Is NAT2 Gene Polymorphism Associated with Vitiligo?

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Abstract

Background: N-acetyltransferase-2 (NAT2) is a phase II xenobiotic enzyme that plays an important role against oxidative stress-mediated reactive oxygen species protection. Polymorphism in specific genotypes of NAT2 may lead to an imbalance in antioxidant systems and may influence the pathogenesis of vitiligo. We conducted this study to see the association between NAT2 gene polymorphism and risk of vitiligo. We looked into whether single-nucleotide polymorphisms (SNP) at positions 857, 481, and 590 of the coding region of the NAT2 gene play as a risk factor for vitiligo among north Indian people.

Materials and Methods: In this study, we assessed 100 patients with vitiligo and 160 healthy individuals as controls. Genomic DNA was extracted from human peripheral blood and polymerase chain reaction-restricted fragment length polymorphism was done to identify the single nucleotide polymorphism at positions 857, 481, and 590 of the coding region of the NAT2 gene.

Results: In this study, we observed a significant higher risk with slow acetylator genotypes of NAT2 (OR = 2.85; 95% CI = 1.68-4.84, P value < 0.001) for the vitiligo. Furthermore, in the association between NAT2 acetylator genotypes with percentage of body surface area (BSA) of disease, we observed that slow acetylator genotypes of NAT2 has significant higher risk with low grade of disease (1%–10% >11%–30% >30% of BSA).

Limitations: A major limitation of this study was the small sample size and warrants further investigation on a large epidemiological study to confirm these findings.

Conclusions: Our preliminary data indicate that NAT2 slow acetylator genotype exhibits significant association for the risk of vitiligo, especially in disease predisposition and initiation.

Key Words: Acetylator genotype, n-acetytransferase-2 gene, oxidative stress, vitiligo

Introduction

Vitiligo is a multifactorial polygenetic disorder of the skin characterized by depigmented patches due to loss of the melanocytes. It is caused by the complex interaction between genotypic and phenotypic environment of an individual. Worldwide, its incidence ranges from 0.1% to 3% in general population.[1,2] However, in Indian subcontinent, India has the highest prevalence, varying from 0.25% to 2.5%,[3] There is no sex predilection for vitiligo, as such both sexes are affected equally.[1,4] Exact pathogenesis of vitiligo is still unclear, however, more persuasive evidences are genetic, environmental, oxidative stress, biochemical, and immunological factors that seem to act together or individually to generate a lenient environment for the melanocyte loss.[5-11]

Recent studies show that besides the immunological and pathogenic mechanisms, oxidative stress may be an important contributor to the pathogenesis of melanocyte death.[6,7] The imbalance between oxidative stress and antioxidation seems to induce excess production of reactive oxygen species (ROS) in the epidermal layer of skin, which may be an important pathogenic factor of vitiligo.[6,7,9,11] Xenobiotic phase I (cytochrome P450s) and phase II enzymes (i.e. glutathione S-transferases [GSTs], N-acetyltransferases [NAT1 and NAT2]) play a major role in the biotransformation and protection against environmentally exposed exogenous and endogenous toxicants, i.e. drugs, carcinogens, hair dyes and several others.[10-14] N-acetyltransferase metabolizes wide range of xenobiotic compounds that may be responsible for ROS production and melanocyte damage via the acetylation process.[10,11] It catalyzes the metabolic inactivation or activation of environmentally

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How to cite this article: Srivastava DS, Aggarwal K, Singh G. Is NAT2 gene polymorphism associated with vitiligo?. Indian J Dermatol 2020;65:173-7.

Received: August, 2018. Accepted: December, 2018.
exposed compounds, such as plethora of hydrazine and arylamines by the pathways of n- or o-acetylation.[10-14]

NAT2 polymorphically expresses at a number of tissues such as liver and epidermis.[15] Humans express two forms of N-acetyltransferases: NAT1 and NAT2, both genes are polymorphic. Now, 24 alleles in NAT1 and >59 alleles in NAT2 have been identified.[16] A recent review described the nucleotide and amino acid changes associated with various alleles and deduced phenotype from NAT2 genotypes was generated.[17,18] The NAT2 slow and fast acetylator phenotypes can be generated by existing SNPs in the coding region of the NAT2 gene, which influences the affinity for the substrate and catalytic activity of the protein. Consequently, the presence of the different alleles in each individual genome produces a broad range of metabolic phenotypes from fully active fast acetylator phenotype to the less active slow acetylator phenotype and it may vary in different populations due to different ethnic groups.[19-20]

Out of several NAT2 alleles, three mutant alleles (NAT2*5, NAT2*6, and NAT2*7), which can be identified by the detection of the mutations at positions 481, 590, and 857 of the coding region of the NAT2 gene, have been shown to be associated with slow acetylator genotypes. Recent studies have shown that variation in NAT2 gene may modify the risk of various diseases, such as cancer,[17] psoriasis,[20] systematic lupus erythematosus,[21] contact allergy,[22] Alzheimer’s, Parkinson’s, diabetes mellitus, rheumatoid arthritis, and autoimmune diseases.[23] Search until August 22, 2018, in electronic databases, such as Medline (1966–Present), EMBASE (1980–Present), or in Google engine by typing of key words, i.e. NAT2 gene polymorphism and risk of vitiligo or genetic polymorphism of NAT2 gene for susceptibility of vitiligo disease, does not reveal any report on NAT2 with vitiligo risk was available in electronic database.

