1. Introduction

Schizophrenia is a common, genetically heterogeneous disorder with a lifetime prevalence of approximately 1% in the general population. Most candidate-gene studies have derived their functional authority from neuropharmacological studies suggesting that abnormalities in monoamine neurotransmission—in particular, dopaminergic and serotonergic systems—play a role in the aetiology of schizophrenia. Overall, the results presented in this extensive body of literature are disappointing [34].

Evidence of a susceptibility locus for schizophrenia, mapping to chromosome 8p22-21, has been reported by a number of research groups [4–6,17,27–30,35,39,40]. Other studies have either offered less significant statistical support or have been negative [20,44]. Both endogenous [41,13] and exogenous [26,14] neurotoxic substances have been involved in the genesis of schizophrenia.

N-acetyltransferases (NAT) have been linked to the detoxification of several dietary and occupational arylamine carcinogens, primarily functioning as phase II conjugation enzymes. There are two genes encoding functional NAT in humans (NAT1 and NAT2) on chromosome 8p22 within 400 kb from each other [33]. Their corresponding proteins are enzymes that are involved in the metabolism and detoxification of drugs and other foreign chemicals and might be involved in brain pathology through abnormal detoxification, secondarily affecting the brain [7].

NAT2 is polymorphic, metabolises aromatic amines, and is involved in the phase II metabolism of several compounds, for example caffeine and tobacco. This enzyme has been used in several studies to relate acetylation capacity to disease, and an association between NAT2 activity and susceptibility to cancer [1,15,19,25,38], Parkinson’s disease [8,2] or systemic lupus erythematosus [43] has been described.

The NAT2 gene has been well characterised and 13 single nucleotide polymorphisms (SNPs) have been characterised in it. Permutation and combination of one to four of these comprise 29 different alleles which have been correlated with the slow, intermediate, and rapid acetylation phenotypes [9]. Of these, two missense substitutions (T341C and G590A) appear to be the most critical for determining the acetylator status in Caucasians [3,18,31,32]. However, previous data suggest a linkage disequilibrium between 341-C, 481-T, and 803-G [31,3,11,37,21] so the 481-T mutation is sufficient for detecting the “slow acetylator” (SA) allele designated NAT2*5B [31]. Slow acetylation of NAT2 is observed in a high proportion of Caucasians (40–70%) while this is less common amongst Asians (10–30%) [9,22]. “Slow acetylators” (SA) are less efficient than “rapid acetylators” (RA) at metabolising numerous chemical carcinogens and toxins [22].

Given the evidence of a susceptibility locus for schizophrenia, mapping to chromosome 8p22-21 and the failure of Blaveri et al. [7] to demonstrate association between a polymorphism of the NAT1 gene and schizophrenia we focused on other candidate 8p22 gene, the NAT2, closely linked to NAT1. In this study, we investigate the association between two SNPs (C481T and G590A) [42,10] in the NAT2 gene and schizophrenia.
2. Subjects and methods

2.1. Population

Two hundred and twenty-eight outpatients with schizophrenia [mean age = 35.6 years, range 15–69 years; 60.5% males] from the region of Asturias (Northern Spain; total population: 1 million) were enrolled in the study. All patients had a diagnosis of schizophrenia according to DSM-IV criteria (diagnoses were determined by experienced psychiatrists using the Spanish version of the Structured Clinical Interview for DSM-IV—SCID-I—). Two hundred unselected healthy individuals (Mini-International Neuropsychiatric Interview—MINI—DSM-IV criteria) [mean age = 45 years, range 25–75 years; 75% males] from the same Caucasian population were analysed. A higher age of the controls as compared with schizophrenic outpatients indicates them to be true controls.

All cases and controls were of Caucasian Spanish origin, shared similar sociodemographic profiles and were comparable as regards the geographical origin of their families. All controls and patients showing a history of drug or alcohol abuse were excluded from the study. One hundred and twenty-five (54.8%) patients and 65 (32.5%) controls were current smokers. Current smoking is defined as current daily smoking [16]. Informed consent was obtained from all individuals enrolled in the study. The study was conducted according to the provisions of the World Medical Association Declaration of Helsinki, and institutional approval of the study was granted [45]. The study was also subject to and in compliance with national Spanish legislation.

