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BRAIN RESEARCH

Research Report

A 2-base pair deletion polymorphism in the partial duplication of the α7 nicotinic acetylcholine gene (*CHRFAM7A*) on chromosome 15q14 is associated with schizophrenia

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ARTICLE INFO

Article history:
Accepted 14 July 2009
Available online 23 July 2009

Keywords:
Nicotinic acetylcholine receptor
CHRFAM7A
CHRNA7
Association study
Deletion
Schizophrenia
Duplication
P50

ABSTRACT

Multiple genetic linkage studies support the hypothesis that the 15q13–14 chromosomal region contributes to the etiology of schizophrenia. Among the putative candidate genes in this area are the α7 nicotinic acetylcholine receptor gene (*CHRNA7*) and its partial duplication, *CHRFAM7A*. A large chromosomal segment including the *CHRFAM7A* gene locus, but not the *CHRNA7* locus, is deleted in some individuals. The *CHRFAM7A* gene contains a polymorphism consisting of a 2 base pair (2 bp) deletion at position 497–498 bp of exon 6. We employed PCR-based methods to quantify the copy number of *CHRFAM7A* and the presence of the 2 bp polymorphism in a large, multi-ethnic population. The 2 bp polymorphism was associated with schizophrenia in African Americans (genotype $p=0.005$, allele $p=0.015$), and in Caucasians (genotype $p=0.015$, allele $p=0.009$). We conclude that the presence of the 2 bp polymorphism at the *CHRFAM7A* locus may have a functional significance in schizophrenia.

1. Introduction

Understanding the genetic determinants of schizophrenia will lead to more promising treatments for this devastating psychiatric disorder, which afflicts approximately 1% of the world population. This disease has demonstrated a substantial genetic component in twin, family and adoption studies, raising hopes that genetic linkage studies could identify predisposing genes of large to moderate effect (Cardno et al., 1999; Cardno and Gottesman, 2000; Leo, 2006; Wynne et al., 2006). To date, definitive linkage results have been difficult to achieve. However, chromosomal rearrangements on 15q11–14 are associated with neurodevelopmental syndromes. Intrachromosomal recombination events that map to 15q11–14 have been implicated in Prader–Willi/Angelman syndrome (Christian et al., 1998; Robinson et al., 1998). Sharp et al.

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doi:10.1016/j.brainres.2009.07.041

recently reported a recurrent microdeletion at 15q13.3 that is associated with mental retardation and seizures (Sharp et al., 2008) and large recurrent, but rare, microdeletions at 15q11.2 and 15q13.3 are associated with schizophrenia (Stefansson et al., 2008). Rare chromosomal deletions and duplications in several locations in the genome, including chromosomes 15, 22, and 1 have also been found to be associated with schizophrenia (International Schizophrenia Consortium, 2008). As these rearrangements are associated with neurocognitive dysfunction, the region is likely to be important in brain development. The α7 nicotinic cholinergic receptor gene (*CHRNA7*) and its partial duplication (*CHRFAM7A*) map within this dynamic region (Baron, 2001; Freedman and Leonard, 2001; Linthorst and Reul, 2008). Within the large deletion and duplication events described above, this segmental duplication containing *CHRNA7* also has complex rearrangements. The purpose of the current study was to determine whether the partial duplication, *CHRFAM7A*, is associated with schizophrenia or its physiological correlate, the P50 gating deficit.

Genetic analysis of *CHRNA7* has been complicated by a partial duplication, now designated *CHRFAM7A*, which maps 1.6 Mb centromeric to the gene (Gault et al., 1998; Riley et al., 2002) (see Fig. 1). This appears to be a relatively recent event unique to humans; it is not carried by closely related primates (Locke et al., 2003). In this duplication, exons 5–10, intervening introns, and the 3′ untranslated region of *CHRNA7* are conserved (>99% nucleotide identity). In place of exons 1–4, novel exons termed D′, D, C, B and A are fused 700 bp upstream of *CHRNA7* exon 5 (GenBank AF029838) (Gault et al., 1998). The breakpoint occurs in an *alu* sequence within intron 4. Riley et al. (2002) also described another exon, E, lying between exons A and B. Exons C–A are the result of a partial duplication of a putative kinase-like gene (*ULK4*) on chromosome 3p21. Axons C–A also map to several additional loci in the chromosome 15q13–14 region. Exons D′ and D map to no fewer than 5 distinct loci on chromosome 15, but not to chromosome 3. Their origin is unknown.

