CHRFAM7A copy number and 2-bp deletion polymorphisms and antisaccade performance

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Abstract

Chromosome 15q13–q14 harbours the gene for the α7 nicotinic acetylcholine receptor subunit (CHRNA7) and a related gene (CHRFAM7A) which arises from a partly duplicated portion of CHRNA7. Recent evidence suggests that CHRFAM7A is a locus with a possible role in schizophrenia and cognitive functioning. We studied an antisaccade task as a fronto-parietal measure of executive function that reflects risk for schizophrenia. Association of CHRFAM7A genotype with antisaccade performance was assessed in 103 healthy Caucasian individuals. No significant associations of 2-bp deletion or CHRFAM7A copy number with antisaccade performance parameters were observed. The failure to observe an association between antisaccade performance and polymorphisms in CHRFAM7A gene is consistent with specificity of the gene effects on hippocampal and memory functions as previously demonstrated.

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Introduction

Convergent findings from a number of approaches suggest that the cholinergic system may play a role in schizophrenia. For example, an altered neuronal nicotinic acetylcholine receptor system may contribute to the pathophysiology of schizophrenia (Freedman et al. 1995) and the nicotinic acetylcholine receptor α7 subunit has been implicated in the genetics of this condition. Further, nicotine consumption, through cigarette smoking, is increased in schizophrenia patients and their biological relatives (Lyons et al. 2002) compared with the general population. Given that cognitive deficits in schizophrenia benefit from nicotine administration (Barr et al. 2008; Depatie et al. 2002), smoking might represent a form of self-medication (Kumari & Postma, 2005).

The antisaccade task is a fronto-parietal measure of executive function that reflects risk for schizophrenia (Hutton & Ettinger, 2006). In this task, the subject first fixates a central stimulus and then makes a saccade away from a peripheral target to its mirror position. Correct performance on this task requires suppression of the reflexive saccade towards the target and transformation of the stimulus location into a volitional motor command mediated by frontal cortex, posterior parietal cortex, basal ganglia and superior colliculus (SC) (Hutton & Ettinger, 2006). Patients with schizophrenia, their relatives, and individuals at risk for psychosis show impaired antisaccade performance (Nieman et al. 2007; Petrovsky et al. 2008), supporting the status of the task as a schizophrenia endophenotype. Endophenotypes are biological markers thought to represent a simpler and more direct reflection of genetic risk for an illness than the heterogeneous illness phenotype itself.

So far, little is known about the genetics of antisaccades, but it can be hypothesized that genetic polymorphisms relating to the cholinergic system might play a role in inter-individual performance
differences. It has been shown that antisaccades are influenced by cholinergic modulation. Nicotine improves performance in schizophrenia patients (Depatie et al. 2002) and healthy subjects (Rycroft et al. 2006), similar to effects seen on other cognitive tasks (Barr et al. 2008). At present, little is known about the molecular mechanisms by which cholinergic agents influence antisaccade performance. Agonists of the nicotinic acetylcholine receptors (nAChRs) stimulate the activity of these receptors, thereby perhaps directly allowing enhanced attention or functioning on this task. However, it is also possible that stimulation of nAChRs evokes the release of other neurotransmitters such as dopamine which in turn might lead to altered performance. One way of investigating whether nAChRs play a role in antisaccade performance would be to search for functional nAChR genes which might be responsible for at least part of the differences between individuals in antisaccade behaviour. To our knowledge, this has not been investigated.

