Higher scores of self reported schizotypy in healthy young males carrying the COMT high activity allele

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The gene for COMT is located on chromosome 22q11, an area that has been implicated in the pathogenesis of schizophrenia through linkage studies and the detection of deletions in schizophrenics and velocardiofacial syndrome patients that often present psychotic symptomatology. Additionally catechol-O-methyl transferase activity has been found increased in schizophrenia and a functional polymorphism in the COMT gene itself has been associated with the disease, as well as with aggression in patients. We tested the hypothesis that COMT genotype for the functional Val158Met might contribute to the variance of self reported schizotypy and aggression scores in the normal population. We genotyped 379 healthy 18- to 24-year-old male individuals who had completed the PAS, SPQ and AQ questionnaires. Our results showed that self-reported schizotypy scores in both questionnaires were significantly related to COMT genotype (\( P = 0.028 \) for the PAS and \( P = 0.015 \) for the SPQ) with individuals homozygous for the high activity allele showing the highest scores. No significant differences were detected for AQ scores. We conclude that the COMT genotype for the functional Val158-Met polymorphism is correlated to self-reported schizotypy in healthy males. This finding is in the same direction as reported findings on schizophrenia and it adds to the list of evidence that COMT or a nearby gene in linkage disequilibrium is involved in the pathogenesis of the disease.

Keywords: catechol O-methyltransferase; genes; chromosomes; human; pair 22; questionnaires; schizotypy; aggression; schizophrenia

Introduction

There are many lines of evidence suggesting the presence of a schizophrenia-related gene on the long arm of chromosome 22. Several linkage studies for schizophrenia have reported positive findings in the 22q area.¹–⁶ Additionally velocardiofacial syndrome (OMIM No. 192430), a clinical entity characterized by cleft palate, cardiac anomalies, typical facies, learning disabilities, and a high rate of psychosis among patients and their relatives,⁷,⁸ is caused by a deletion at 22q11. Furthermore, Karayiorgou et al⁹ have identified 22q deletions in schizophrenic patients.

22q11.2 harbors the gene for catechol-O-methyltransferase (COMT), an enzyme that plays an important role in the metabolism of catecholamines including dopamine.¹⁰ The dopaminergic pathways have long been thought to play an important role in the pathogenic mechanisms of schizophrenia. Erythrocyte COMT activity has been studied in schizophrenic patients and was found elevated in some,¹¹–¹³ though not all studies.¹⁴–¹⁶ COMT activity has been found to have a normal variability, with a trimodal distribution of low, intermediate and high levels.¹⁷,¹⁸ A G to A transition at codon 158 of the COMT gene, resulting in a valine-to-methionine substitution, has been identified and found to be associated with differential COMT activity¹⁹,²⁰ and the two alleles have been termed low (Met158) and high (Val158) activity. Several studies have shown the high activity allele to be preferentially transmitted to the schizophrenic offspring,²¹–²³ although other studies have found no association with the disease.²⁴–²⁶

Schizophrenia is a complex disorder with variable phenotypic expressions. Several studies confirm a strong genetic component but it appears most likely that many different genes are involved and environmental factors also contribute to the expression of the disease in those susceptible.²⁷ Schizotypal personality often precedes the development of schizophrenia²⁸ and it is considered by some to be a low end of the schizophrenia spectrum. It has also been found to be present in high frequency in schizophrenics’ relatives (reviewed by Webb et al²⁹), suggesting a common genetic background with the disease. Although many studies have shown aggregation of schizotypal personality
in schizophrenics’ families, when positive or negative symptoms are examined there is lack of consistency in the findings. Nevertheless there have been reports of significant heritability for schizotypal personality and it has been suggested that positive and negative symptoms in schizophrenia correlate with the corresponding symptoms in schizotypal relatives. It therefore seems likely, that schizotypal personality represents a condition attributable to some (but possibly not all) of the genes that predispose to schizophrenia, as it is a common but not a consistent premorbid finding. Schizotypal personality also appears to have a higher penetrance since it is often found in non-affected family members. Both these characteristics, if true, may considerably augment the power of linkage and genetic association studies when schizotypy, which is the related continuous measurable trait, is the phenotype used and not the disease itself. Although the association study is an advantage, as it allows the study of its variation in relationship to the genotype, as opposed to measuring genotype frequencies in subgroups defined by arbitrary cut-off points. On the other hand ‘pencil and paper’ methods are often not considered very reliable, certainly not as reliable as clinical evaluation. Nevertheless the nature of these methods makes them very objective as scores are not assigned by the investigator but they are derived by a common for all subjects scoring system. In addition to this, they can be administered to large numbers of individuals far easier than clinical evaluation. The possibility of errors in the assessment due to the use of questionnaires is present and it could introduce random noise in the results, which could mask possible findings. This is balanced though by the potential to use larger samples.

