Association of CYP2C9 Genetic Variants with Vitiligo

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Background: Vitiligo is a depigmenting skin disorder in which genetic factors play an important role. Objective: To examine the association of CYP2C9 *1/*2/*3 gene polymorphism with vitiligo. Methods: In this case controlled study, 95 Saudi patients with vitiligo (50 men and 45 women), with a mean age of 27.3 years, were analyzed. Patients were compared to 86 healthy controls from the same locality (76 men and 10 women), with a mean age of 20.1 years. In all participants, DNA was extracted and processed for characterization of CYP2C9 *1/*2/*3 gene variants using real time-polymerase chain reaction. Results: Vitiligo patients have a significantly higher CYP2C9 *3 allele carriage rate compared to controls (32.7% versus 4.7%, \( p = 0.00 \), odds ratio = 9.9, 95% confidence interval = 3.3 ~ 29.6). On the other hand, frequencies of CYP2C9 *2 genotypes and alleles did not show any significant difference between vitiligo cases and controls. When the frequencies of CYP2C9 genotypes were compared among subgroups of age, gender, family history, and disease patterns, the cases with positive consanguinity had significantly higher frequencies of homozygous genotypes than others (\( p = 0.029 \)). Conclusion: CYP2C9 *3 allele carriage is probably associated with vitiligo susceptibility.

Keywords: CYP2C9, Genetic polymorphism, Vitiligo

INTRODUCTION

Vitiligo is an acquired skin depigmentation that affects all races but is far more disfiguring in blacks. The precise cause of vitiligo is unknown. An autoimmune process targeting melanocytes is considered to mediate its pathogenesis. Consistent with this hypothesis histological studies have reported the absence of melanocytes in the affected skin. In addition to cellular immunity, multiple autoantibodies against melanocyte antigens including various enzymes and other substances have been detected in the sera of some patients with vitiligo. Since genetic factors appear to play a role, 20% to 30% of patients were reported with a positive family history of the disorder. Nevertheless, many vitiligo patients have neither a family history of vitiligo nor a history of other autoimmune diseases. Consequently, many other hypotheses have been proposed to explain the pathogenesis of this disorder, including an inadequate defense from the toxic effects of free radicals and exposure of industrial chemicals. These effects were hypothesized to be controlled by the variable expression of cytochrome P450 (CYP or P450) genes that encode a superfamily of multi-functional monooxygenases, which comprise more than 6,000 individual enzymes. CYPs play a major role in the metabolism of foreign lipophilic compounds, including drugs and chemical carcinogens, as well as endogenous compounds such as steroids, fat-soluble vitamins, fatty acids, and biogenic amines. In addition, CYP expression and activity can be influenced by various factors such as genetic variations, presence of inhibitors or inducers, and disease states with differential tissue-specific expression pattern including the
The polymorphisms of important CYP450 genes such as CYP2C9, CYP2C19, CYP2D6, and CYP2E1 have been studied extensively in a large number of populations and showed a significant heterogeneity in the frequency of different alleles/genotypes and consequently in the resulting metabolizer phenotypes. Cytochrome P450/2C9 (CYP2C9) is primarily localized in the liver but can be expressed in other tissues like the skin. This enzyme belongs to the subfamily cytochrome 2C, which comprise CYP2C9 and 3 isoenzymes, 2C8, 2C18, and 2C19. The CYP2C9 gene is polymorphic and within the inactive alleles 2C9*2, *3, *6, *15, and *25, only *2 and *3 occur more frequently in Caucasians. In 2C9 *2 (rs 1799853) the amino acid arginine Arg 144 is replaced by Cys while in 2C9 *3 (rs 1057910) Ile 359 is replaced by Leu. Variations in the polymorphic and within the inactive alleles/genotypes and consequently in the resulting metabolizer variants of CYP2C9 was isolated from the peripheral blood using a MagNA Pure LC instrument (LC DNA Isolation Kit LV; Roche Molecular Biochemicals, Mannheim, Germany). Oligonucleotide primers and fluorescence-labeled hybridization probes were designed for amplification and sequence-specific detection of both CYP2C9 *2 and 2C9 *3 (TIB MolBiol, Berlin, Germany). The master mix used in the PCR reaction contained 2 μl of a 10× mixture of LightCycler FastStart DNA master hybridization probes, 5 mM MgCl2 (final concentration), 1 μM final concentration of primers, 0.075 μM final concentration of specific primers, and 0.2 μM final concentration of hybridization probes. Real time PCR was done using LightCycler instrument (Roche Diagnostics, Mannheim, Germany). The specificity of the amplified product was confirmed by corresponding melting curve analysis.

