

## Association of Family History of Type 2 Diabetes with COMT Gene Polymorphism (I/D) in Pakistani Population

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### Abstract

The Catechol-O-Methyl Transferase (COMT) gene polymorphism (I/D of C nucleotide at base position 900) has been previously associated in the brain disorders and inflammatory reactions but some studies also showed its presence in the development of type 2 diabetes (T2D) and kidney disease. So, the present study aimed to find association of I/D polymorphism with T2D, and its associated factors like family history and nephropathy (End Stage Renal Disease, ESRD) patients in a small group of Pakistani Punjabis.

The results identified a significant ( $p=0.02$ ) correlation of the 900 I/D C polymorphism with family history of diabetes, as it was found that greater number (74%) of patients having I allele had a positive family history of T2D. This has not been previously reported in Pakistani Punjabi population; however, this preliminary finding requires further validation studies.

**Keywords:** Type 2 diabetes; Family history; Nephropathy; Pakistani

### Abbreviations

COMT: Catechol-O-Methyl Transferase; T2D: Type 2 Diabetes; ESRD: End Stage Renal Disease; PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism; ADA: American Diabetes Association; DM: Diabetes Mellitus; DN: Diabetic Nephropathy; CDC: Centers for Disease Control; MB-COMT: Membrane-bound Catechol-O-Methyl Transferase; Hb: Haemoglobin; ANOVA: ANalysis Of Variance; BMI: Body Mass Index.

### Introduction

The catechol-o-methyl transferase (COMT) gene is an important member of the dopaminergic pathway. Although it is associated with the mental functioning and abnormalities especially parkinson disease [1-3]. But recently, this pathway has been investigated in some studies for its association with diabetes and kidney disease on the basis of hypertension [4-6]. Moreover, dopamine also acts as a natriuretic hormone and is implicated to play a role in the development and function of the kidney. Such role of dopamine has been supported from the functional studies using the COMT inhibitor in the diabetic kidney disease in rats [7,8]. Genetic association studies of the dopamine receptor genes and COMT were linked to essential hypertension [4,9]. Furthermore, dopamine also controls the activity of Angiotensin II which is the key component of Renin Angiotensin Aldosterone System (RAAS) pathway. So, the relationship of dopamine and Angiotensin II could be found in controlling the vascular tone, sodium ion balance and renal injury [9]. If this enzyme is blocked as is evident from some experiments in mice, it can provide protection from the development of diabetic nephropathy [8].

of various pathophysiological pathways, the dopamine system is also an important modulator of the renal differentiation, and is very important for the regulation of salt metabolism and in turns hypertension in addition to the schizophrenic and personality traits disorder [1,2,4]. Dopamine acts as natriuretic hormone by the inhibition of the  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  and other sodium transporters in several tubular segments. The Catechol-O-Methyl Transferase

(COMT) is one of the important genes of the dopaminergic pathway with a main function of COMT is to remove toxic metabolites from the body. Owing to importance of COMT, it is important to study the function and genetics of COMT in humans to devise targeted therapies for patients with diabetes and kidney related problems.

The COMT gene is located on the long (q) arm of chromosome 22 at position 11.21. A genome - wide linkage scan for genes controlling urinary albumin excretion revealed the linkage of chromosome 22 with diabetic nephropathy [10]. One of the insertion/deletion (Ins/Del) single nucleotide polymorphism of C nucleotide in this gene at base position 900 (i.e. 900 I/D C) has been positively correlated with nephropathy and hyperglycemia [5]. As this gene is located in the proximal tubular epithelial cells of the kidney where the dopamine synthesis takes place. Moreover, due to its location it is also involved in regulating blood pressure [6]. An association of dopaminergic pathway genes with kidney diseases among type 2 diabetics was investigated in Asian Indian population, and the results showed positive correlation of COMT gene polymorphism (900 I/D C) with chronic renal insufficiency [5]. By inhibiting dopamine metabolizing enzyme, COMT (EC 2.1.1.6) has been found to effectively abolish glomerular hypertension, and reduce progression to glomerulosclerosis by inhibiting  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  activity [7,8].

Thus, due to the significant role of the COMT gene polymorphism in kidney functions, hypertension and diabetes. Therefore, in this pilot study, we investigated the susceptibility COMT insertion/deletion

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(I/D) polymorphism in diabetics through case-control study in Pakistani Punjabi population.

## Material and Methods

A total of 191 subjects divided in four groups were included in this study divided in to diabetics, nephropathy and control groups (Description of groups in supplementary data). These samples were collected from schools, public places and in collaboration with hospitals (DHQ and Allied hospital). All subjects were above 35 years of age, for healthy controls the subjects having no hypertension, diabetes and family history was included. The diagnosis of diabetes was made according to American Diabetes Association criteria. The individuals having systolic and diastolic blood pressures above 120/80 mmHg were considered as hypertensive. After explaining the aims of the study, a written informed consent was obtained from each subject, and the study was approved by the institutional ethics review committee.

### Anthropometric and biochemical analysis

Various demographic and anthropometric measurements were taken along with blood samples from the subjects for DNA extraction and for biochemical analysis. Clinically important analytes such as hemoglobin (Hb), blood glucose, urea, creatinine, cholesterol, and triglycerides were measured on a semi-automated clinical chemistry analyzer (Microlab 300, Merck)

### Genotyping

The genotyping for COMT gene I/D polymorphism (900 Ins/Del C) was carried out by PCR coupled with Restriction Fragment Length Polymorphism (RFLP). Three different genotypes were observed II, DD and ID for COMT gene polymorphism (I/D). The already reported primers [5] were used for carrying out the PCR for amplification of COMT gene.