Taking into account these observations, we set out to test whether the genotype for the Val158Met functional polymorphism in the COMT gene on chromosome 22q11.2 is related to the schizotypy scores of normal subjects in two well established questionnaires, the Schizotypal Personality Questionnaire (SPQ) and the Perceptual Aberration Scale (PAS). The SPQ was used to measure overall schizotypy without accounting for both positive and negative symptoms. The PAS was used because it is a widely accepted scale and it has been shown to present a significant heritability and to give higher scores among schizophrenics’ relatives. Other scales were not used due to examination time limitations. Additionally, because a correlation between the COMT genotype and violence in Schizophrenic patients has been reported, we analyzed for the same subjects their scores for the Buss–Perry Aggression Questionnaire, in order to study possible associations of the COMT genotype with aggression in our non-clinical sample.

Materials and methods

Subjects
Our 379 unrelated subjects were a random portion of 2075, 18- to 24-year-old male airforce recruits, who participated in a larger study (the ASPIS, Stefanis et al, submitted for publication) which, among other cognitive and oculomotor tests, involved administration of the Perceptual Aberration Scale, the Schizotypal Personality Questionnaire and the Aggression Questionnaire. The selection of only part of the sample was necessary for technical reasons and it was done in a random fashion, according to the last digit of an alphabetically assigned identification number. The Greek airforce recruits are an age-defined male population of Greek citizens. No other criteria except their personal preference and the needs of the airforce are used for their selection, although there is an observed tendency for them to be of a slightly higher socioeconomic status than ground forces recruits. The male only sex was a necessary restriction. This possibly limits the application of our findings, but it might also provide additional power in case there is a sex-specific effect, a possibility that is consistent with various findings in the schizophrenia literature. Our goals and procedures were explained to the subjects and written informed consent was acquired.

Questionnaires
The Perceptual Aberration Scale, the Schizotypal Personality Questionnaire and the Aggression Questionnaire were translated into Greek by two independent bilingual translators and back translated by two other bilingual translators. The back translations were communicated to the authors of the questionnaires for review and were accepted as accurate. In addition four validity questions from the Temperament and Character Inventory (TCI) were included and individuals found to respond unreliably were excluded from the analysis.

DNA extraction
DNA was extracted from mouthwash as follows: 15 ml of sterile saline solution was supplied and the subjects were instructed to perform a rigorous mouthwash for approximately 20 seconds. The sample was centrifuged at 1440 g for 15 min at 4°C. The pellet was resuspended in 2 ml Lysis buffer (2% SDS, 0.1 M NaCl, 0.05 M tris-HCl (pH 8.0) 1 mM EDTA) and incubated at 37°C for 16 h with 2 mg of Proteinase K. Proteins were precipitated and removed with the addition of 1 ml of 6 M NaCl, and centrifugation at 2160 g for 20 min at 4°C. DNA was precipitated from the supernatant with ethanol.

Mouthwash was chosen in order to obtain a better procedure acceptance rate and to avoid possible denials related to psychological profile (eg fear of the needle). It has been shown in large samples that there are no genotyping discrepancies between DNA obtained from blood and mouthwash.
**COMT typing**
Typing was performed by PCR amplification, digestion with the restriction enzyme NlaIII and 3% agarose electrophoresis as described by Kirov et al.\(^4\)\(^5\) Agarose gels were scored independently by two researchers (DA and IH) blind to the schizotypy and aggression scores of the subjects. Alleles were assigned as L (low activity, NlaIII site present) and H (high activity, NlaIII site absent).

**Statistical analysis**
Basic statistics and regression analyses were performed for the three quantitative variables. The subjects were then assigned into three groups according to their genotype (Val/Val (HH), Val/Met (LH) or Met/Met (LL)) and one way Analysis of Variance (ANOVA), using the genotype as the grouping variable and the scores in the PAS, SPQ and AQ as dependent variables, was performed. Additionally, a chi-square test between genotype subgroups are shown on Table 1. Correlation analysis showed highly significant correlations between the three variables (PAS vs SPQ \(r = 0.64\), PAS vs AQ \(r = 0.33\), SPQ vs AQ \(r = 0.48\), \(P < 0.001\) in all cases).

