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ARTICLE

Association between the 2-bp deletion polymorphism in the duplicated version of the alpha7 nicotinic receptor gene and P50 sensory gating

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There is considerable evidence implicating the 15q13.3 region in neuropsychiatric disorders, with the α7 nicotinic receptor gene CHRNA7 the most plausible candidate. This region has multiple duplications and many copy number variants (CNVs). A common CNV involves a partial duplication of CHRNA7 (CHRFAM7A), which occurs in either orientation. We examined the distribution of these alternative genomic arrangements in a large cohort of psychiatric patients, their relatives and controls using the 2-bp deletion polymorphism as a marker for the orientation of CHRFAM7A. We investigated three common alleles for association with psychosis and with the P50 sensory gating deficit, which is strongly associated with psychosis and strongly linked to 15q13.3. We found significant within-family association with P50 (empirical $P = 0.004$), which is robust to population stratification. Most of the effect came from the 2-bp deletion allele, which tags the variant of CHRFAM7A in the same orientation as CHRNA7. This allele is associated with the presence of the P50 sensory gating deficit (empirical $P = 0.0006$). Tests comparing within-family and between-family components of association suggest considerable population stratification in the sample. We found no evidence for association with psychosis, but this may reflect lower power using this phenotype. Four out of six previous association studies found association of different psychiatric phenotypes with the same 2-bp deletion allele.

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INTRODUCTION

The 15q13.3 region has been implicated in several neurological and psychiatric disorders. The P50 sensory gating deficit, one of the best supported endophenotypes of schizophrenia and bipolar disorder, is strongly linked to this region,[1] as are two idiopathic epilepsies.[2,3] Of the many attempts to demonstrate linkage of schizophrenia and bipolar disorder to this region, two studies showed linkage to bipolar disorder,[4,5] but several found either weak[6–10] or no linkage to schizophrenia.[11–13] Both schizophrenia and bipolar disorder have also shown association with 15q13.3 markers.[14] The most plausible candidate gene in 15q13.3 for the major psychoses and epilepsies is CHRNA7, the α7 nicotinic acetylcholine receptor gene.[15,16] However, 15q13.3 is a complex region in the midst of many large segmental duplications.[17,18] These are highly variable, resulting in several different copy number variants (CNVs).

One common CNV includes part of CHRNA7 (exons 5–10), which is duplicated in the hybrid gene CHRFAM7A that occurs in most individuals. We found weak evidence for association of the absence of one copy of CHRFAM7A with psychosis in a large Scottish sample.[19] When present, CHRFAM7A can occur in either orientation, with one orientation strongly associated with the common 2-bp deletion polymorphism in exon 6,[20] found only in CHRFAM7A (Supplementary Figure 1a). We found no association of the 2-bp deletion with schizophrenia in the above study, but an association with schizophrenia has since been reported.[21] Other studies have found that the 2-bp deletion is associated with bipolar disorder,[22] P50 sensory gating deficit[23] and deficits in episodic memory,[24] another endophenotype proposed for schizophrenia. Recent genome-wide scans for CNVs showed that much rarer CNVs at 15q13.3, usually involving deletion of ~2 Mb from CHRFAM7A to CHRNA7, are overrepresented in many neurological and psychiatric disorders. These deletions are very rare in the general population (~0.02%), but more common in schizophrenia, autism/developmental disorders and some forms of intellectual impairment (~0.2–0.3%) and even more common in idiopathic generalized epilepsy (~1.0%).[25–28] These ~2-Mb microdeletions lead to the loss of CHRNA7 and five other genes (Supplementary Figure 1a), but smaller variants that show a similar range of phenotypes remove only CHRNA7 and one other

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gene.[29] These observations strongly implicate the CHRNA7 region in schizophrenia and other neuropsychiatric disorders.

P50 sensory gating is an EEG signal that is believed to reflect the ability to filter out repetitive stimuli and prevent information overload.[30–32] The P50 wave is generated in a conditioning-testing paradigm where the first stimulus activates or conditions the inhibition phenomenon, whereas the second tests its strength. Normally, individuals reduce the second (test) response relative to the first (condition) waves. The diminished test P50 wave is thought to be due to activation of inhibitory neural circuitry by the conditional P50 stimuli.[1,33–35] Compared with controls, patients with schizophrenia and psychotic bipolar disorder have a relatively larger P50 response to the second stimulus, from only 20–50% suppression.[31,36–47] Clinically unaffected first-degree relatives of patients with schizophrenia and psychotic bipolar disorder also have poor P50 suppression, suggesting that this might act as a marker of genetic risk for these disorders,[46–53] as it is heritable.[54–57]

We have examined the effects of CHRFAM7A CNV/2 bp deletion variants on the major psychoses and the P50 sensory gating deficit. We utilized a large group of families, twin pairs and controls including several family members diagnosed with schizophrenia or psychotic bipolar disorder, where P50 measurements were made.

