Author’s response to reviews

Title: Schizophrenia susceptibility and NMDA-receptor mediated signalling: an association study involving 47 tagSNPs of DAO, DAOA, PPP3CC, and DTNBP1 genes

Authors:

Emilio Sacchetti (genetica@fatebenefratelli.it)
Catia Scassellati (cscassellati@fatebenefratelli.it)
Alessandra Minelli (alessandra.minelli@med.unibs.it)
Paolo Valsecchi (labgen@fatebenefratelli.it)
Cristian Bonvicini (cbonvicini@fatebenefratelli.it)
Patrizio Pasqualetti (patrizio.pasqualetti@afar.it)
Alessandro Galluzzo (scat_08@hotmail.com)
Rosaria Pioli (rpioli@fatebenefratelli.it)
Massimo Gennarelli (gennarelli@fatebenefratelli.it)

Version: 2 Date: 26 May 2012

Author’s response to reviews: see over
Dan Rujescu,
University of Munich LMU
Editor of BMC Medical Genetics

Dear Dan Rujescu,

Appended please find our manuscript entitled “Schizophrenia susceptibility and NMDA-receptor mediated signaling: an association study involving 47 tagSNPs of DAO, DAOA, PPP3CC, and DTNBP1 genes” by Emilio Sacchetti*, Catia Scassellati*, Alessandra Minelli, Paolo Valsecchi, Cristian Bonvicini, Patrizio Pasqualetti, Alessandro Galluzzo, Rosaria Pioli, Massimo Gennarelli.

In the revision of this version of the manuscript, it has been added, as suggested, the description of the enrolment and diagnosis of the patients in the study, and that the study had received appropriate ethical approval and the subjects had given written informed consent. This has been inserted in the Methods section (pages 7-8; line 8) in italics.

Recent studies supported associations between four NMDA-receptor-mediated signalling genes (DAO, DAOA, PPP3CC and DTNBP1) and schizophrenia susceptibility, even though with contrasting results.

In an attempt to replicate these findings for the first time in Italian population, a panel of 47 tagSNPs was analysed in the more representative case-control sample of 879 subjects. The primary analyses did not demonstrate associations between schizophrenia and any of the studied DAOA, DAO, PPP3CC, or DTNBP1 single SNPs. A haplotype analysis revealed an association in the allele frequency for the estimated PPP3CC CAG triplotype in the SNP window rs4872499 T/C-rs11780915 A/G-rs13271367 G/A (pcorrect=0.001). Similarly, the clustered genotype frequencies of the estimated/phased CAG triplotype differed between cases and controls (p=0.004), with the carriers having a higher frequency in the control population (p=0.002).

For all further secondary analyses, the patient samples were divided into more homogeneous subgroups according to sex, diagnostic subtypes and age at onset. In relation to gender, the analysis of single SNPs evidenced a protective effect in males of rs11780915 and rs13271367 in PPP3CC gene (pcorrect=0.02 and pcorrect=0.04 respectively). Moreover the estimated/phased GT diplotype (rs2070586A/G-rs3741775G/T) carriers of the DAO gene were more highly represented in female controls (p=0.017), as were the estimated/phased CAG triplotype carriers of the PPP3CC gene in females (p=0.01). In addition, we performed an interaction analysis, and a 66% (p=0.003) lower risk of developing schizophrenia for female (CAG+GT) carriers versus non-CAG or -GT carriers was observed. For DTNBP1, we found a protective effect in males for the rs6459409 (p=0.02) and the estimated/phased CT diplotype (rs6459409-rs9476886) carriers (p=3x10^{-4}). In relation to diagnostic subtypes, the estimated/phased DAO GT diplotype and PPP3CC CAG triplotype female carriers were found to show RRR values of 0.52 and 0.54 lower risk for a paranoid phenotype respectively.

Finally, no associations were observed after the evaluation of the age of onset for any of the genes investigated.

Thus DAO, PPP3CC, and DTNBP1 genes, might be differentially involved in schizophrenia susceptibility according mainly to gender and gene interaction mechanisms. The present results underline the need for more systematic use of the phenotype “dissection” strategy and the search for interaction effects to strengthen the informative power of genetic association studies.
We would greatly appreciate it if you could consider this manuscript for publication as research article in **BMC Medical Genetics**.

All authors declare that the material is original research, has not been previously published and has not been submitted for publication elsewhere while under consideration.

Thanking you in advance for your kind attention,

Yours sincerely

Massimo Gennarelli:
Genetic Unit, I.R.C.C.S. “San Giovanni di Dio” – Fatebenefratelli, Via Pilastroni, 4 – 25125 Brescia, Italy. Tel.: +39 030 3501453; Fax: +39 030 3533513 E-mail address: gennarelli@fatebenefratelli.it