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Compound heterozygosity for a hemizygous rare missense variant (rs141999351) and a large CNV deletion affecting the *FSTL5* gene in a patient with schizophrenia

Reports of associations between some large and rare copy number variations (CNVs) and schizophrenia have considerably enriched the debate on the genetics of schizophrenia. Although CNVs represent plausible risk factors, their presence in unaffected individuals is likely to implicate the involvement of additional genetics and/or environmental factors in the development of schizophrenia, as exemplified by the double-hit hypothesis. In the case of CNV deletions, the second hit might be, for instance, a point mutation unmasking a recessive allele.

This last possibility is sustained by data of a 58-year-old man with a DSM-IV-TR diagnosis of paranoid schizophrenia and no evidence of Axis I or II disorders. The patient was identified as a carrier of a large deletion during the CNV screening of a cohort of Italian schizophrenia patients using the SNP-array technology (Magri et al., 2010). Briefly, genotyping was achieved with the Affymetrix Human Mapping GeneChip 6.0 arrays, intensity data were analyzed using the BRLMM-P Plus algorithm and CNVs identified using the segmentation algorithm implemented in the Affymetrix Genotype Console (GTC) 3.0.1 (for more detailed information, see Magri et al. (2010)). This analysis identified in the patient an about 2,300 kb deletion on chromosome 4q32.1-32.2 (chr4:160,666,009-162,968,318 hg19 assembly). The deletion encompasses the *FSTL5* (follistatin-like 5) gene, extending from intron 2 to approximately 1,640 kb downstream the 3' end of the gene and removing nearly all the coding sequence. As a result of this finding, we planned a mutational analysis of the *FSTL5* gene in the patient. The Sanger sequencing of the 15 coding exons of the gene and their intronic flanking regions revealed a T > C transition at position 1358 of the cDNA (c.1358T > C NM_020116; chr4:162,421,268A > G hg19 assembly), resulting in a methionine to threonine substitution at position 453 (p.Met453Thr) of the amino acid sequence (NP_064501). This substitution was predicted to be "damaging" by SIFT (http://sift.jcvi.org) and "disease-causing" by MutationTaster (http://www.mutationtaster.org) servers.

We then screened 164 patients with schizophrenia and 246 control subjects for the c.1358T > C transition. The mutation was found in heterozygosity in a second patient and in one control. No CNVs in the *FSTL5* gene, including small deletions/insertions, or other pathologic CNVs were detectable by Affymetrix array analysis in both these subjects; moreover, *FSTL5* sequencing did not evidence any potentially damaging point mutation, in addition to the c.1358T > C missense mutation.

The c.1358T > C transition leading to the p.Met453Thr substitution is reported in the dbSNP database (http://www.ncbi.nlm.nih.gov/snp/) as rs141999351 single nucleotide variant (SNV), with a global minor allele frequency of 0.0006. None homozygous genotype for this variant is present in the 1000 Human Genome (Phase 3 release, n=2504 individuals; http://browser.1000genomes.org) as well as in the Exome Variant Server (release ESP6500SI-V2, n=6503 individuals; http://evs.gs.washington.edu/EVS/) databases.

Given its rarity, the identification of the rs141999351 missense variant in a patient who is *de facto* hemizygous for the *FSTL5* gene suggests that it could contribute to the development of the disorder, acting through a recessive effect.

The *FSTL5* gene is one of the five members (*FSTL1–5*) of the follistatin-like gene family, whose common feature is to encode for secreted extracellular glycoproteins with partial homology to follistatin, a well-known modulator of activin and other transforming growth factor-β (TGF-β) superfamily members (Welt et al., 2002). So far, the functional role of *FSTL5* has been poorly characterized. The high level of expression in the spinal cord tissue of early embryonic mice suggested its role in the development of the neurodorsal spinal cord and/or in the axonal guidance of dorsal root ganglion (Masuda et al., 2009). However, as retrieved from the Atlas database (http://www.ebi.ac.uk/gxa), *FSTL5* expression is not limited to the spinal cord and it has been observed in different areas of the developing and adult central nervous system. These findings indicate that *FSTL5* plays relevant roles in the organization and development of the brain, likely modulating the activity of TGF-β superfamily members. Interestingly, a dysregulation in TGF-β signaling pathway has been repetitively observed in schizophrenia. In particular, postmortem studies have shown an up-regulation of the TGF-β signaling cascade in the hippocampus (Benes et al., 2007) and in the pyramidal neurons of schizophrenia subjects (Pietersen et al., 2014).

In conclusion, although functional studies would be required, the co-occurrence in the same patient with schizophrenia of two rare alterations in the *FSTL5* gene (the rs141999351/p.Met453Thr missense variant and a CNV deleting almost the whole gene) suggests that these two mutational events may be relevant for the development of the disorder and indicates a possible role of *FSTL5* in the development of schizophrenia.

Conflict of interest

The authors declare no conflicts of interest.

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