METHODS

The sample

The sample included 871 Caucasian individuals from the national Maudsley Family and Twin Psychosis Studies. A subgroup of these ($N = 445$) underwent EEG recordings from which were derived the P50 suppression ratios, computed as the ratio of testing to conditioning wave amplitudes (T/C) expressed as a percentage. Patients satisfied DSM-IV criteria for schizophrenia, schizoaffective disorder or psychotic bipolar disorder. Their unaffected first-degree relatives were free of any psychotic illness whereas the controls had no personal or family history of psychosis. Subjects were excluded if they had a neurological disorder, head injury with loss of consciousness exceeding 10 min or diagnosis of alcohol or substance dependence in the previous 12 months. The studies were approved by the Joint South London and Maudsley and the Institute of Psychiatry NHS Research Ethical Committee or the South East Research Ethics Committee. All participants gave written informed consent.

Clinical assessments and P50 measurements

Clinical assessment, P50 measurements and analysis were performed as described in detail elsewhere.[31]

Genotyping

Genotyping for the CHRFAM7A CNV/2 bp deletion polymorphisms required two sequential assays.[19] Presence of the 2-bp deletion was determined by limited cycle PCR using ABI3130 and Genemapper v3.0 software (Life Technologies Limited, Paisley, UK). Each sample was assayed at least twice to identify three genotypes (13, 23 and 33) where the 2-bp deletion is present, with alleles defined as 1 = null CHRFAM7A, 2 = wild-type CHRFAM7A and 3 = CHRFAM7A with 2 bp deletion (Supplementary Figures 1a, b). Samples without a 2-bp deletion were assayed by a Taqman assay (Life Technologies Limited) to determine the copy number of CHRFAM7A (at least three determinations per sample) to identify the remaining three genotypes (11, 12 and 22).

Genotyping was performed and analyzed 'blind' to patient identity. Several patients had more than one DNA sample. These and monozygous (MZ) pairs accounted for 606 genotyped samples, which were compared to assess genotyping reproducibility, giving an error rate of 6%. Duplicate or MZ pairs discordant for genotype were excluded.

Statistical analyses

Significance of association of the CNV/2 bp deletion polymorphisms with clinical groups was performed by χ^2-tests, including only one twin from each MZ pair concordant for psychosis but excluding discordant MZ pairs. In initial analyses, these tests were also applied to P50, as two categorical groups: P50 T/C ratios >60 *versus* $<40\%$, and including one MZ twin per pair using each average T/C ratio. Family-based association analyses were performed on categorical phenotypes by the transmission disequilibrium test (TDT) using Transmit (https://www-gene.cimr.cam.ac.uk/staff/clayton/software/).[58] Transmit uses parental genotypes, when present, but can use siblings to derive possible parental genotypes when absent. As Transmit will not accept twinships, dizygous (DZ) twins were entered as siblings while one twin per MZ pair were entered as above. To perform tests for association on continuous phenotype scores we used QTDT (http://www.sph.umich.edu/csg/abecasis/QTDT/),[59,60] which incorporates a modified form of TDT. QTDT will construct possible parental genotypes where absent and accepts all types of sibships. It can utilize components variance modeling, which we used as recommended for samples including families with multiple offspring.[60] QTDT assesses both between-family and within-family components of association to provide three tests of association: 'total' combines both components but is invalid in the presence of population stratification, 'within' gives the within-family component and is robust to stratification and 'stratification' compares the components to test for stratification.

RESULTS

The sample

The sample comprises patients diagnosed with a major psychosis (mainly schizophrenia or bipolar disorder), their families (including co-twins), controls and control twin pairs, all of whom were white Caucasians. Table 1 shows the demographic matching between patients, relatives and controls. As is often seen with studies of psychosis, the patient group had significantly more males than the control group. The P50 subset illustrates the well-established strength of association between P50 and psychosis,[46,61,62] with very strong associations between patients and controls ($P = 5 \times 10^{-9}$), and between relatives and controls ($P = 6 \times 10^{-6}$).

Association study by patient group

Comparison of the distribution of the three alleles among the three patient groups showed no significant association (Table 2). The distributions of the six genotype frequencies (see Supplementary Table 1) were all in Hardy–Weinberg equilibrium (HWE), and there was also no significant association. As case–control association studies are vulnerable to population stratification, we investigated possible family-based association. This was performed by TDT, which compares the transmission of alleles from heterozygous parents to affected offspring. As shown in Table 3 (top), there was no evidence for family-based association with psychosis.

