Impact of COMT H108L, MAOB int 13 A>G and DRD2 haplotype on the susceptibility to Parkinson’s Disease in South Indian subjects

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In view of documented evidence demonstrating the association of dopaminergic metabolism and neurotransmission with Parkinson’s disease (PD), a case-control study was conducted to investigate the impact of particular polymorphisms in the catechol O-methyl transferase (COMT) H108L, monoamine oxidase B (MAOB) int 13 A>G, dopamine transporter 1 (DAT1) A1215G, dopamine receptor D2 (DRD2) Taq1A, DRD2 Taq1B and DRD2 Taq1D genes on the susceptibility to PD. PCR-RFLP method was used for the genetic analysis. The COMT H108L polymorphism increased PD risk by 1.4-fold (95%CI: 1.02-1.98), whereas reduced risk was observed with MAOB int 13 A>G polymorphism (OR: 0.77, 95%CI: 0.51-0.99). Multifactor dimensionality reduction analysis showed gene-gene interactions between these two loci that resulted in loss of the protective role of MAOB G-allele in the presence of COMT L-allele. DAT1A1215G polymorphism in the exon 9 was not associated with PD. Individually, DRD2 polymorphisms showed null association. However, all-variant haplotype of DRD2 locus i.e. T-G-T haplotype showed 29.8-fold risk for PD compared to all-wild haplotype i.e., C-A-C haplotype (95%CI: 6.85-130.4). To conclude, genetic variants of COMT, MAOB and DRD2 loci modulate susceptibility to PD in South Indian subjects.

Keywords: Parkinson’s disease, Dopamine, Catechol O-methyl transferase, Monoamine oxidase, Dopamine receptors, Polymorphism.

Parkinson’s disease (PD) is a progressive neurodegenerative disease with complex etiology. Different studies have shown that exposure to environmental agents is the main causative factor for sporadic PD¹,². However, recent study has shown that environmental triggers in association with genetic changes can alter individual’s susceptibility to this disease³.

The major hallmark of PD is the degeneration of dopamine neurons that innervate a part of the basal ganglia known as the striatum. This degeneration results in disordered movement. Genetic variations in dopamine transporter and receptor genes, as well as the enzymes involved in dopamine metabolism, such as catechol O-methyl transferase (EC. 2.1.1.6; COMT) and monoamine oxidase B (EC.1.4.3.4; MAOB) can alter an individual’s susceptibility to the disease⁴. Both COMT and MAOB act synergistically in the degradation of dopamine. COMT methylates dopamine to 3-methoxy tyramine, which is further oxidized to the final end product homovanillic acid by MAOB⁵.

COMT is a ubiquitous enzyme that inactivates neurotransmitters, catechol hormones and drugs, such as levodopa. A functional polymorphism of COMT gene, characterized by amino acid change at codon 108 (valine to methionine) has been studied with relevance to PD⁶. Transition of G to A at position 1947 of COMT gene results in the substitution of methionine for valine at codon 108 that alters the enzyme from the highly active H-form to the lower activity L-form, thereby rendering the protein thermolabile⁷,¹⁰. Decreased activity of COMT would result in the metabolism of dopamine to neuromelanin and augment neuronal cell death by enhancing cytotoxicity⁹. The L-form or variant allele

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Abbreviations: COMT, catechol O-methyl transferase; DRD2, dopamine receptor 2; DRD4, dopamine receptor 4; DAT1, dopamine transporter 1; MAOB, monoamine oxidase; MPTP, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine; MDR, multifactor dimensionality reduction analysis; PD, Parkinson’s disease; RFLP, restriction fragment length polymorphism; ROS, reactive oxygen species; VNTR, variable number tandem repeat.
of COMT (COMT L) is shown to be involved in the pathogenesis of PD in Japanese population, whereas studies on Asians and Causacians have failed to demonstrate any association\textsuperscript{10,11}. MAOB is a key metabolic enzyme involved in the inactivation of dopamine intraneuronally. It is also involved in the pathogenesis of PD by the activation of exogenous neurotoxins, such as 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) and production of reactive oxygen species (ROS), which are deleterious to dopaminergic cells\textsuperscript{12}. An intronic polymorphism (intron 13 A/G) in MAOB has been widely studied and its association with the disease has been reported in Caucasians\textsuperscript{13,14}, whereas no such association has been observed in a Japanese population\textsuperscript{15}.

