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The impairment of GABAergic pathway as one of the driver forces in the etiopathogenesis of schizophrenia: evidence from functional studies and gene-set enrichment analyses

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Recent genetic findings on schizophrenia (SZ) point to the disruption of excitatory glutamatergic signaling and of inhibitory GABAergic modulation as one route to pathogenesis of SZ. Recently, whole exome sequencing of patients affected by SZ and with high levels of autozygosity allowed us to identify some ultra-rare homozygous mutations. Among these, there was a novel missense substitution (c.391A>G), mapping in the glutamate acid decarboxylase 1 (GAD1) gene (NM_000817). GAD1 encodes for GAD67 enzyme, that catalyzes the production of gamma-aminobutyric acid (GABA) from L-glutamic acid. To clarify the role of ultra-rare homozygous variants in GABAergic genes as risk factors for SZ, we performed a genetic study of the c.391A>G mutation. Moreover, we looked for the enrichment of ultra-rare homozygous variant in 124 GABAergic genes in a cohort of 4,969 cases and 6,245 controls of the Psychiatric Genomics Consortium (DbGaP: phs000473.v1.p2).

Pedigree analysis revealed that the c.391A>G mutation segregated with SZ phenotype accordingly to a recessive model of inheritance. Biochemical assays revealed that the amino acid substitution induced by the mutation reduced GAD67 enzymatic activity by ~30%, while western blot and proximity ligation assays (PLAs) suggested that this impairment was due to a reduced homodimerization of GAD67, rather than to a reduced protein level. Interestingly, PLAs highlighted that the mutation impairs homodimerization only when present in a homozygous state. This corroborates the recessive effect suggested by the pedigree analysis.

The screening of 4,969 SZ patients did not highlight any other homozygous or compound heterozygous subject for ultra-rare variants in GAD1. However, when the analysis was extended to genes implicated in the GABAergic pathway, the frequency of patients with ultra-rare homozygous mutations (0.1%) was significant higher than in controls (0.02%), (p-value = 0.033).

In conclusion, GABAergic signalling deficit in SZ has always been considered an adaptive response to a broad range of biological and environmental factors. This study suggests that the impairment of GABAergic system could be a driver event due to ultra-rare homozygous mutations, adding new insights on the role of GABAergic pathway in SZ.