Mutation screening in human brain mRNA for both *CHRNA7* and *CHRFAM7A* identified 33 polymorphisms (Gault et al., 2003). Thirty of these polymorphisms are single nucleotide polymorphisms (SNPs). Three insertion/deletion polymorphisms were identified, including a 2 bp deletion polymorphism (2 bp polymorphism) in exon 6 that maps exclusively to *CHRFAM7A*. In addition, some chromosomes are missing the *CHRFAM7A* locus and rare individuals are missing both copies.

Riley et al. (2002) suggested that the *CHRFAM7A* locus is the result of a primary duplication of the full-length *CHRNA7* gene, followed by a non-homologous deletion of sequences contained in the more centromeric copy of *CHRNA7*. The absence of the *CHRFAM7A* gene on some chromosomes would represent a later deletion of the primary duplication event due to further recombination. An alternative explanation, suggested by Flomen et al. (2008) is that the absence of *CHRFAM7A* represents an ancestral sequence that did not undergo duplication of *CHRNA7*. Further, *CHRFAM7A* exists in two orientations with reference to *CHRNA7* (Flomen et al., 2008). The 2 bp polymorphism in exon 6 of *CHRFAM7A* is in strong linkage disequilibrium with this polymorphic inversion of *CHRFAM7A* and surrounding loci. On chromosomes containing the 2 bp polymorphism, *CHRFAM7A* is almost always in the same orientation as *CHRNA7*; at loci that do not contain the 2 bp polymorphism, *CHRFAM7A* is almost always in the opposite orientation. Thus, numerous recombination events over time have created the complex and fragmented region observed today.

The *CHRNA7* gene is genetically linked to schizophrenia and its endophenotype, the P50 auditory gating deficit. The P50 endophenotype, a neurophysiological auditory gating deficit, is commonly seen in schizophrenics and their first degree relatives (Freedman et al., 1991). This trait involves inhibition of response to repetitive auditory stimuli and can be measured by means of auditory evoked potentials in a paired pulse paradigm. Scalp electrodes record waves with a 50 millisecond latency (P50) following paired auditory stimuli delivered 0.5 s apart. In a normal response, the subject decreases the amplitude of the second response (test response) compared to the response to the first stimulus (conditioning response) through the action of an inhibitory neuronal pathway. The results are reported as the P50 test-conditioning (T/C) ratio (Freedman et al., 1991). The endophenotype is normalized by smoking in schizophrenic patients (Leonard et al., 1998a) and is strongly linked to the D15S1360 dinucleotide repeat marker within intron 2 of the *CHRNA7* gene on chromosome 15q14 (Freedman et al., 1997). Other markers in this region have shown weaker linkage to schizophrenia as a disease (Freedman et al., 2001; Gejman et al., 2001; Leonard et al., 1998b; Liu et al., 2001; Neves-Pereira et al., 1998; Riley et al., 2000; Tsuang et al., 2001; Xu et al., 2001).

A role for the α7 nicotinic receptor in mediating the P50 response is strongly supported by pharmacological and biochemical data, both in humans and in animals (Adler et al., 1998; Leonard et al., 2001). The snake toxin α-Bungarotoxin (α-BTX) preferentially binds to the α7 receptor in the CNS. α-BTX binding levels are decreased in schizophrenic postmortem hippocampus, where the P50 response is mediated (Freedman et al., 1995). α-BTX binding is also decreased in cingulate cortex and in the reticular nucleus of the thalamus in schizophrenia, while *CHRNA7* protein levels are reduced in prefrontal cortex (Court et al., 1999; Guan et al., 1999; Marutle et al., 2001). The proximal promoter of *CHRNA7* contains functional SNPs that decrease transcription and are associated with schizophrenia (Leonard et al., 2002). This is consistent with the observation that *CHRNA7* expression is aberrant in schizophrenia.

Multiple lines of evidence indicate the involvement of genes in the 15q13–14 locus in schizophrenia, and the *CHRFAM7A* locus itself is polymorphic. We determined the copy number and 2 bp polymorphism status of *CHRFAM7A* quantitatively and evaluated them as risk factors for schizophrenia and for increased P50 T/C ratio in a large multi-ethnic population of Caucasian, African American and Hispanic descent.

2. Results

Southern blot was used on an initial set of samples and fluorescent in situ hybridization (FISH) was used on a subset of these to confirm and visualize the findings on Southern