The downstream physiological consequences of dopaminergic neurotransmission are binding of dopamine to specific receptors present on the post-synaptic nerve terminal. Dopamine exerts motor and cognitive function through the interaction with specific receptors of G-protein coupled receptor family\textsuperscript{16}. Based on biochemical, pharmacological and physiological criteria the five different subtypes of dopamine receptors, grouped into 2 sub-families D1 and D2\textsuperscript{17,18}. Of these, dopamine receptors D2 (DRD2) and D4 (DRD4) belong to D2 family that mediate signaling effect and modulate motor behavior and activity of nigrostriatal neurons\textsuperscript{19}.

A polymorphism of DRD2 gene located in the 3’flanking region known as Taq1A (rs1800497) has been studied worldwide for its association with PD\textsuperscript{20}. The T-allele of DRD2 (rs1800497) shows reduced receptor density\textsuperscript{21} and a reduction of dopaminergic activity in central nervous system\textsuperscript{22}. Two other polymorphisms of DRD2 in the regulatory region Taq1B or the intronic polymorphism Taq1D have also been studied with respect to PD. The T-allele of Taq1A (DRD2A) polymorphism has been reported to be associated with PD in the Norwegian population\textsuperscript{20} and a study on the Italian population has also found a positive correlation with G allele of Taq1B (DRD2B)\textsuperscript{23}. In Asian populations, the association of DRD2 with PD is not yet investigated, however Taq1A and Taq1B are found to be in strong linkage disequilibrium\textsuperscript{24}.

Dopaminergic neurotransmission is mainly regulated at the presynaptic nerve terminal by reuptake of dopamine from the synapse mediated by dopamine transporter\textsuperscript{25}. A variable number tandem repeat (VNTR) polymorphism in the 3’-untranslated region and A1215G polymorphism in exon 9 of the DAT1 gene has been studied with respect to PD\textsuperscript{26,27}. Association studies on these genetic polymorphisms have showed contrary results, probably due to differences in ethnicity and environmental exposures. Studies from Indian population are sparse and none of them have conducted a comprehensive analysis of genetic variants of dopamine metabolism and transport in PD. Here, we report a case-control study to identify individual, as well as cumulative impact of polymorphisms in COMT, MAOB, DAT1, DRD2 genes on the susceptibility to PD in South Indian subjects.

Materials and Methods

Subjects

A total of 150 unrelated Parkinson’s disease (PD) patients in the age group of 55.7 ± 10.5 yrs were recruited from the Department of Neurology, Nizam’s Institute of Medical Sciences (NIMS), Hyderabad, India. Cases were diagnosed and confirmed by consultant neurologist based on UK Parkinson’s disease rating scale (UKPDRS). Presence of two of the four cardinal signs i.e. resting tremor, cogwheel rigidity, bradykinesia and postural instability was regarded as inclusion criteria for the diagnosis. All cases of secondary Parkinsonism were excluded from the study. Controls comprising 200 age, gender and ethnicity-matched healthy individuals free of any neurological disease were recruited in the study. Demographic data related to age, gender, and smoking history was taken. The study was approved by the Institutional Ethical Committee of Nizam’s Institute of Medical Sciences, Hyderabad, India (EC/NIMS/1289/2011). Informed consent was obtained from all the subjects (or from dependents) participated in the study.

Whole blood samples (6 ml) were collected in EDTA vacutainers from all the subjects (cases and controls) and plasma was separated immediately following centrifugation at 3500 rpm for 10 min and stored at -70°C until analysis. Genomic DNA was isolated from the blood using a standard protocol\textsuperscript{28}.

Genetic analysis

Genetic polymorphisms in dopamine metabolizing and transport genes were analyzed by PCR-restriction fragment length polymorphism (RFLP) method. RFLP was used to determine COMT rs4680 G>A, MAOB rs1799836 intron13 A>G polymorphism,
DAT1 rs6347, DRD2A rs1800497, DRD2B rs1079597 and DRD2D rs1800498 single nucleotide polymorphism (SNPs) using specific restriction enzymes. The optimized reaction conditions and primers used are shown in Table 1.

**Statistical analysis**

Deviation from Hardy-Weinberg equilibrium was tested using Chi square test for observed and expected frequencies. Fischer exact test was conducted on a 2 x 2 contingency table in which data was computed based on the presence or absence of the variable in cases and controls. Odds ratio (OR) and 95% confidence interval (CI) were calculated to predict the risk associated with variant genotypes. Using multiple logistic regression analysis, confounding variables, such as age, gender and smoking were adjusted to rule out independent association of each variable.

All the statistical analysis was done by Statpages.org. Gene-gene interactions were studied using multifactor dimensionality reduction analysis (MDR) version 2.0 beta 8.4. MDR builds an interaction graph based on entropy estimates which facilitates interpretation of the relationship between variables. Haplotype analysis and linkage disequilibrium statistics were performed using web-based SHEsis software.