**Statistical analysis**

Statistical analysis was performed using the statistical software program SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Comparisons between cases and controls' genotype and allele frequencies were done using the chi-square test and odds ratio (with 95% confidence intervals). In addition, conformity to the Hardy Weinberg law of genetic equilibrium was tested among cases and controls using the chi square test through the assessment of the difference between the frequencies of the observed and the expected genotypes. A p-value <0.05 was considered statistically significant.

**RESULTS**

Normal controls showed 5 different genotypic variants including CYP2 C9 *1/*1, *1/*2, *1/*3, *2/*2, *2/*3, and *3/*3 with frequencies of 67.4%, 22.1%, 3.5%, 5.8%, 1.2%, and 0.0% respectively. Vitiligo patients had significantly higher CYP2C9 *3 allele carriage rate (both *1/*3, *2/*3, and *3/*3 genotypes) compared to controls (32.7% vs. 4.7%, p=0.00, odds ratio [OR]=9.9, 95% confidence interval [CI]=3.3 ~ 29.6). This was confirmed by the higher CYP2C9 *3 allele frequency among patients compared to controls (16.8% vs. 2.3%, OR=8.1, 95% CI=2.8 ~ 23.4, p=0.0; Table 1). Interestingly, statistical analysis of the vitiligo cases excluding the focal type that is liable for mistyping, according to a recent classification, has confirmed the previous results indicating a significantly higher CYP2C9 *3 allele frequency among cases compared to controls (17.12% vs. 2.3%, OR=8.4, 95% CI=2.8 ~ 24.8, p=0.0; data not shown). On the other hand, frequencies of CYP2C9 *2 genotypes and alleles did not show any significant difference between vitiligo cases and controls (p>0.05; Table 1). Conformity to the Hardy
Table 1. Frequency of CYP2C9 *1/*2/*3 polymorphism in vitiligo cases compared to controls

| CYP2C9 locus *2 | CYP2C9 locus *3 | Final genotype | Case (n=95) | Control (n=86) |
|-----------------|-----------------|----------------|-------------|---------------|
| *1/*1           | *1/*1           | *1/*1          | 46 (48.4)   | 58 (67.4)     |
| *1/*2           | *1/*1           | *1/*2          | 16 (16.8)   | 19 (22.1)     |
| *1/*1           | *1/*3           | *1/*3          | 29 (30.5)   | 3 (3.5)       |
| *2/*2           | *1/*1           | *2/*2          | 2 (2.1)     | 5 (5.8)       |
| *1/*2           | *1/*3           | *2/*3          | 1 (1.1)     | 1 (1.2)       |

HWE (CYP2C9 locus *2)

\[ \chi^2 = 82.7, \ p \leq 0.001 \]

HWE (CYP2C9 locus *3)

\[ \chi^2 = 1.5, \ p \geq 0.05 \]

Table 2. Frequency of CYP2C9 *2 and *3 alleles and genotypes in Saudi cases of vitiligo related to their demographic data and the pattern of the disease

|                  | Homozygous normal (*1/*1) | Heterozygous mutant (*1/*2 & *1/*3) | Homozygous mutant (*2/*3 & *2/*2 & *3/*3) | p-value |
|------------------|---------------------------|------------------------------------|------------------------------------------|---------|
| Age (yr)         |                           |                                    |                                          |         |
| ≤20 (n=33)       | 15 (46.9)                 | 16 (46.9)                          | 2 (6.3)                                  | 0.77    |
| >20 (n=62)       | 31 (51.7)                 | 29 (45.0)                          | 2 (3.3)                                  |         |
| Gender           |                           |                                    |                                          |         |
| Male (n=50)      | 27 (56.3)                 | 21 (39.6)                          | 2 (4.2)                                  | 0.45    |
| Female (n=45)    | 19 (43.2)                 | 24 (52.3)                          | 2 (4.5)                                  |         |
| Family history   |                           |                                    |                                          |         |
| Positive (n=39)  | 20 (52.6)                 | 16 (39.5)                          | 3 (7.9)                                  | 0.30    |
| Negative (n=56)  | 25 (48.1)                 | 29 (50.0)                          | 1 (1.9)                                  |         |
| Consanguinity    |                           |                                    |                                          |         |
| Positive (n=31)  | 21 (67.7)                 | 8 (25.8)                           | 2 (6.5)                                  | 0.029†  |
| Negative (n=64)  | 25 (41.7)                 | 37 (55.0)                          | 2 (3.3)                                  |         |
| Pattern          |                           |                                    |                                          |         |
| Focal (n=22)     | 12 (63.2)                 | 10 (36.8)                          | 0 (0.0)                                  | 0.58    |
| Vulgaris (n=52)  | 25 (48.1)                 | 25 (48.1)                          | 2 (3.8)                                  |         |
| Acrofacial (n=20)| 9 (45.0)                  | 9 (45.0)                           | 2 (10.0)                                 |         |
| Universal (n=1)  | 0 (0.0)                   | 1 (100.0)                          | 0 (0.0)                                  |         |

Values are presented as number (%). †Mark indicates significant difference, p<0.05.

