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Neuregulin-1 haplotype HAP_{ICE} is associated with lower hippocampal volumes in schizophrenic patients and in non-affected family members

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Abstract

The neuregulin-1 (NRG1) gene on chromosome 8p has been suggested as a potential susceptibility gene for schizophrenia. The exact way in which genetic variation in NRG1 might impact on this susceptibility for the disorder is a focus of current research. The present study aimed at investigating the possible relationship between a putative NRG1 at-risk haplotype (HAP_{ICE}) and hippocampal volumes in schizophrenic patients and their healthy first-degree relatives. We genotyped 30 schizophrenic patients and 52 non-affected family members with regard to the presence or absence of the NRG1 haplotype HAP_{ICE}. Structural magnetic resonance imaging was used to determine hippocampal brain volumes in the same subjects. Patients and relatives carrying haplotype HAP_{ICE} both had smaller relative hippocampal volumes as compared to patients or relatives who did not carry this haplotype. These findings provide first direct evidence for a link between NRG1 genetic variation and hippocampal volume reductions in schizophrenic patients and non-affected relatives. This preliminary evidence may help to guide further research into the pathophysiological pathways that underlie this genetic susceptibility for schizophrenia.

Keywords: NRG1; Genetic susceptibility; Schizophrenia; Hippocampus; Neuroimaging

1. Objectives of the study

Schizophrenia is a debilitating psychiatric disorder which has been shown to be substantially heritable (Cannon et al., 1998). Recently, the neuregulin-1 (NRG1) gene on chromosome 8p has been identified as a potential susceptibility gene for schizophrenia (Stefansson et al., 2002). Different haplotypes of this gene (as well as alleles of individual SNPs and microsatellites) have been found to be significantly more frequent in schizophrenic patients than in healthy controls (Corvin et al., 2004; Li et al., 2004; Stefansson et al., 2003; Tang et al., 2004; Williams et al., 2003; Yang et al., 2003; Zhao et al., 2004). These associations were clustered in two regions, one in the 5′ regulatory domain of the gene, and one further downstream. While the associated haplotypes are varying between investigated populations, the core at-risk haplotype first described in the icelandic population (HAP_{ICE}) has been replicated in two independent case-control studies involving either Scottish or UK and Irish subjects (Stefansson et al., 2003; Williams et al., 2003). In a systematic review Tosato et al. (2005) included a summary of HAP_{ICE} association results in about 4500 subjects, and concluded that

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[1] Each author has participated sufficiently in the work to take public responsibility for the content.

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HAP_{ICE} confers a small, but significant risk for schizophrenia. Likewise, a very recent meta-analysis by Li and colleagues provided further support for an association of several NRG1 polymorphisms and NRG1 haplotypes with schizophrenia.

NRG1 is a large gene that gives rise to at least 15 distinct transcripts which are derived from four principal isoforms, types I–IV. These NRG1 isoforms reflect differing transcription initiation sites and alternative splicing. NRG1 mRNA and protein is detected in neurons of many areas of developing and adult human brain, including hippocampus, cerebellum, neocortex, and some subcortical nuclei (Law et al., 2004). The exact functions of this gene and of the multiple transcripts expressed by this gene are still a matter of current research efforts. However, there is good evidence that NRG1 has effects on neuronal and glial functions that appear to be disturbed in schizophrenia. This includes neural developmental processes like neuronal migration, synaptogenesis and myelination, as well as neurotransmission and synaptic plasticity, e.g. recruitment of NMDA, GABA-A and alpha-7 nicotinic receptor, and long-term potentiation (for reviews, see Falls, 2003; Stefansson et al., 2004). Nevertheless, it is still unclear how genetic variation in NRG1 might impact on susceptibility for schizophrenia.