**Results**

The genotype distribution of all the variants was in accordance with Hardy-Weinberg equilibrium, except for MAOB, which was X-linked. Variant allele frequency of COMT rs4680 and MAOB rs1799836 in cases and controls (COMT: 37.3% vs. 29.5; MAOB 32.3 vs. 40.3) showed significant association with the disease. Logistic regression analysis showed significant independent association of COMT L-allele with PD risk (OR: 1.4, 95% CI: 1.02-1.98, \(p = 0.05\)) and significantly reduced risk with MAOB G-allele (OR: 0.71, 95% CI: 0.51-0.99, \(p = 0.05\)) (Table 2).

Dominant genetic model confirmed the protective role of MAOB (rs1799836) AG and GG genotypes against PD (OR: 0.59, 95% CI: 0.37-0.93). The minor allele frequency of dopamine transporter 1 (DAT1) and receptor gene variants were found to be equally distributed in cases and controls [DAT1 (22% vs

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Location</th>
<th>Primer sequence (5’…………. .3’)</th>
<th>(T_m)°C</th>
<th>Amplicon size</th>
<th>Restriction enzyme</th>
<th>Restriction site</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT</td>
<td>Codon 108</td>
<td>TCGTGGACGGCCTGATTCAGG AGGTCTGACAACGGGTCAGGC</td>
<td>55</td>
<td>217</td>
<td>NlaIII</td>
<td>+ (A allele)</td>
</tr>
<tr>
<td>MAOB</td>
<td>Intron 13</td>
<td>GGAACCTCCTTACACACAGG GACTGCGAGTTTCATCTCTC</td>
<td>54</td>
<td>232</td>
<td>Tsp45I</td>
<td>- (G allele)</td>
</tr>
<tr>
<td>DAT1</td>
<td>A1215G</td>
<td>CATCATCTACCAGGAAGGC CAGGGTGACGGATCATGA</td>
<td>62</td>
<td>83</td>
<td>DdeI</td>
<td>- (G allele)</td>
</tr>
<tr>
<td>DRD2A</td>
<td>3' UTR</td>
<td>ACCCTCCTGATGTCATA CAGGGTGACGGATCATGA</td>
<td>57</td>
<td>310</td>
<td>TaqI</td>
<td>- (T allele)</td>
</tr>
<tr>
<td>DRD2B</td>
<td>Intron1</td>
<td>GATACCCACTCTCAAGAGTC GATGTTGAGAATTACGCCAGG</td>
<td>57</td>
<td>457</td>
<td>TaqI</td>
<td>- (G allele)</td>
</tr>
<tr>
<td>DRD2D</td>
<td>Intron 2</td>
<td>CCCAGCAAGGAGGAGAGGGA GACAAGTACTTGGTAAGCATG</td>
<td>60</td>
<td>419</td>
<td>TaqI</td>
<td>- (T allele)</td>
</tr>
</tbody>
</table>

\(T_m\): Annealing temperature; ‘+’: indicate site created, ‘-’: indicate site destroyed

Table 2—Minor allele frequency distribution in cases and controls

<table>
<thead>
<tr>
<th>Polymorphism studied</th>
<th>MAF</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR (95% CI)</th>
<th>P value</th>
<th>Adjusted OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT rs4680</td>
<td>37.3</td>
<td>39.5</td>
<td>29.5</td>
<td>1.42(1.02-1.98)</td>
<td>0.03*</td>
<td>1.37(0.99-1.88)</td>
<td>0.05*</td>
</tr>
<tr>
<td>MAOB rs1799836</td>
<td>32.3</td>
<td>40.3</td>
<td>31.2</td>
<td>0.71(0.51-0.99)</td>
<td>0.04*</td>
<td>0.78(0.60-1.00)</td>
<td>0.05*</td>
</tr>
<tr>
<td>DAT1 rs6347</td>
<td>22</td>
<td>16.8</td>
<td>22.8</td>
<td>1.39(0.93-2.09)</td>
<td>0.114</td>
<td>1.34(0.92-1.95)</td>
<td>0.13</td>
</tr>
<tr>
<td>DRD2 rs1800497</td>
<td>35</td>
<td>31.2</td>
<td>31.2</td>
<td>1.19(0.85-1.66)</td>
<td>0.34</td>
<td>1.26(0.89-1.78)</td>
<td>0.18</td>
</tr>
<tr>
<td>DRD2 rs1079597</td>
<td>32</td>
<td>29.6</td>
<td>29.6</td>
<td>1.11(0.78-1.56)</td>
<td>0.60</td>
<td>1.15(0.82-1.63)</td>
<td>0.41</td>
</tr>
<tr>
<td>DRD2 rs1800498</td>
<td>34</td>
<td>37</td>
<td>37</td>
<td>0.91(0.65-1.27)</td>
<td>0.63</td>
<td>0.83(0.59-1.18)</td>
<td>0.29</td>
</tr>
</tbody>
</table>