2.2. RFLP analysis of the NAT2 gene

Genotyping was performed blind to the clinical data and in duplicate, with patients and control subjects analysed side by side to eliminate errors in genotyping. Genomic DNA was extracted from peripheral white blood cells obtained from each subject, using conventional phenol-chloroform extraction and ethanol precipitation protocol. The C to T change at position 481 was identified by KpnI digestion of a 290 bp PCR fragment obtained with primers 5′ TGTCGATGCTGGGTCTG GAA 3′ and 5′ ATGAAGATGTTGGAGACGT 3′ (annealing temperature 62 °C). These PCR primers were derived from the NAT2 sequence (EMBL accession number X14672). After digestion with restriction endonuclease KpnI, the 481-T and 481-C alleles are visualised as fragments of 290 and 170 + 120 bp, respectively. The G to A change at nucleotide 590 destroys a TaqI site. DNA from patients and controls was amplified with the NAT2 primers described above and digested with restriction endonuclease TaqI for analysis. The 590-A and 590-G alleles are visualised as fragments of 290 and 230 + 60 bp, respectively. The SNPs were finally addressed to alleles NAT2*4 (none), NAT2*5B (481-T), and 6*B (590-A) [22].

2.3. Statistical analysis

Observed frequencies were compared to those expected according to the Hardy-Weinberg equilibrium through a $\chi^2$ test. Differences between allele and genotype frequencies were assessed using a $\chi^2$ test. Odds ratios (ORs) and their Cornfield 95% confidence intervals (95% CIs) were also calculated. SPSS/PC+ software was used for the statistical analyses. The Genecounting Program [12] was used to compare estimated haplotype frequencies in patients and controls and to test for differences with a likelihood ratio test (LRT) as well as to calculate the pair-wise linkage disequilibrium (LD) between all pairs of markers.

3. Results

The mean age of the schizophrenic group was 35.6 (11.7) [Males: 60.5%, mean age (S.D.): 34.73 (10.55); Females: 39.5%, mean age (S.D.): 36.96 (13.40)]. The most common schizophrenia subtype was paranoid (71.9%), followed by residual (11.0%), undifferentiated (6.1%), hebephrenic (4.4%), simple (3.1%), catatonic (0.4%), and unspecified (3.1%). The mean age at onset of illness was 26.2 (9.9) years. The mean time of evolution of the illness was 9.4 (8.6) years. All patients were outpatients at the time they participated in the study. Most of the patients (88.6%) were on current atypical antipsychotic treatment (mostly olanzapine, risperidone, and quetiapine) and 11.4% were receiving typical antipsychotics.

Both groups (cases and controls) showed Hardy–Weinberg equilibrium for the analysed genetic variability. Our data shows a linkage disequilibrium between both polymorphisms, according to $R$ values (Cramer’s $V$) [Patients: Cramer’s $V$ = 0.583, $P < 0.00001$; Controls: Cramer’s $V$ = 0.480, $P < 0.00001$]. The distribution of the genotypes for the C481T and G590A polymorphisms in schizophrenics and controls is summarised in Table 1. The genotype distribution of NAT2 C481T and NAT2 G590A is similar between patients

<table>
<thead>
<tr>
<th>Alleles</th>
<th>NAT2 C481T</th>
<th>NAT2 G590A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype Patient frequencies [N (%)]</td>
<td>TT 55 (24.1%)</td>
<td>19 (8.3%) A</td>
</tr>
<tr>
<td></td>
<td>TC 111 (48.7%)</td>
<td>83 (36.4%) AG</td>
</tr>
<tr>
<td></td>
<td>CC 62 (27.2%)</td>
<td>126 (55.3%) GG</td>
</tr>
<tr>
<td>Control frequencies [N (%)]</td>
<td>TT 30 (15.0%)</td>
<td>9 (4.5%) AA</td>
</tr>
<tr>
<td></td>
<td>TC 105 (52.5%)</td>
<td>81 (40.5%) AG</td>
</tr>
<tr>
<td></td>
<td>CC 65 (32.5%)</td>
<td>110 (55.0%) GG</td>
</tr>
</tbody>
</table>

χ² (df) 5.78 (2) $P$ value 0.286

### Alleles

| Patient frequencies [N (%)] | T 221 (48.5%) | 121 (26.5%) A |
| Control frequencies [N (%)] | C 235 (51.5%) | 335 (73.5%) G |
| $\chi^2$ (df) 4.48 (1) | $P$ value 0.034 |
| OR (95% CI) 1.34 (1.02–1.76) | 1.10 (0.81–1.49) |
and controls ($\chi^2 = 5.78$, df = 2, $P = 0.055$) and $\chi^2 = 2.86$, df = 2, $P = 0.239$, respectively). However, more individuals homozygous for the 481-T were found in patients than in controls ($\chi^2 = 5.57$, df = 1, $P = 0.018$; OR = 1.93, 95% CI = 1.16–2.51).

The frequency of homozygous for the 590-A was similar in both groups ($\chi^2 = 2.56$, df = 1, $P = 0.10$; OR = 1.93, 95% CI = 0.85–4.37). The 481-T allele was present at frequencies of 0.49 and 0.41 in patient and control populations, respectively ($\chi^2 = 4.48$, df = 1, $P = 0.034$; OR = 1.34; 95% CI = 1.02–1.76) (Table 1). The 590-A frequencies were 0.27 and 0.25 in patients and controls, respectively ($\chi^2 = 0.36$, df = 1, $P = 0.551$; OR = 1.10; 95% CI = 0.81–1.49) (Table 1).

