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SHORT COMMUNICATION

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Association between catechol-*O*-methyltransferase polymorphism and vitiligo

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Vitiligo is an acquired idiopathic depigmentation disorder affecting about 2% of the world's population [1]. Although the pathogenesis of this cosmetically disfiguring disease has still not been completely clarified, many investigators believe that an autoimmune reaction may play an important role [2]. Oxidative stress has been implicated in vitiligo as a causative agent [3, 4, 5, 6]. Picardo et al. have proposed that abnormal release of catecholamines from autonomic nerve endings may play an aetiological role in the onset and development of vitiligo through an overproduction of toxic radicals in the microenvironment of melanocytes [7]. Catechol-*O*-methyltransferase (COMT) is a ubiquitous enzyme that catalyses the *O*-methylation of biologically active or toxic catechols and plays a major role in the metabolism of drugs and neurotransmitters. The gene that codes COMT has been suggested to be on 22q11 [8]. In melanocytes, COMT can prevent the formation of toxic *o*-quinones during melanine synthesis [9]. Recently, it has been found that epidermal homogenates from vitiligo patients express higher levels of COMT activity than homogenates from healthy controls [10].

A common biallelic polymorphism in the COMT gene that determines the level of enzyme activity has been associated with neuropsychiatric disorders. COMT activity is genetically polymorphic in human red blood cells and liver. The genetic polymorphism results in a three- to fourfold difference in COMT activity. The differences in COMT activity are due to a G-A substitution at codon 158 of the membrane-bound forms of COMT, which corresponds to codon 108 of the soluble or cytoplasmic form, leading to a valine-methionine substitution. A valine at codon 108/158 results in the heat-stable, high-activity COMT variant (H), whereas a methionine at this position results in the heat-labile, low-activity variant (L) [11]. The two alleles (Val 108/158 or H, and Met 108/158 or L), and the three genotypes (Val 158/Val 158 or H/H, Val 158/Met 158 or H/L and Met 158/Met 158 or L/L) can be identified using a restriction fragment length polymorphism (RFLP) analysis based on a polymerase chain reaction (PCR) using the restriction enzyme Nla III [12].

The aim of this study was to determine whether COMT polymorphism might be involved in the aetiopathogenesis of vitiligo and whether there is a relationship between COMT polymorphism and clinical type of vitiligo. The study groups comprised 50 vitiligo patients and 66 control subjects. The diagnosis of vitiligo was based on a thorough physical examination, examination with Wood's light (UVA light with a wavelength of 351 mm), and evaluation of the patient's medical history. The patient's gender, age, and clinical type of disease (vitiligo vulgaris, acrofacial vitiligo or universal vitiligo) were documented.

Blood samples of approximately 7 ml were collected into Vacutainer tubes containing EDTA. DNA was extracted from peripheral blood leucocytes, and a PCR-based RFLP assay was performed to detect the presence of the G→A transition at position 1947 in COMT (accession no. Z26491). PCR was used to amplify a 185-bp fragment of genomic DNA containing the polymorphism. Briefly, the primer sequences were 5′-GGAGCTGGGGGCCTACT-GTG-3′ (forward) and 5′-GGCCCTTTTTCCAGGTCTG-ACA-3′ (reverse). PCR was performed in a 50 µl volume with 20–100 ng DNA, 100 µ*M* dNTPs, 20 pmol of each primer, 1 m*M* $MgCl_2$, 20 µ*M* Tris-HCl, pH 8.6, 50 µ*M* KCl, 0.2% (w/v) bovine serum albumin and 1 U Taq polymerase (MBI Fermentas, Vilnius, Lithuania).

Amplification was performed on an automated thermal cycler (Techne Genius, Cambridge, UK). The PCR condi-

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Table 1 COMT allele and genotype frequencies in vitiligo patients and control subjects

Genotype	Vitiligo patients, *n* (%)[a]	Control subjects, *n* (%)[b]	*P* value (Z-test)	OR (95% CI)
H/H	12 (24)	22 (33.3)	0.3562	0.6316 (0.2764–1.4434)
Male	6 (12)	9 (13.7)	0.3974	
Female	6 (12)	13 (19.7)	0.1337	
H/L	27 (54)	30 (45.5)	0.1810	1.409 (0.674–2.945)
Male	13 (26)	18 (27)	0.4390	
Female	14 (28)	12 (18.2)	0.1046	
L/L	11 (22)	14 (21.2)	0.3160	1.048 (0.429–2.557)
Male	5 (10)	10 (15.2)	0.2064	
Female	6 (12)	4 (6.1)	0.1295	

[a] *n*=50
[b] *n*=66

Table 2 COMT allele frequencies in vitiligo patients and control subjects

COMT allele	Vitiligo patients, *n* (%)[a]	Control subjects, *n* (%)[b]	*P* value	OR (95% CI)
H (Val 158)	39 (78)	52 (78)	0.4566	0.955 (0.391–2.330)
L (Met 158)	38 (76)	44 (66.7)	0.1371	0.632 (0.276–1.443)

[a] *n*=50
[b] *n*=66

tions were 3 min for initial denaturation at 94ºC, 35 cycles at 94ºC for 1 min for denaturation, 1 min at 60ºC for annealing and 1 min at 72ºC for extension, followed by 7 min at 72ºC for final extension. The resulting PCR products were subjected to restriction digestion for 3 h at 37ºC using 5 U Nla III (BioLabs). The digest products were resolved at 100 V for 20–30 min on a 4% NuSieve 3:1 Agarose (FMC BioProducts) containing 0.5 μg/ml ethidium bromide. A 100-bp marker (100 bp DNA ladder; MBI Fermentas) was used as a size standard for each gel lane. The gel was visualized under UV light using a gel electrophoresis visualizing system (Vilber Lourmat, Marne La Vallée, France). The COMT-HH genotype was represented by 114, 36 and 35-bp fragments, COMT-LL by 96, 35, 36 and 18-bp fragments, and COMT-HL by 114, 96, 36, 35 and 18-bp fragments. The 18-bp fragment was difficult to visualize because of its small size and comigration with the similarly sized primer residue; however, detection of this fragment was not critical for genotype determination. Two independent reviewers, who were unaware of the case/control status, based genotyping upon scoring of the results.