COMT genotyping showed that the two alleles, H and L were present at overall frequencies of 53.4% and 46.6% respectively. The three genotype frequencies were LL: 21.3% (81/379), LH: 50.4% (191/379), HH: 28.2% (107/379) not deviant from that expected for genotypes in Hardy–Weinberg equilibrium.

Higher mean scores were observed for all variables in individuals homozygous for the high activity allele (see Table 1 for means and SD for the three groups). Analysis of variance (ANOVA) showed that the differences observed for the PAS and SPQ scores between the three possible genotypes were statistically significant, while the differences observed in the AQ were not. More specifically HH homozygotes had significantly higher scores than LH heterozygotes (PAS: \(P = 0.005\), SPQ: \(P = 0.024\)) and combined non-HH individuals (PAS: \(P = 0.012\), SPQ: \(P = 0.006\)) in both questionnaires. HH homozygotes also had significantly higher scores for the SPQ than LL homozygotes (\(P = 0.006\)) while significance limits were not reached for the PAS, possibly due to the small number of individuals in the homozygosity groups. The differences between LL and LH individuals were not statistically significant.

In order to investigate the global effect of genotype on schizotypy we also used a general linear model analysis. This analysis allowed us to examine the effect of genotype on both PAS and SPQ scores in a multivariate analysis. The rationale for this approach was that both PAS and SPQ actually measure a single underlying trait of schizotypy and they are highly correlated (Pearson \(r = 0.65\); \(P < 0.001\)). By using this analysis we could get an estimate of the total effect of genotype on schizotypy (measured with two independent variables, PAS and SPQ) and also estimate the specific effects of genotype on each one of these variables. We used a general linear model analysis where genotype was the single categorical predictor variable and logPAS and SPQ were the dependent variables. Log PAS was used in order to normalize the distribution of PAS scores which was, as mentioned above, skewed. Four subjects’ scores could not be used as they were 0 for PAS and could not be log transformed. It must be noted that two of those were in the HH group and two in the HL group and therefore their omission had a small positive effect on the reported values for this analysis. The Wilks test for the general effect of genotype in this model was highly significant (\(F = 4.20\), \(P < 0.002\)). The model \(r\) for the specific effect of genotype on logPAS was 0.18, and the \(r^2\) was 0.034, indicat-

|          | Mean ± SD (n) | Mean LL ± SD (n) | Mean HL ± SD (n) | Mean HH ± SD (n) | F       (P)          |
|----------|---------------|------------------|------------------|------------------|---------|----------------|
| PAS      | 7.55 ± 5.07 (366) | 7.61 ± 5.74 (77) | 6.95 ± 4.8 (186) | 8.61 ± 4.88 (103) | \(F = 3.60\) \(\text{ns}\) |
| SPQ      | 28.06 ± 12.07 (365) | 25.81 ± 12.37 (77) | 27.48 ± 12.02 (185) | 30.77 ± 11.57 (103) | \(F = 4.22\) \(\text{P = 0.015}\) |
| AQ       | 81.84 ± 16.09 (334) | 79.72 ± 15.27 (74) | 81.77 ± 16.95 (166) | 83.65 ± 15.07 (94) | \(F = 1.23\) \(\text{P = ns}\) |

SD, standard deviation; n, number of observations; *difference significant below the 0.05 level (ANOVA); ns, not significant.
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Discussion

It has been proposed by many that in order to solve the schizophrenia puzzle, it might be fruitful to examine phenotypes that are strongly related to the disease and appear in high frequencies in relatives of the patients. These traits, termed endophenotypes might be associated to fewer genes and seem to present a higher penetrance than the disease itself, providing better targets for genetic studies. A number of such endophenotypes have been proposed, such as differences in cognitive and occulomotor functions, inhibition of the P50 evoked potentials etc.46 Some of these, like eye tracking and occulomotor functions, inhibition of the P50 evoked potentials etc.46 have already been used for linkage analysis and provided positive results (chromosome 6p21–23,47 and chromosome 15: alpha-7 nicotinic acetylcholine receptor gene locus,48 respectively). Also Myles-Worsley et al,49 using both antisaccade and P50 as a combined inhibitory phenotype, found evidence of linkage on chromosome 22q, close to the COMT gene locus. Interestingly positive linkage findings in all these regions have also been reported for schizophrenia.50,51

Schizotypal personality often precedes schizophrenia and is also present in high frequency in schizophrenics’ relatives.52 Therefore schizotypy which is the relevant quantifiable personality trait shares some of the same properties with endophenotypes. Nevertheless, so far there are no reported studies attempting to associate schizotypy with candidate genes, or to use it for linkage analysis.