The \( \alpha_7 \) nicotinic acetylcholine receptor subunit gene (CHRNA7) represents a promising starting point for this work. CHRNA7 is widely expressed in the central nervous system and maps to 15q13–q14. Markers at or near CHRNA7 are strongly linked to another endophenotype of schizophrenia, the electrophysiological measure P50 (Freedman et al. 1997; Leonard et al. 2002). Deficits in P50 sensory gating are normalized by nicotine (Freedman et al. 1997) and have been shown to be jointly linked with antisaccades to a locus on chromosome 22q11–12 (Myles-Worsley et al. 1999). Weaker linkage to schizophrenia itself was demonstrated by Freedman et al. (1997), while the prevalence of functional CHRNA7 promoter polymorphisms was also greater in schizophrenia (Leonard et al. 2002). Most recently, Stefansson and colleagues (2008) found a significant association of schizophrenia with a deletion in chromosome region 15q13.3, between breakpoints BP 4 and 5, which removes CHRNA7 and several other genes, underlining the importance of this region in schizophrenia.

In most individuals the CHRNA7 gene is partially duplicated, giving rise to a hybrid gene, CHRFAM7A, which contains exons 5–10 of CHRNA7 joined to four exons from an unrelated gene, FAM7A. Chromosomes both with and without the CHRFAM7A duplcon have been identified, indicating a copy number variation (CNV) with respect to exons 5–10 (Flomen et al. 2006). Reduced copy number has previously shown weak association with psychosis (Flomen et al. 2006). Where present, CHRFAM7A exists as a polymorphic inversion in either direct or inverted orientation with respect to CHRNA7 (Flomen et al. 2008). Additionally, it contains a polymorphic 2-bp deletion within exon 6, which has been associated with deficits in P50 sensory gating (Raux et al. 2002) and episodic memory performance (Dempster et al. 2006). The 2-bp deletion is in strong linkage disequilibrium with the direct orientation of CHRFAM7A in respect to CHRNA7 (Flomen et al. 2008), which may therefore be the actual variant responsible for the above associations.

It is unclear how variants of CHRFAM7A might affect these psychosis endophenotypes as it is unknown whether it is translated or whether it is expressed in the same neurons as CHRNA7. Its translation products may interact with those of CHRNA7, acting in a dominant negative manner, which would be prevented by the 2-bp deletion polymorphism truncating the CHRFAM7A product. Alternatively, CHRNA7 expression may be modulated by CHRFAM7A expression (e.g. by competition for transcriptional factors), which might be influenced by its orientation (Flomen et al. 2008). Interestingly, microdeletion at 15q13.3, which is associated with schizophrenia (Stefansson et al. 2008), may be more likely to occur by non-allelic homologous recombination when CHRFAM7A is in a direct orientation with respect to CHRNA7 in the duplicated segments (Makoff & Flomen, 2008).

While both P50 and episodic memory are functions thought to rely on hippocampal integrity, associations of CHRFAM7A genotype with tests of fronto-parietal cognition have not been studied. The aim of this study was to explore whether variants in the nicotinic receptor gene account for variance in a fronto-parietal schizophrenia endophenotype. We therefore investigated the possible association between the CHRFAM7A CNV with its associated 2-bp deletion/inversion polymorphism and antisaccades. We restricted this preliminary investigation to healthy individuals as this allows the study of gene–cognition relationships in the absence of clinical and treatment confounds. It also takes into account the previous observation of stronger CHRFAM7A genotype effects on cognition in healthy compared to schizophrenia individuals (Raux et al. 2002).

Method

Subjects

Healthy volunteers were recruited through advertisements at the University of Bonn and around the local community. Participants provided information on age, gender, ethnicity, handedness, smoking status (smoker, non-smoker), years spent in full time education, and paternal as well as maternal socioeconomic status.
were instructed to look at the target while in the centre of the monitor for a random duration of up to 12 min, and, subsequently, in one of four peripheral locations (100–2000 ms). Participants were given a health questionnaire to provide information about their general physical health. In addition, participants were asked to complete four standardized personality questionnaires: the Rust Inventory of Schizotypal Cognitions (RISC; Rust, 1988), the World Health Organization (WHO) Adult ADHD Self-Report Scale (ASRS; Kessler et al. 2005), the Obsessive–Compulsive Inventory (OCI; Foa et al. 2002), and the neuroticism scale of the Eysenck Personality Questionnaire – Revised (EPQ-R; Eysenck & Eysenck, 1991) to ascertain possible effects of genotype on personality traits. Ethical approval of the local ethics committee was obtained and volunteers provided written informed consent.