Association study by P50

Allele frequencies for individuals with P50 data are shown in Table 4. In a preliminary analysis, we compared the allele distributions between individuals with a T/C ratio $>60\%$ (generally regarded as abnormal) with those $<40\%$ (within the normal range). As shown in Table 4, there was no significant difference between allele frequencies. The distributions of genotype frequencies are shown in Supplementary Table 2, with all genotypes in HWE.

As with comparisons between patient groups, this type of association analysis can be compromised by population stratification. Furthermore, it does not utilize all available power of the sample as (a) it reduces the full range of T/C ratios to two discrete groups and (b) it does not utilize the family relationships. To overcome these limitations, we analysed the data by QTDT, which compares transmission of each allele from heterozygous parents with offspring scored with the full range of T/C ratios.

Table 1 Details of sample, all of whom were white Caucasians

	Affected patients	*Unaffected relatives*	*Unaffected controls*	*Totals*
Complete sample	248	258	365	871
[a]MZ twins	87 (29)	29 (0)	[b]175 (87)	[b]291 (145)
[a]DZ twins	16 (1)	14 (0)	102 (51)	132 (66)
DSM-IV diagnosis	169 schizophrenia 66 bipolar 10 schizoaffective 3 other psychosis			
Mean age (±SEM)	39.0±1.5	47.8±1.9	39.9±1.3	42.0±0.9
Age range	17–74	16–83	19–72	16–83
Males: females	154:94	113:145	116:249	383:488
P50 sample	141	108	196	445
[a]MZ twins	56 (19)	18 (0)	[b]78 (38)	[b]152 (74)
[a]DZ twins	7 (0)	5 (0)	67 (32)	79 (37)
DSM-IV diagnosis	85 schizophrenia 49 bipolar 6 schizoaffective 1 other psychosis			
Mean age (±SEM)	40.2±2.1	43.0±2.5	38.1±1.8	39.9±1.2
Age range	21–65	19–63	19–64	19–65
Males: females	90:51	50:58	65:131	205:240
[c]P50 T/C ratio	65.4±8.2	57.6±7.1	38.6±4.7	51.7±3.9

Abbreviations: DZ, dizygous; MZ, monozygous; T/C, ratio of testing to conditioning wave amplitudes.
[a]Number of twins for each group with number of twin pairs shown in brackets.
[b]Includes one triplet.
[c]Significance by *t*-tests: patients *versus* controls: $P=5\times10^{-9}$; patients *versus* relatives: $P=0.2$; relatives *versus* controls: $P=6\times10^{-6}$.

Table 2 Comparison of allele frequencies by patient group

Allele	*Affected patients*	*Unaffected relatives*	*Unaffected controls*
1	44 (0.13)	55 (0.14)	54 (0.11)
2	141 (0.42)	157 (0.41)	226 (0.47)
3	151 (0.45)	170 (0.45)	206 (0.42)
Totals	336	382	486

Alleles defined as 1 (null *CHRFAM7A*), 2 (wt *CHRFAM7A*), 3 (*CHRFAM7A* with 2 bp deletion).
Global significance for overall allele frequency comparisons: patients *versus* controls: $\chi^2=1.87$, 2 degree of freedom (df), $P=0.4$; relatives *versus* controls: $\chi^2=3.48$, 2df, $P=0.18$.

Table 3 Comparison of transmissions to offspring affected with psychosis or T/C ratio >60%

Allele	*Phenotype*	*Observed transmissions*	*Expected transmissions*	[a]*Significance* (χ^2, *df*, P)
1	Psychosis	42 (0.13)	42 (0.13)	$\chi^2=0.01$, 1df, $P=0.9$
2		136 (0.43)	136 (0.43)	$\chi^2=0.00$, 1df, $P=1.0$
3		142 (0.44)	142 (0.44)	$\chi^2=0.00$, 1df, $P=1.0$
Totals		320	320	
1	P50 T/C>60%	22 (0.11)	26 (0.13)	$\chi^2=2.31$, 1 df, $P=0.13$
2		82 (0.40)	85 (0.42)	$\chi^2=0.74$, 1df, $P=0.4$
3		100 (0.49)	93 (0.46)	$\chi^2=3.76$, 1df, **$P=0.05$**
Totals		204	204	

Abbreviations: df, degree of freedom; T/C, ratio of testing to conditioning wave amplitudes.
For psychosis, global significance: $\chi^2=0.01$, 2df, $P=1.0$.
For P50, global significance: $\chi^2=4.41$, 2df, $P=0.11$.
[a]Significance determined by transmission disequilibrium test.
Nominally significant *P*-value shown in bold.