There are many studies linking schizophrenia with the COMT gene locus, the COMT functional polymorphism, and COMT activity as we mentioned in more detail in our introduction. We report here an association between the COMT functional polymorphism and self-reported schizotypy as measured by two well-established questionnaires. Our result is in agreement with the previous positive findings on schizophrenia, adding to the list of positive findings associating COMT with the disease. Nevertheless other studies have failed to find association and linkage between COMT and schizophrenia. The differences we observe here as well as the reported linkage and association results of others with schizophrenia indicate that the effect of the COMT genotype although present is not very strong. A weaker effect is easy to miss in association studies and hard to detect in linkage studies, depending greatly on the family panel that is used. The most likely scenario for schizophrenia is that more than one gene with variable contributions is involved in its pathogenesis.27 The large number of positive findings to which we contribute here, make it likely that COMT, or a nearby gene in linkage disequilibrium with this polymorphism, is one of them. Further verification of these results will be necessary. Multiple well studied established associations of genes with schizophrenia could lead to a better understanding of the disease and the candidate pathways for pharmacological intervention and possibly the development of a polymorphism panel with a significant predictive value.

Our data indicate that the COMT genotype is associated with schizotypy scores in both questionnaires used. The SPQ, a questionnaire that shows a normal distribution in the sample, shows higher scores for HH homozygotes, intermediate scores for HL heterozygotes and lower schizotypy scores for LL homozygotes. Additionally, the top 10% scorers in both questionnaires have a significantly higher frequency of the HH genotype than the bottom 10% scorers. Although the PAS questionnaire shows higher scores for HH homozygotes, heterozygotes show lower scores (yet not significantly) from LL homozygotes. The sample is not large enough to clearly establish the significance of this unexpected inter-group variation, and the two questionnaires are not measuring exactly the same parameter, therefore conclusions cannot be drawn from this observation. It appears that PAS scores behave in a somewhat more complicated way than SPQ scores in relation to phenotype, a finding that could be related to the fact that PAS scores demonstrate a skewed distribution towards low scores. Nevertheless it is a consistent finding in both questionnaires, that HH homozygotes show higher schizotypy scores.

We report here performing ANOVA for three different ‘paper and pencil’ phenotypes using the COMT genotype as an independent variable. Additional comparisons have also been performed without positive results using our data from cognitive and occulomotor tests. The significance level of our finding cannot withstand correction for multiple comparisons and should therefore be interpreted with caution and as an adjunct to the existing literature on the subject. Caution is also
necessary as positive association results are often due to population admixture and stratification. Our population is one of Greek nationals selected with the criterion of age. The vast majority of male Greek nationals serve in the Greek armed forces and there are no set criteria for assignment in the airforce. Therefore our sample represents well the Greek population of this age stratum. Although as with most European populations, a certain extent of admixture is possible, it is unlikely that it is large enough to account for our results.

Regarding the reported association between the low affinity allele and aggressive and antisocial behavior in schizophrenics, our study did not detect any significant differences in aggression scores between genotypes in our sample. The small, but not significant difference that was present, was in the opposite direction than that reported. Additionally no difference was observed in aggression scores between genotypes among high scorers (10%) in schizotypy (although this subgroup is too small to allow for conclusions). Therefore, the reported finding might be confined to patients and not be present in our non-clinical sample.

The dopamine hypothesis for schizophrenia suggests that there is an excess of dopamine activity associated with the disease. COMT is an enzyme that is involved in the O-methylation and degradation of catecholamines including dopamine. Finding the high activity allele to be associated with schizophrenia might seem to be opposite to what one would expect. Ours, and others’ experimental results are however consistent regarding the direction of the association. Dopamine regulation does not occur through the same mechanisms in all brain areas. COMT appears to be of greater importance in dopamine activity regulation in the prefrontal cortex,3,4,5 in areas involved in cognitive functions. Cognitive function is enhanced by dopamine in schizophrenia: report of potential linkage for schizophrenia on chromosome 22q; Part 3. Am J Med Genet 1995; 60: 172–173.


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