3.19$, $P = 0.0065$ respectively. Significance at $P < 0.01$). Other two genes such as *ADAMTS2*, and *RFX2* although not reaching statistical significance also presented a tendency to be overexpressed when compared with control values.

In conclusion, these data represent one of the first preclinical validations of alterations in gene expression previously observed in clinical samples. This set of genes, whose expression is regulated in blood samples by exposure to antipsychotic drugs in schizophrenia patients and dysregulated in the frontal cortex by prenatal insults in mouse models, might provide new and interesting information about mechanisms of effective action of antipsychotics. These findings may ultimately lead to the identification of new therapeutic targets for treatment of schizophrenia and other neurodevelopmental psychiatric conditions.

Acknowledgments

This study was funded by NIH R01 MH084894 and R01 MH111940 grants and SAF2010-20840-C02-01/02 and SAF2013-46292-R from MINECO (Spain).

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Fig. 1.

Expression of ADAMTS2, CD177, CNTNAP3, ENTPD2, RFX2 and UNC45B genes in frontal cortex of adult mice born to influenza virus-infected mothers (filled bars) and controls (opened bars) determined by qRT-PCR assays ($n = 8$ animals per group). Data are mean $\pm$ SEM (** $p < 0.01$). Student's t -test.