**Genotyping**

DNA was extracted from venous blood. The 2-bp deletion genotype and copy number of CHRFAM7A were determined as described previously (Flomen et al. 2006).

The genotyping yielded five groups, consisting of subjects with one copy with no deletions (1C0D), one copy with one deletion (1C1D), two copies with no deletions (2C0D), two copies with one deletion (2C1D), and two copies with two deletions (2C2D). We did not observe any examples of the rare 6th genotype (subjects having 0 copies of CHRFAM7A).

**Oculography**

Eye movements were recorded using infrared oculography (IRIS Skalar 6500; Skalar Instruments GmbH, Germany) of the left eye and sampled at 500 Hz as described previously (Ettinger et al. 2003). Participants were seated 57 cm from a 17-inch monitor with their heads on a chinrest. The target was a white dot (0.3° diameter) presented on a black background. A 3-point calibration (0°, ±12°) was carried out, followed by 60 antisaccade trials. A trial consisted of the target in the centre of the monitor for a random duration of 1000–2000 ms and, subsequently, in one of four peripheral locations (±6°, ±12°) for 1000 ms. Participants were instructed to look at the target while in the centre and to the exact mirror image location when it jumped to the side.

Data analysis (Eyemap, AMTech GmbH, Germany) involved automatic detection of saccades using criteria of minimum amplitude (1°), velocity (30°/s), and latency to target (100 ms), and individual categorization into directional correct antisaccades and reflexive error saccades. Antisaccade latency (ms), reflexive saccade error rate (% reflexive saccades over total number of valid trials), antisaccade gain (% saccade amplitude over target amplitude), and antisaccade spatial error were calculated. Spatial error was obtained by subtracting the target amplitude from saccade amplitude and dividing the result by the target amplitude. The absolute value of this term reflects the residual error and was then averaged across all saccades and multiplied by 100.

**Statistical analyses**

Statistical analyses were conducted using SPSS version 15.0 (SPSS Inc., USA). Genotype (1C0D, 1C1D, 2C0D, 2C1D, 2C2D) was used as independent variable and socio-demographic (age, education, paternal and maternal SES) and antisaccade (error rate, latency, gain, and spatial error) variables were used as dependent variables in separate univariate analyses of variance (ANOVA). We also examined the relationship between genotype and gender using χ² test. Finally, we included smoking status (smoker, non-smoker) as an additional independent variable in the ANOVA model and investigated whether smoking status was associated with genotype (using χ² test).

**Results**

Socio-demographic and antisaccade variables are summarized in Table 1. A total of 111 participants completed the study. There were eight genotyping failures, leaving a final sample size of n=103 (57 males; age 25.87±5.50 yr; 17.64±3.36 yr education; paternal SES =3.12±0.90; maternal SES =2.83±1.02; 27 smokers). All participants were Caucasian. The genotype groups did not differ in any socio-demographic variable or smoking status (all p>0.20). The genotype distribution did not significantly differ from Hardy–Weinberg equilibrium (χ² = 4.51, df = 3, p = 0.21).

Analyses of antisaccade variables revealed no association with genotype for the combined copy number/2-bp deletion assay (all p>0.37). Grouping subjects by 2-bp deletion alone (n=31 without deletions, n=72 with at least one deletion) and by copy number alone (n=28 with one copy, n=75 with two
copies) in separate analyses did not yield any significant effects ($p > 0.34$ and $p > 0.59$, respectively). The effect sizes for the analyses of antisaccade variables are presented in Table 2. Finally, given the known effects of smoking on antisaccade performance (Rycroft et al. 2006) and the possibility of an interaction between an acetylcholine-related genotype and nicotine consumption, smoking status was added as an independent variable into the model. Genotype effects remained unchanged, and there were no significant main or interaction effects involving smoking status ($all p > 0.18$).