Brain volume reductions of the hippocampal formation have been repeatedly observed in both schizophrenic patients and their healthy family members (Narr et al., 2002; O'Driscoll et al., 2001; Seidman et al., 2002; van Erp et al., 2004; van Erp et al., 2002) and have been proposed to represent a promising endophenotype for schizophrenia (Zobel and Maier, 2004). It has been suggested that NRG1 might contribute to disturbances in hippocampal structure and function in schizophrenia as it seems to modulate inhibitory GABAergic transmission in the hippocampus (Okada and Corfas, 2004). Indirect support for this pathogenetic role of NRG1 comes from studies showing that NRG1 is highly expressed in CA3 pyramidal neurons projecting to CA1 and that NRG1 accumulates at various central synapses including the hippocampal CA1 molecular layer (Chaudhury et al., 2003; Law et al., 2004). Furthermore, altered expression both of NMDA receptor subunits (Gao et al., 2000) and of the neuregulin-1 transcripts (Law et al., 2006) has been observed in the hippocampus of schizophrenic patients. Moreover, in animal experiments as well as in cell cultures NRG1 expression has been demonstrated to influence hippocampal synapse formation and function both in the short-term, e.g. by alteration of synaptic electrophysiology (Kwon et al., 2005; Roysommuti et al., 2003), and in the long-term, e.g. by induction of structural changes like enhanced neurite arborisation (Gerecke et al., 2004).

The aim of the present study was to investigate in schizophrenic patients and their non-affected biological relatives whether the putative NRG1 risk haplotype HAP_{ICE} is related to hippocampal brain volumes as determined by in vivo structural magnetic resonance imaging.

2. Materials and methods

2.1. Subjects

We studied 30 patients with diagnosis of schizophrenia according to ICD-10 and DSM-IV criteria and 52 non-affected family members from 14 uni-affected and 18 multi-affected families. All subjects were Caucasian. In the group of schizophrenic patients there were 19 males and 11 females, mean age was 34.5 years (s.d. = 10.1 years, range = 19–54 years). The group of non-affected family members consisted of 28 males and 24 females, mean age was 47.4 years (s.d. = 15.8 years, range = 18–74 years). Exclusion criteria were dementia, neurological illness, brain traumas, brain tumors and substance abuse. All subjects were recruited at the Departments of Psychiatry, Universities of Duesseldorf and Bonn, and gave written informed consent to participate in the study after having obtained complete description of the study. The investigation was carried out in accordance with the latest version of the Declaration of Helsinki, and the study was approved by the ethics committees of the Universities of Duesseldorf and Bonn.

2.2. Genetic analyses

The DNA was isolated from whole blood or permanent cell lines derived from Epstein-Barr virus-transformed lymphocytes with a Qiagen Blood- and Cell-Culture kit. 12.5 ng of DNA each was used for TaqMan genotyping assays. Genotyping of SNP8NRG221533 was performed using a Taqman Assay by Design (Applied Biosystems, Darmstadt, Germany) as described previously by Schwab et al. (2005). Genotyping of microsatellite markers 478B14-848 and 420M9-1395 was performed as described in Schwab et al. (2000) using primers as described by Stefansson et al. (2002). The program FAMHAP (Becker and Knapp, 2004) was used to estimate haplotype frequencies.

We investigated the effect of the presence or absence of the Icelandic NRG1 core at-risk haplotype (HAP_{ICE}) (Li et al., 2006; Stefansson et al., 2002; Tosato et al., 2005). This haplotype is based on seven markers, which can be identified by a set of just three informative marker loci, one single nucleotide polymorphism (SNP) and two microsatellite markers. These three markers (SNP8NRG221533, NRG1_478B14-848 and NRG1_420M9-1395) span a region of approximately 300 kB within the NRG1 gene. The at-risk variant of this haplotype ("G 0 0" = HAP_{ICE}) consists of the G nucleotide at SNP8NRG221533 and the most frequent allelic variants of microsatellites NRG1_478B14-848 and NRG1_420M9-1395.

Furthermore, we explored the isolated effects of each of the three constituent marker loci, by testing the influence of the SNP8NRG221533 G allele, and of the most frequent variants at each of the two microsatellites NRG1_478B14-848 and NRG1_420M9-1395.