Table 4 Comparison of allele frequencies by P50 T/C ratios

Allele	*All T/C ratios*	*T/C ratios >60%*	*T/C ratios <40%*	[a]*Significance*
1	84 (0.12)	28 (0.12)	38 (0.13)	$\chi^2=0.27$, 1df, $P=0.6$
2	298 (0.44)	95 (0.40)	132 (0.46)	$\chi^2=2.06$, 1df, $P=0.15$
3	296 (0.44)	115 (0.48)	116 (0.41)	$\chi^2=3.17$, 1df, $P=0.07$
Totals	678	238	286	

Abbreviations: df, degree of freedom; T/C, ratio of testing to conditioning wave amplitudes.
[a]Global significance for allele frequency comparisons T/C ratios >60% *versus* <40%: $\chi^2=3.18$, 2df, $P=0.2$.

QTDT has three tests of association: 'total', 'within' and 'stratification' (see Methods). The tests of total association were not significant (Table 5). By contrast, the tests of within-family association, which are robust to population stratification, were clearly significant. The empirical measurement of significance for global association ($P=0.004$, based on 10 000 permutations) is much stronger than the calculated value ($P=0.02$), and is regarded as more reliable[60] as it makes no assumptions regarding normality of distribution or degrees of freedom (Table 5). When each allele was considered alone, allele 3 (empirical $P=0.0006$) and, to a much lesser extent, allele 1 (empirical $P=0.03$) were both significant. The tests for stratification were also significant for global association (empirical $P=0.04$) and allele 3 alone (empirical $P=0.004$). We also modeled the effects of psychosis diagnosis, sex or age as covariates, which made very little difference to the tests of association (Supplementary Table 3).

To provide model-free evidence for the within-family association found for P50 and to identify its direction, we dichotomized the P50 scores as for the initial analysis and performed TDT using Transmit. Unsurprisingly, the loss of twin data (used in QTDT) and the loss of the full range of P50 scores caused considerable loss of power.

Table 5 Quantitative transmission disequibrium test results for P50 T/C ratio

Allele	Association test	[a]Calculated significance	[b]Empirical significance
All	total	$\chi^2=4.29$, 2df, $P=0.12$	
1		$\chi^2=2.44$, 1df, $P=0.12$	
2		$\chi^2=0.74$, 1df, $P=0.4$	
3		$\chi^2=3.44$, 1df, $P=0.06$	
All	within	$\chi^2=7.62$, 2df, **$P=0.02$**	**$P=0.004$** (10,000 permutations)
1		$\chi^2=4.35$, 1df, **$P=0.04$**	**$P=0.03$** (10,000 permutations)
2		$\chi^2=0.62$, 1df, $P=0.4$	
3		$\chi^2=7.07$, 1df, **$P=0.008$**	**$P=0.0006$** (100,000 permutations)
All	stratification	$\chi^2=4.06$, 2df, $P=0.13$	**$P=0.04$** (1,000 permutations)
1		$\chi^2=2.09$, 1df, $P=0.15$	$P=0.13$ (1,000 permutations)
2		$\chi^2=0.24$, 1df, $P=0.6$	
3		$\chi^2=4.34$, 1df, **$P=0.04$**	**$P=0.004$** (10,000 permutations)

Abbreviation: T/C, ratio of testing to conditioning wave amplitudes.
[a]Calculated significance was derived from the differences in log likelihood scores (χ^2) and degrees of freedom (df) between the null and alternative models.
[b]Empirical significance was derived from multiple permutation tests, number of tests as shown. Nominally significant *P*-values shown in bold.

Nonetheless, nominally significant association with allele 3 was detected ($\chi^2=3.76$, 1 degree of freedom (df), $P=0.05$; Table 3, bottom). These results support the QTDT within-family tests of association and show that the direction is of elevated P50 scores associated with increased transmission of allele 3.

DISCUSSION

Within-family tests provide evidence for association of the CHRFAM7A variants with P50 T/C ratio, with most of the association between elevated ratios and raised allele 3 (2 bp deletion) frequencies. However, χ^2-tests and tests combining between- and within-family associations, that do not control for stratification, were not significant, although such tests considering allele 3 alone almost reached nominal significance. This pattern of findings could indicate a type I error in the within-family tests of association. However, they may also be explained by population stratification for the genetic variants investigated here, which would invalidate between-family tests, whereas within-family tests remain robust. Supporting this, we found that tests of population stratification for these genetic variants were significant, indicating that only the within-family association test is valid and suggesting that significant between-family association may have been masked by population stratification.[59] Similar results were found for other associations.[63,64]

Although our cohort were all white Caucasians, some genetic heterogeneity within this ethnic group would not be surprising. Investigating the sample with a panel of ethnically specific SNPs might clarify this issue, although CHRFAM7A variants investigated here show a wide allele frequency variation between ethnicities,[21] suggesting that stratification effects may be particularly pronounced for these variants. Although population stratification is a plausible explanation for the difference between within- and between-family tests of association, other factors may be involved.