MAF, Minor allele frequency; Crude OR, crude odds ratio; 95% CI, 95% confidence interval, Adjusted OR: odds ratio adjusted for age, sex and smoking status using logistic regression analysis; *: statistically significant.
16.8%), DRD2A (35% vs 31.2%), DRD2B (32% vs 29.2%) and DRD2D (34% vs 37%), indicating no association with PD (Table 3).

MDR analysis predicted significant gene-gene interactions between COMT and MAOB in increasing PD risk ($P_{\text{interaction}}=0.003$) (Table 4). Interaction graphing showed higher entropy estimate for COMT, suggesting epistatic modulatory effect of COMT H108L over MAOB polymorphism in nullifying its protective role (Fig. 1A, B).

Haplotype analyses of dopamine receptor gene variants showed strong linkage disequilibrium between DRD2A and DRD2B (D’=0.82) and mild linkage disequilibrium with DRD2D (D’=0.41-0.5) (Table 5). All-variant haplotype of dopamine receptor DRD2 T-G-T was associated with increased risk (OR: 29.8, 95% CI: 6.85-130.4), when compared with all-wild haplotype i.e. C-A-C (OR: 1.58, 95% CI: 1.15-2.17) (Table 6), as revealed by using the SHEsis software.
of PD is reported in Taiwanese. In Polish population, combined haplotype of MAO-B (variant) and COMT (heterozygous) is also predicted to be associated with four-fold risk in female PD patients. Our results were consistent with these observations and showed synergistic association of dopamine metabolizing gene polymorphisms like COMT V108M and MAO-B rs1799836 in elevating the risk of PD in the Indian population.

In silico studies of COMT to determine the structural and functional changes conferred by COMT V108M variant have shown that substitution of valine to methionine alters one of the α-helical structure of COMT, resulting in distortion of SAM binding site due to its close proximity with the polymorphic site, leading to the reduced activity of COMT.

Dopamine and other catecholamines are metabolized by COMT and MAOB. Low activity of COMT may cause a shift of catecholamine metabolism towards MAOB, leading to increased oxidative stress which is toxic to nigrostriatal dopaminergic neurons. Variant forms of both the enzymes might result in increased metabolism of dopamine to neuromelanin, enhancing the formation of cytotoxic radicals, contributing to neuronal degeneration, thereby elevating PD risk.

We observed no association of dopamine transporter 1 gene polymorphism (DAT1A1215G) with PD. Our results were in agreement with previous report on the lack of association between this polymorphism with PD. Further, the three different dopamine receptor (DRD2) gene polymorphisms (Taq1A, Taq1B and Taq1D) were also found to have no significant association independently with PD. The genotype frequency of Taq1B and Taq1D was observed to be equally distributed within the cases and controls. Studies on Norwegian population have shown an increased PD risk associated with DRD2 Taq1A polymorphism, whereas studies in different populations have reported no significant association. A study on Italian population has found a positive association of Taq1B polymorphism with PD.

Haplotype analyses across the various populations have found strong linkage disequilibrium at the DRD2 locus. In the current study, strong linkage disequilibrium was observed between the DRD2A and DRD2B alleles, when compared to DRD2D allele. The all-variant haplotype T-G-T of DRD2A, B and D showed decreased risk of PD, whereas gene-gene interactions predicted elevated risk in association with COMT H108L. The synergistic association between the MAOBG allele and COMT in increasing the risk
compared to all wild-haplotype C-A-C. The haplotype T-G-C showed protective phenotype, but risk was found to be increased (29.8-fold) in the presence of variant alleles in all the loci (T-G-T). Our observation was consistent with a study on Asian population, which predicted strong linkage disequilibrium between DRD2A and DRD2B.

In conclusion, the present study demonstrated the association between the genetic variants of dopamine metabolizing genes COMT and MAOB in modulating the risk of PD in South Indian population. Due to the complex etiology of PD, studies on multiple genetic loci involving different genotype combinations might provide insight towards the susceptibility of PD rather than single gene studies. However, further large-scale association studies are required to confirm the findings of this study in Indian population.

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References