### Statistical analysis

All the statistical analysis was performed by using SYSTAT 11 for windows software (version 11, Systat Software Inc., Chicago, Illinois, USA) and Graphpad Instat (version 3.50). All the genotypes were tested for Hardy-Weinberg equilibrium.

## Results

COMT genotypes (II, ID, DD) were, firstly amplified by PCR by and then amplicons were separately subjected to restriction enzyme based RFLP assay. Figure 1 shows the PCR amplicon of 279 bp size of the target region of COMT gene, which was subjected for the identification of COMT genotypes (II, ID or DD). Therefore, these PCR products were further restricted by a restriction enzyme to reveal the genotypes, and the results are shown in Figure 1 with II (279 bp), DD (171 bp and 108 bp) and ID (279 bp 171 bp and 108 bp) genotypes in some of the representative samples from the studied subjects.

Baseline characteristics of all four groups were tested for the association with COMT gene polymorphism. There were no significant differences among the four groups for all of the parameters as Body Mass Index (BMI), blood pressure, hemoglobin, blood glucose, urea, creatinine, cholesterol, and triglycerides. All the results were not significant with reference to the genotypes (results not presented here).

The genotype and allele frequencies for COMT gene polymorphism were in accordance with Hardy-Weinberg equilibrium. The allele frequency for I was 0.40 and 0.60 for D allele. The genotype frequency for II was 29 (15.4%), for ID was 96 (50.3%) and for DD was 66

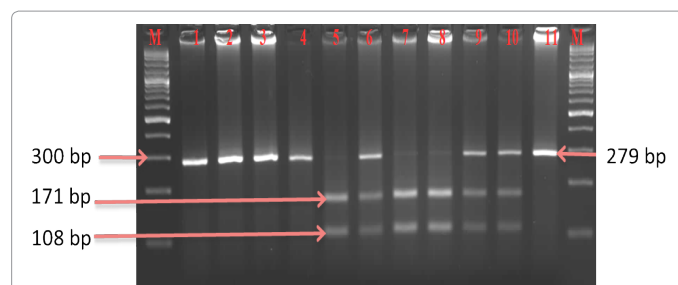
(34.3%) in the study subjects. However, there was no significant difference between the genotype and allele frequency among the three groups (G2-D, G3-DN and G4-N) versus controls (G1-C) (Results in supplementary data).

The Chi square analysis for the association of COMT polymorphism (I/D) with family history of diabetes, nephropathy and hypertension was also performed (Table 1). An important though preliminary novel finding from this analysis was a significant correlation of the COMT gene polymorphism with the family history of disease. No such correlation has been previously reported for this gene at least in Pakistani population. From the Chi-square analysis (Table 1) for COMT polymorphism, it is evident that the high percentage (74%) of subjects with family history of diabetes had II genotype.

## Discussions

In this study the COMT gene (i.e. 900 I/D C) is tested for its association with diabetes and nephropathy along with related parameters e.g. hypertension and family history of disease.

Previously, the association of COMT gene with diabetes and nephropathy has been reported in a study conducted in the Asian Indian population which was found to be positively associated with diabetic nephropathy [5]. Owing to its importance in metabolic disorders and hypertension, the COMT was selected as candidate gene for polymorphism of genotypes in a small group of Pakistani Punjabi population. As the population of this region was shared between the two countries (India and Pakistan) prior to independence and both regions have similar environmental and genetic setup. Additionally, the association of common clinical parameters was assessed for this COMT polymorphism with diabetes and nephropathy in Pakistani Punjabi population.



Lanes M on both ends contain the 100bp DNA ladder, Lane 1 contains the PCR product without the restriction enzyme, Lanes 2,3,4 and 11 contains the II genotype, Lane 5, 7, and 8 contains DD genotype and Lanes 6, 9 and 10 contain the ID genotype on 2.5% agarose gel.

Figure 1: PCR-RFLP based Genotypes of COMT gene polymorphism (I/D).

Status		II	ID	DD	*p value
Family history of diabetes	Yes	20 (74.0%)	45(48.3%)	41 (64.1%)	0.02
	No	7 (25.9%)	48(51.6%)	23 (35.9%)	
**Hypertension	Yes	10 (37.0%)	40 (44.0%)	35 (53.0%)	0.31
	No	17 (62.9%)	51(56.0%)	31 (46.9%)	
***Nephropathy	Yes	12 (44.4%)	51 (52.6%)	36 (53.7%)	0.70
	No	15 (55.6%)	46 (47.4%)	31 (46.2%)	

\*p value is considered as significant when  $p \leq 0.05$ . \*\*Hypertension: when systolic and diastolic blood pressure was more than 120/80 mm Hg. \*\*\*Nephropathy: when patients have end stage renal disease and were on dialysis. Comparison for yes/no in the same category of status was calculated by the chi square analysis.

Table 1: Effect of COMT gene polymorphism (I/D) on family history of diabetes, hypertension and nephropathy.

## Conclusions

The main finding of this study was a positive correlation of family history of diabetes with COMT (900 I/D C) polymorphism, which has not been previously reported. Since, this is a preliminary finding from a small number of samples; it is therefore, suggested that a large number of samples should be analyzed in future to authenticate this result.

As such association studies will be helpful in minimizing the disease risk in genetically predispose individuals. And also in Pakistan as the risk of diabetes and hypertension is very high so such studies will be helpful for minimizing the disease burden of diabetes and reducing the cost of the disease.

If such an association is established, it will help to minimize the risk of development of disease in genetically predisposed individuals by earlier interventions.

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