Data from the personality questionnaires are presented in Table 1. There were no associations with genotype for the combined copy number/2-bp deletion assay ($all p > 0.48$). Grouping subjects by 2-bp
deletion alone and by copy number alone also did not reveal any significant effects ($p > 0.25$ and $p > 0.22$, respectively). Finally, there were no effects of smoking status or interactions of smoking status with genotype on questionnaire variables (all $p > 0.05$).

**Discussion**

This is, to our knowledge, the first study to explore the association between CHRFAM7A and measures of executive function. We selected the antisaccade task as it represents a marker of risk for schizophrenia with well-defined fronto-parietal neural correlates and because performance can be modulated by cholinergic agents (Hutton & Ettinger, 2006). The CHRFAM7A gene was selected as it is a promising locus in the cholinergic system with regard to schizophrenia and cognition (Dempster *et al.* 2006; Raux *et al.* 2002).

In the present study, no significant association of CHRFAM7A copy number or 2-bp deletion/inversion polymorphism with antisaccade performance parameters was observed. However, it should be noted that given the relatively small sample size of the present study, we can only exclude a large effect size. It is possible that CHRFAM7A does in fact impact on aspects of executive function and future studies with larger samples are required to address this question. A power calculation found that >600 subjects are required to detect effects of $d = 0.2$ with >80% power.

Failure to detect association may actually be consistent with neurophysiological and cognitive specificity of the effects of this genotype, given that previous studies have shown evidence for association with episodic memory and P50 suppression (Dempster *et al.* 2006; Raux *et al.* 2002). Although episodic memory and P50 suppression involve widespread neural correlates and CHRFAM7A is not expressed exclusively during hippocampal formation, the results suggest a more specific effect on hippocampally mediated memory and inhibitory functions. In this context it is important to note that the hippocampus primarily mediates long-term memory processes, while working memory relies more heavily on fronto-parietal networks (however, see Weinberger *et al.* 1992, for evidence of an association between hippocampus and working memory).

We did not observe any significant associations of the CHRFAM7A polymorphisms with questionnaire measures of personality traits indexing variation in schizotypy, neuroticism, attention deficit hyperactivity disorder, and obsessive–compulsive disorder. The lack of association with personality traits is unlikely to be due to measuring error as the questionnaires used here have established reliability. The same applies to the antisaccade task, for which high test–retest reliabilities and internal consistencies have been reported (Ettinger *et al.* 2003).

Both smokers and non-smokers were included in the present study. Smoking status was not associated with genotype and did not affect antisaccade or personality variables, nor did it mediate genotype associations with these measures.

Given that the antisaccade task is a schizophrenia endophenotype with good heritability, future investigations of the specific molecular genetic mechanisms underlying inter-individual differences are important. There is strong evidence for cholinergic influences on antisaccades, so in addition to assessing the CHRFAM7A CNV and 2-bp deletion/inversion polymorphism genotype in a much larger sample, it would be worthwhile to investigate other polymorphisms in cholinergic system genes associated with schizophrenia or frontal lobe functioning, including CHRFAM7A and CHRNA.

A related issue concerns the possible role of CHRFAM7A and other cholinergic genotypes in the effects of cholinergic manipulation on neurocognitive performance. Even in the absence of a main effect of genotype on behavioural performance as in the present study, it is possible that there are modulating pharmacogenetic effects of this polymorphism that become apparent in pharmaceutical challenge studies. Similarly, it would be of interest to investigate whether this polymorphism mediates inter-individual differences in the effects of smoking withdrawal on cognitive performance (Powell *et al.* 2002).

The main limitation of the present study is the small sample size. Given the power calculations presented here, multi-centre collaborative efforts will be required to demonstrate the operation of small-gene effects on performance. Future studies may also wish to examine possible associations of CHRFAM7A on standard neuropsychological tests of different domains of executive function and memory.

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Statement of Interest
None.

References


