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# Dual contribution of *NR2B* subunit of NMDA receptor and *SK3* $Ca^{2+}$-activated $K^+$ channel to genetic predisposition to anorexia nervosa

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**Abstract**

Since identification of the genetic component in anorexia nervosa (AN), genes that partake in serotonergic and dopaminergic systems and in hormonal and weight regulation have been suggested as potential candidates for AN susceptibility. We propose another set of candidate genes. Those are genes that are involved in the signaling pathway using NMDA-R and SK channels and have been suggested as possible effectors of NMDA-R driven signaling. The role of NMDA-R in the etiology of schizophrenia has already been substantiated on various levels. Several studies based on population and family groups have implicated SK3 in schizophrenia and more recently in AN as well. Our study group consisted of 90 AN family trios. We examined the transmission of two potentially functional polymorphisms, 5073T>G polymorphism in the gene encoding the NR2B subunit of NMDA-R and CAG repeats in the coding region of *SK3* channel gene. Using HHRR and TDT approaches, we found that both polymorphisms were preferentially transmitted to AN offspring (TDT yielded $\chi^2 = 5.01$, $p = 0.025$ for *NR2B* 5073G alleles and $\chi^2 = 11.75$, $p < 0.001$ for *SK3* L alleles including >19 repeats). Distribution analysis of the combined *NR2B*/*SK3* genotypes suggests that the contribution of both polymorphisms to AN risk is independent and cumulative (OR = 2.44 for *NR2B* GG genotype and OR = 3.01 for *SK3* SL and LL genotypes, and OR = 6.8 for the combined *NR2B*/*SK3* genotypes including high-risk alleles). These findings point to the contribution of genes associated with the NMDA-R signaling pathway to predisposition and development of AN.


*Keywords:* Anorexia nervosa; *N*-Methyl-D-aspartate glutamate receptor; NR2B (GRIN2B); SK3 (hSKCa3, KCNN3); Haplotype-based relative risk; Transmission disequilibrium test

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## 1. Introduction

Anorexia nervosa (AN) is a disorder of complex etiology in which inherent as well as external factors may play a role in AN susceptibility. Contribution of the genetic factors to AN risk is estimated at 58% at least and some studies estimates are even as high as 88% [reviewed in Bulik et al. (2000)]. However, little is known about the identity of specific genes and their relative contribution. Moreover, few genetic factors have been found to be specific to AN and no single factor has been shown to be necessary or sufficient to express the phenotype. The present study is based on the notion that gene products that partake in the signaling pathway involving the *N*-methyl-D-aspartate type ionotropic glutamate receptor (NMDA-R) are potential substrates of AN pathophysiology. Molecular understanding of the role of NMDA-R signaling in cognitive and mental functions materialized from numerous studies that demonstrated that NMDA-R is critical in processes of neuronal migration and synaptogenesis during pre- and post-natal brain development (Malenka and Nicoll, 1993; Rakic et al., 1994; Wu et al., 1996), as well as from findings of behavioral and cognitive deficits associated with NMDA-R hypofunction produced by pharmacologic (Javitt and Zukin, 1991; Malhotra et al., 1997) and genetic manipulations (Mohn et al., 1999; Miyamoto et al., 2001). Subunit composition is an important determinant of NMDA-Rs electrophysiological, biochemical and functional properties (Sucher et al., 1996), therefore we focused on NR2B (GRIN2B) containing NMDA-Rs which were associated with age-dependent neuronal plasticity and memory formation (Tang et al., 1999), and were also associated with feeding behavior and related physiological functions (Stanley et al., 1996, 1997; Khan et al., 1999), which makes them relevant to this study. Furthermore, since manifestation of AN could be related to the presence of polymorphisms in the *NR2B* gene, we studied a distinctive group of 90 AN family trios using haplotype-based haplotype relative-risk (HHRR) and transmission disequilibrium (TDT) approaches. Within this framework, we examined the distribution of 5073T>G single-nucleotide polymorphism (SNP) (rs890) localized in the untranslated exon 13 of the *NR2B* gene at 12p13.1. Although functional implications of rs890 are still obscure and have not been clearly demonstrated, it is the current view that the 3′ untranslated gene region (3′UTR) may contain elements that play a role in post-transcriptional regulation of gene expression and translation (Wilkie et al., 2003). Recent studies also indicate that rs890 is associated with obsessive-compulsive disorder (OCD) (Arnold et al., 2004), oppositional defiant disorder (ODD) (Comings et al., 2000) and schizophrenia (Di Maria et al., 2004).

The choice of the other candidate gene, the small-conductance $Ca^{2+}$-activated $K^+$ (SK3 and KCNN3) channel, relied on a rationale ensuing from several neurophysiological and population-based studies. SK channels, being responsible for the medium and slow after-hyperpolarization current components, are considered to be determinants of neuron firing patterns and membrane excitability (Sah, 1996; Pedarzani et al., 2001). According to one of the theories suggested by Gargus et al. (1998), SK channels may provide one of the endogenous mechanisms that produce, either directly or in dopamine-dependent manner, the hypofunction of NMDA-R signaling, and thus may also contribute to the development of neurological or psychiatric disorders. Several studies support this notion by demonstrating that moderate expansions of coding CAG repeats in *SK3* gene at 1q21.3 are associated with schizophrenia (Chandy et al., 1998; Bowen et al., 1998; Dror et al., 1999) and with the presence of ataxia (Figueroa et al., 2001). As mentioned earlier, a family-based study (Koronyo-Hamaoui et al., 2002) reported that longer CAG repeats in *SK3* are also associated with AN. These results were also reproduced in a case-control series (Koronyo-Hamaoui et al., 2004). We can now report on the basis of an extended cohort of AN family trios that both polymorphisms in *NR2B* and *SK3* are over-represented in AN and may contribute to the inherited risk of AN, and thus may be involved in a key pathway underlying predisposition to AN and, possibly, other psychopathologies.

## 2. Materials and methods

### 2.1. Patients and parents

Patients ($n = 90$ females; age $16.3 \pm 2.7$ years; body mass index (BMI) $14.73 \pm 1.83$ kg/m$^2$) were recruited through Israeli clinics specializing in eating disorders. The study was approved by the Institute Review Board and an informed consent was obtained from all participants and their parents. All participants underwent a structured interview (SCID-P or K-SADS). All families were of Jewish origin and the majority (70%) of Jewish-Ashkenazi extraction. Diagnoses of AN and other axis I disorders were established according to DSM-IV criteria. Subjects with past or present physical illness, schizophrenia and schizophreniform or delusional disorders were excluded from the study. Participants met the diagnostic criteria of AN subtype and co-morbidity for at least two years prior to genotyping. Most of the patients were diagnosed as restrictive type (80%). Lifetime co-morbidity was diagnosed in about half of the patients, co-morbid mood disorder in 44%, OCD in 20% and other anxiety disorders in 16%. Mood and/or anxiety disorders were diagnosed in about 20% of the mothers and 14% of the fathers. This diagnostic distribution