All statistical tests were carried out using SPSS 10.0 for Windows. The Chi-square test was used to compare either allelic frequencies or the genotype frequencies of COMT in patients with vitiligo and control subjects. The Z-approximation test was used to compare two independent proportions. *P* values <0.05 were considered statistically significant. The strength of association was estimated by calculating the odds ratios (ORs) and 95% confidence intervals (95% CIs) from the 2×2 table data.

Of the 50 patients, 35 (70%) suffered from vitiligo vulgaris, 13 (26%) acrofacial vitiligo, and 2 (4%) the universal form. The mean age of the control subjects was 23±6 years and of the patients was 35±16 years. In all, 50 vitiligo patients (24 male, 26 female) and 66 control subjects (37 male, 29 female) were genotyped at the COMT locus. Genotype and allele counts and frequencies for the patients and controls are presented in Tables 1 and 2.

The distributions of the H/H, H/L, and L/L genotypes were 24%, 54%, and 22%, respectively, in the vitiligo patients. No differences in COMT-HH, COMT-HL and COMT-LL polymorphism was detected between vitiligo patients and the control subjects (OR_1 0.6316, OR_2 1.409 and OR_3 1.048). However, the COMT-LL genotype was significantly associated with acrofacial vitiligo (*P*=0.047). HH and HL genotypes were not associated with acrofacial vitiligo (*P*=0.393 and *P*=0.506, respectively). Other clinical types of vitiligo were not associated with COMT genotypes. Both male and female allelic and genotypic frequencies of the vitiligo patients were similar to those of the control group (*P*=0.976, χ^2=0.048; Table 1). The allelic frequencies of the vitiligo patients resembled those of the control group (Table 2).

COMT is involved in the metabolism of some neurotransmitters and catecholamines [13]. COMT is of particular importance in preventing the formation of toxic *o*-quinones during melanin synthesis; thus, it plays a regulatory role in melanin synthesis [9]. Autodestruction by intermediates of melanin metabolism has been implicated in the aetiology of vitiligo [9, 14]. We found a significant association between the COMT-LL genotype and acrofacial vitiligo. Shelley and Öhman have observed that elevated levels of catecholamines produced by nerve endings in close proximity to the epidermis in vitiligo can damage all epidermal cells or even cause direct bleaching, thereby aggravating the depigmentation process in vitiligo [15]. Picardo et al. have proposed that abnormal release of catecholamines from autonomic nerve endings may play an aetiological role in the onset and development of vitiligo through overproduction of toxic radicals in the microenvironment of melanocytes [7]. It may be suggested that low enzyme activity leads to an increase in toxic *o*-quinones, which later results in depletion of melanocytes in acrofacial vitiligo. Our findings indicate that the lower enzyme

activity COMT genotype might be related to the clinical type of vitiligo.

Acrofacial vitiligo is the most persistent type and is mostly unresponsive to traditional treatments. These patients might be successfully treated with autologous transplantation methods [16, 17]. This polymorphism may be responsible for the resistance to treatment of acrofacial vitiligo. In contrast to our findings, Le Poole et al. have observed increased enzyme activities in vitiliginous epidermis compared with control epidermis [10]. No apparent differences in COMT-HH, COMT-LL and COMT-HL polymorphisms were detected between our vitiligo patients and the control group. Most previous studies have dealt with increased catecholamines and beta-2 adrenoceptors, high levels of monoamine oxidase A (MAO-A) and COMT in vitiligo [18, 19, 20, 21, 22]. Only Bamshad et al. have found no difference in COMT activity in the skin of vitiligo patients [23]. Since both MAO-A and COMT show increased expression and activity in vitiliginous skin, one can speculate that high and low COMT polymorphism in vitiligo is irrelevant. COMT allelic and genotypic frequencies are similar in male and female vitiligo patients. There was also no male/female difference in the distribution of alleles and COMT genotypes in the controls. Our findings and those of previous studies indicate that COMT expression and activity may be associated with vitiligo rather than COMT polymorphism.

It is now accepted that melanocyte destruction in vitiligo is an autoimmune phenomenon [24]. Oxidative stress followed by an immune response has been implicated in several immunological diseases as the primary event [25, 26]. Oxidative stress has been implicated in vitiligo as a causative agent [3, 4, 5, 6]. Schallreuter et al. have observed low catalase levels in the epidermis of patients with vitiligo. A decrease in catalase activity would be expected to increase the concentration of hydrogen peroxide, and these radicals can lead to autoimmune melanocyte destruction [27]. COMT is of particular importance in preventing the formation of toxic *o*-quinones [10]. We may speculate that in vitiligo the formation of toxic *o*-quinones cannot be prevented because of the low enzyme activity, and as a result, autoimmune melanocyte destruction is triggered. However, in our study, the low activity form of COMT was found to be associated only with acrofacial vitiligo.

This is the first report concerning COMT polymorphism in vitiligo patients. Further studies are now required to elucidate the potential role of COMT in the genetic aetiology of vitiligo. Further analysis of various phenotypic subtypes and extended samples may clarify the current findings, eventually resulting in a better understanding of the genetic components of vitiligo.

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