One potential source of error could be incorrect genotype calls, which are known to increase type I errors in family-based association studies.[65,66] Therefore, the observed within-family association might be because of genotype errors that we estimated at 6%. Using simulated error rates up to 10% in SNPs, type I errors were found to be highest where both alleles had very different frequencies and minimal with alleles of equal frequency,[65] with similar observations for multiallelic polymorphisms.[66] Our strongest association was due to an excess of allele 3 transmissions from 13 and 23 heterozygotes. Allele 3 frequency is close to the combined frequencies of the other two alleles, where the error-induced type I error rate is expected to be low. This suggests that genotype errors are unlikely to account for the significant within-family association.

Unlike P50, the psychosis phenotype showed no evidence for association with the CHRFAM7A alleles investigated. This is consistent with the hypothesis that endophenotypes may confer greater power to detect association than the disease phenotype, where the variance is spread over a wider range of genetic and environmental factors. Furthermore, the psychosis phenotype is not amenable to quantitative family-based studies such as QTDT, which under certain circumstances may also have more power than categorical association tests.

Of the six previous studies involving CHRFAM7A variants and either a psychosis phenotype or endophenotype,[19,21–24,67] four found a significant association with the 2-bp deletion. However, there is no consistency with the phenotype involved. Raux *et al*[23] found a higher frequency of the 2-bp deletion allele in individuals with a high P50 T/C ratio, but no association with schizophrenia. Our data support these results. By contrast, Sinkus *et al*[21] found no association with P50, but significant association with schizophrenia in both Caucasians and African-Americans. Dempster *et al*[24] found an association between the same allele and episodic memory deficits, a possible endophenotype of schizophrenia. Finally, Hong *et al*[22] found an association between the 2-bp deletion and bipolar disorder. It is possible that at least some of these four studies may be detecting the same genetic effect as in the present study.

The biological effect of the 2-bp deletion in CHRFAM7A is difficult to infer as the role of CHRFAM7A, found uniquely in humans, is poorly understood. The gene is transcribed, as mRNA sequences have been detected.[17,68] Two recent studies demonstrated that co-expression of CHRFAM7A with CHRNA7 significantly reduced acetylcholine currents.[69,70] This appeared to be a posttranslational effect, and the presence of the 2-bp deletion in exon 6 of CHRFAM7A further reduced these currents.[70] The CHRFAM7A sequence predicts two different potential proteins that contain some α7 nicotinic receptor amino-acid sequence, one being prevented by the 2-bp deletion, whereas the other is only possible in its presence. However, there is no evidence for either protein.

Another possibility is that the 2-bp deletion is linked to a functional polymorphism. Previously, we found that CHRFAM7A commonly exists in either orientation (Supplementary Figure 1a) and that the 2-bp deletion is strongly associated with CHRFAM7A in the same orientation as CHRNA7.[20] This suggests that the 2-bp deletion tags the orientation of CHRFAM7A, although this does depend on a single study. It is possible that the orientation of the gene might affect its expression, as the DNA environment upstream of the likely location of the CHRFAM7A promoter would be altered by the inversion. However, there is an alternative way in which the orientation of CHRFAM7A might exert a biological effect. The rare 15q13.3 microdeletion is strongly associated with many neuropsychiatric conditions, including schizophrenia.[25–27] This deletion is recurrent and probably arises from non-allelic homologous recombination when two direct repeats misalign during meiosis. Two large direct repeats likely to be responsible include CHRNA7 and CHRFAM7A[71] (see Supplementary Figure 1a). The occurrence of these two genes in the same orientation therefore predisposes, in a very small minority of cases, to the 15q13.3 microdeletion in the next generation. A similar misalignment of these direct repeats might occur during DNA replication. An accumulation of such 15q13.3 microdeletions in key neural cells might mimic some of the

phenotypes associated with patients who have inherited this deletion. If it occurs, it would be limited to individuals with at least one copy of CHRFAM7A in the CHRNA7 orientation. Our finding of an association between the P50 sensory gating deficit with the 2-bp deletion allele provides an indirect evidence for an association with this orientation of CHRFAM7A, which is consistent with this intriguing possible mechanism.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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