Published data suggest that the frequencies of NAT2 polymorphisms differ among different racial and ethnic groups.[17,19,22] Whether NAT2 genetic variants (i.e. NAT2*5, NAT2*7, and NAT2*6 alleles) can help to explain part of the large differences in vitiligo awaits further clarification. Therefore, this study was undertaken to study the following objectives: i) to observe the frequencies of rapid and slow acetylators (NAT2) in vitiligo and control individuals and ii) to study effect of NAT2 acetylators genotype on modifying the vitiligo risk on disease initiation.

Materials and Methods

Sample size for the study was calculated by using the Epi Info 7 software. Approval for the study was obtained from the institutional review board. This study was conducted to see the association between NAT2 gene polymorphism and risk of vitiligo in 100 patients and 160 age- and sex-matched healthy controls. The ethnic origin of the cases and controls were similar. Patients with vitiligo vulgaris were selected in the study. Patients were selected on the basis of a questionnaire administered in the OPD of the institute that included medical records. Case records had been kept for each patient. Data had been systematically recorded as outlined in the proforma, such as gender, family history of disease, age at vitiligo onset, type and severity of the disease (percentage of body surface area [BSA] affected), and history of smoking. BSA was evaluated by the “rule of nines” method. Age- and sex-matched healthy individuals had been selected as control group for the study. Inclusion criteria of the patient group were - 1) age between 18 and 75; 2) patients who never had previous vitiligo treatment, and 3) patients who had stopped taking systemic or topical medication 1 month prior to this study. However, those patients who have been smokers, those with chronic illness, pregnant ladies, or lactating mothers and had other autoimmune diseases, such as thyroid disorder, rheumatoid arthritis, bronchial asthma were excluded from the study.

DNA extraction and NAT2 genotyping

For DNA extraction and genotyping, 5 mL of venous blood was collected in an EDTA vial from patients of study group and control group. Genomic DNA was extracted from blood lymphocytes using the proteinase K and phenol chloroform extraction procedure.[23] The NAT2 genotypes were determined using the polymerase chain reaction (PCR)-RFLP as described previously.[24] PCR product of 1,092 bp was generated by PCR using the following primer: Forward 5’-TCTAGGATGATCACCTGCG-3’, Reverse 5’- GGAAACAATGGGAC-TGGG -3’ in a total volume of 50 µl. PCR was carried out in PTC-thermocycler (MJ Research) for 35 cycles. Following PCR, 7 µl of PCR products was digested with four separate enzymes including Kpn1 (5U; Fermentas) for NAT2*5 allele, at 37°C for 2 h; Taq1 (5U; Fermentas) for NAT2*6 allele, at 56°C for 4 h; and BamH1 (5U; Fermentas) for NAT2*7 allele at 37°C for 2 h. Digested product was run on 2% agarose gel for NAT2*5 and NAT2*7 alleles and 3% agarose gel for NAT2*6 allele [Table 1].

Estimating the frequency of rapid and slow acetylator

The variant and nonvariant alleles were recorded and phenotyping of NAT2 gene in vitiligo and control was done on the basis of NAT2 genotypes. For each group (case and control), frequency of rapid and slow acetylator genotypes were determined. The presence of two mutant alleles was defined as slow acetylator genotype, whereas one or zero mutant allele was segregated as rapid acetylator genotype.[25]
Statistical analysis

Statistical analysis was done with SPSS 20.0 software program. Differences in genotype prevalence and association between vitiligo disease and normal healthy groups were assessed by binary logistic regression model. P value < 0.05 was considered significant for the study. Odds ratios (OR) and its 95% confidence interval (CI) were obtained by summarizing data over three strata for BSA (<10%, 11%–30%, and >30%). We evaluated age-adjusted (confounder OR) and unadjusted ORs and 95% CI by using logistic regression model. Univariate analysis, ORs, and 95% CI were used to describe the strength of association.

Results

The present study included 100 vitiligo patients with a mean age of 30.9 years (± 16.98) and 160 normal healthy individuals as control having a mean age of 28.9 years (± 16.75). The distribution of NAT2 acetylator genotypes in control and vitiligo patients is shown in Table 2. Higher frequency of NAT2 slow acetylator was observed (70.0%) among the patient group as compared with the control (45.0%). It was statistically significant for susceptibility of vitiligo in north Indians (OR = 2.85; 95% CI = 1.68–4.84; P < 0.001).