Weinberg Equilibrium (HWE) was noted in CYP2C9*3 locus variants among cases and controls, which did not show any significant difference between the expected and observed genotypes (p > 0.05). However, CYP2C9*2 variants showed significant difference between expected and observed frequencies of polymorphic genotypes (p < 0.001; Table 1).

Combined CYP2C9 *2 and *3 genotypic variants confirmed the higher frequency of the heterozygous mutant forms *2/*1 and *3/*1 genotypes among cases compared to controls (47.4% vs. 25.6%) and a lower frequency of *1/*1 (48.4% vs. 67.4%). Comparison of the frequencies of CYP2C9 normal, heterozygous, and homozygous mutant genotypes among subgroups of age, gender, family history, and disease patterns indicated insignificant difference. On the other hand, cases with positive consanguinity showed higher homozygous genotypes than others (p = 0.029; Table 2).
DISCUSSION

Cytochrome P450 2C9 has been suggested to be very similar to epoxide hydrolase (sEH), which hydrolyzes a wide variety of endogenous and exogenous epoxides that are believed to be formed by cytochrome P450 epoxideases. Moreover, sEH was found in various tissues including the epithelial cells in the skin. In active vitiligo patients, an increase in oxidative stress in the entire epidermal compartment has been demonstrated; in particular, the imbalance in catalase activity, reduced glutathione, and vitamin E levels was associated with hyperproduction of reactive oxygen species. Oxidative DNA damage in vitiligo patients manifested by DNA breakage was attributed to factors like catalase genetic polymorphisms, reduced glutathione peroxidase activity and increased levels of tetrahydrobiopterins (6BH4 and 7BH4), which are inhibitors of tyrosinase, and phenyl alanine hydroxylase enzymes.

We propose an extensive analysis of all potential forms of exposure to chemicals, drugs, pollutants or radiations for all affected subjects probably using investigative techniques like HPLC. Genome wide association studies indicated that most vitiligo susceptibility loci (more than 20) encode immunoregulatory proteins or melanocyte components that likely mediate immune targeting and genetic relationships among vitiligo, malignant melanoma, and normal variation of eye, skin, and hair color.

Since degradation of drugs in humans is driven by detoxification mechanisms whose efficiency is influenced by genetic mutations, Weise et al. studied the association between type 2 diabetes with mutations in prominent members of the CYP 450 2C9 isoenzyme family. Probable genetic contribution to the occurrence of vitiligo among Saudi subjects is relatively higher in patients with positive family history (41.1%) and consanguinity (33.7%). This study, demonstrated that consanguinity apparently had a role into the appearance of homozygous genotypes although most of them were for the normal or wild type allele with some mutant forms. On the other hand, the distribution of genetic variants of CYP2C9 were not affected by age, gender, family history or pattern of vitiligo among the patients. The gene frequencies related to the CYP 2C*3 allele were in conformity with the HWE. In contrast, frequencies related to allele *2 were not in accordance to the HWE that might be due to higher levels of consanguinity or due to the relatively small sized sample. So, this research probably needs to be investigated in a wider study by including other interactive haplotypes and genetic polymorphisms as suggested by other scientists.

Interestingly, Saudi cases did not show any significant difference from the control subjects in terms of the frequency of their CYP2C9 *2 allelic variants. Similarly, Semiz et al. reported that no significant difference in allele frequencies for CYP2C9 *2, was demonstrated between diabetic and non-diabetic subjects. Recently, Kaur-Knudsen...
et al. reported the findings of large studies on the association between genetic variation in CYP1B1 and CYP2C9 and the risk of disease, and rebutted the hypotheses that these genetic variant influences the risk of tobacco-related cancer, female cancer (as cervical and endometrial cancers), chronic obstructive pulmonary disease, and ischemic vascular disease. Veenstra et al. found that genetic variation in CYP2C9 exons, rather than the promoter or other regulatory regions, is largely responsible for warfarin sensitivity associated with CYP2C9 variants in a European American population. Other studies have reported that CYP2C9 *3 genotype did not affect the required warfarin dose while it was associated with increased risk of bleeding when treated with routine dosage regimen during the initiation of treatment.

In conclusion, this study provides presumptive evidence that CYP2C9 *3 is probably associated with the susceptibility to vitiligo among Saudi subjects regardless of the clinical pattern, gender, and presence of family history. Nonetheless, our study is limited in terms of the relatively small sample size, lack of protein studies or cell culture analyses to fully examine the underlying mechanism of vitiligo pathogenesis.

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