Previous studies have shown that 481-TT and 590-AA individuals have significantly reduced NAT2 activity [23]. Distribution of slow acetylators, defined as those who were homozygous for at least one of the NAT2 SA genotypes or double heterozygotes, was also compared between patients and controls. These frequencies were 59% in patients and 46% in controls ($\chi^2 = 7.53$, df = 1, $P = 0.006$; OR = 1.71; 95% CI = 1.16–2.51) (Table 2). A total of four haplotypes were estimated by Genecounting to have non-zero frequencies. Significant differences in the frequencies of these haplotypes between patients and controls were apparent: LRT = 8.62, df = 3, $P = 0.035$. To identify which individual haplotypes contributed to the overall significance of this heterogeneity test, Genecounting also reported approximate LRT statistics and estimated frequencies in patients and controls for each individual haplotype. However, the estimated frequency of the haplotype that combines the putative “protective” alleles 481-C and 590-G, increased in controls (34%) in comparison to patients (25%) (LRT = 5.90, df = 1, $P = 0.041$; OR = 0.64, 95% CI = 0.43–0.98) (Table 3).

4. Discussion

One way of increasing the probability of finding genes involved in schizophrenia is by examining the candidacy of genes that map in an interval that has already been implicated by prior evidence of genetic linkage [7]. The failure of Blaveri et al. [7] to demonstrate association between a 3’UTR polymorphism of the arylamine NAT1 gene and schizophrenia in a sample of 130 patients with schizophrenia of Caucasian Irish, Welsh, Scottish or English ethnicity, lead us to focus on NAT2, another candidate gene located in 8p22, and very closely linked to NAT1. To our knowledge, this is the first report analysing the association between two NAT2 polymorphisms and schizophrenia. Our data support a possible role in schizophrenia for the NAT2-genotypes.

Significant variations have been found in NAT2 genotype distribution in populations with different ethnic backgrounds [9,32,22,24]. However, in our study, both patients and controls were matched for ethnicity and drawn from a homogeneous population. This was therefore ruled out as an explanation for the differences found.

No correction for multiple testing was made. Bonferroni’s correction is usually used when two loci studied were not in linkage disequilibrium. Moreover, according to Perneger [36], Bonferroni’s correction is too stringent in situations where (i) the multiple phenotypes are not independent and (ii) analyses have been conducted according to a pre-established biological hypothesis.

N-acetyltransferases are important detoxifying phase II enzymes with a ubiquitous expression profile, suggesting a protective role in all tissues exposed to carcinogens or toxins. In this study, we analysed two of the most frequent NAT2 polymorphisms associated with the slow acetylator phenotype. The C to T change at nucleotide position 481 that determines the loss of a KpnI site is a silent mutation [10]. However, chromosomes carrying this mutation also have two missense changes, T to C at position 341 (Ile→Thr) and A to G at position 803 (Lys→Arg), which are responsible for the slow-acetylator phenotype. Analysis of the 341 and 481 polymorphisms showed a complete linkage disequilibrium between 341-C, 481-T, and 803-G in our population (data not shown) [37]. Recent studies have described the lowest acetyltransferase activity for the enzyme with the 341/481/803 changes, also designated as the NAT2*5B allele [11,23]. The second most common NAT2 slow acetylator allele consists of a G to A change at nucleotide 590 (designated NAT2*6B allele). The amino acid 197 (Arg→Glu) change introduced by this mutation reduces NAT2 activity to 24% that of the NAT2 wild-type [23]. Thus, slow acetylators might be more susceptible to lower exposure to environmental carcinogens or neurotoxins, but this hypothesis must be investigated in metabolic studies [8].

The relevance of the possible association between NAT2 genotypes and schizophrenia is as yet unknown. Schizophrenia is a multifactorial disorder, and predisposition to the development of such a disorder is likely to be the result of several genetic polymorphisms. The 40-year search for an endogenous or exogenous psychotomimetic agent that might play a role in schizophrenia has failed thus far. Clearly, additional research is needed in this area, since, at present, several questions regarding the role that neurotoxins play in schizophrenia remain unanswered [41]. On the other hand, although the studied SNPs were not the disease causing variants, they might demonstrate...
linkage disequilibrium with a disease-causing variant present in these genes, leading to allelic association between marker and disease [7].

In conclusion, the data suggest that genetically determined changes in the acetylation status could lead to the impaired ability that schizophrenic patients may have in metabolising neurotoxic substances. However, although interesting, these results required further replication in independent samples to prove the reliability of the findings. Finally, further studies are needed to clarify the biological relevance of these findings.

Acknowledgements

This work was supported by Oviedo University grant NP-01-519-3.

References