We categorized severity of disease (BSA) into three groups (1%–10%, 11%–30%, and >30% of BSA). We observed that NAT2 slow acetylator genotypes were highly significant in low grade of disease (1%–10% >11%–30% >30% of BSA) when compared with controls for the susceptibility of vitiligo in our population as shown in Table 3.

The association between NAT2 acetylator genotypes and gender has been summarized in Table 4. The OR for the slow acetylator genotypes was 2.99-fold higher for male and 2.79-fold higher for female as compared with the controls. It was statistically significant in both groups: male (P = 0.004) and female (P = 0.027).

Discussion

Vitiligo is a multifactorial disorder and its pathophysiology is complex. Most environmental toxicants require metabolic activation to interact with DNA and exert their genotoxic potential that is probably associated with vitiligo risk. This activation is mediated by the action of phase I enzymes, such as cytochrome P4501A1. Furthermore, genotoxic chemicals, including reactive intermediates produced from the above enzymatic reactions undergo biotransformation by phase II (GSTs and NATs) enzymatic defensive mechanisms, into more polar and less toxic metabolites that can be easily excreted from the body.[6,7,10,12,14,17]

NAT2 enzyme plays a very important role in xenobiotic biotransformation and protection against the environmental toxicants (i.e. drugs, carcinogens, hair dyes, and several other toxic irritants), which may be responsible for ROS production and melanocytes loss.[10-14] It catalyzes plethora of hydrazine and arylamines (i.e. paraphenylenediamine) metabolism by the two pathways: n-acetylation (inactivation)/o-acetylation (activation).[14,17,22,26] The NAT2 gene is highly polymorphic and each mutant allele contains a combination of one or more nucleotide substitutions. Out of several NAT2 alleles, three mutant alleles (NAT2*5, NAT2*6, and

| Table 1: Genetic polymorphism site of NAT2 and its different alleles |
| --- |
| Gene | PCR products | Nucleotide change | Restriction enzymes | Digestive-products size (bp) | Alleles |
| --- | --- | --- | --- | --- | --- |
| NAT2 | 1,092 bp | G 857A | BamH1 | 810, 282 | NAT2*7 |
| | | C 481T | Kpn1 | 667, 425 | NAT2*5 |
| | | G 590A | Taq1 | 424, 338 | NAT2*6 |

NAT2: N-acetyltransferase-2

| Table 2: Association between NAT2 genotypes and body surface area of disease |
| --- |
| NAT2 genotypes | Controls (n=160) | Patients (Vitiligo) | 2-Tailed P | Odds ratio (95% CI) |
| --- | --- | --- | --- | --- |
| Fast-acetylator | 88 (55.0%) | 30 (30.0%) | 0.001 | 2.85 (1.68-4.84) |
| Slow-acetylator | 72 (45.0%) | 70 (70.0%) | <0.001 | 2.85 (1.68-4.84) |

NAT2: N-acetyltransferase-2, CI: Confidence interval

| Table 3: Association between NAT2 genotypes and percentage of body surface area of vitiligo disease |
| --- |
| Subjects | NAT2 fast-acetylator | NAT2 slow-acetylator | 2-Tailed P | Odds ratio (95% CI) |
| --- | --- | --- | --- | --- |
| Controls (n=160) | 88 (55.0%) | 72 (45.0%) | 0.001 | 2.85 (1.35-6.01) |
| Cases (n=100) classified by the percentage of body surface area of the disease | 12 | 28 | 0.007 | 2.85 (1.35-6.01) |
| 11%–30% | 10 | 22 | 0.019 | 2.68 (1.26-6.04) |
| >30% | 9 | 18 | 0.029 | 2.44 (1.03-5.76) |

NAT2: N-acetyltransferase-2, CI: Confidence interval
NAT2 slow acetylator genotypes may be an important risk factor in the pathogenesis of disease especially for initiation/predisposition of vitiligo.

Furthermore, correlation of NAT2 genotypes with gender, we observed significant association with slow acetylator genotypes of NAT2 in both sexes for the susceptibility of vitiligo \( (P < 0.05) \) [Table 4]. Earlier published data on vitiligo also strengthen our finding that there is no sex predilection as such for vitiligo and both sexes are affected equally.\(^{[1,4]}\)

In general population, slow allele is present in up to 90% of Arab population,\(^{[18]}\) 40%–60% in Caucasians including Indians,\(^{[21,24,31]}\) 5%–25% in East Asians,\(^{[18]}\) and 74% in south Indians.\(^{[32]}\) In this study, frequencies of NAT2 slow alleles were observed 45% and it has been in accordance to Caucasians\(^{[31]}\) and previous study pertaining to north Indians (44.3%).\(^{[24]}\) Differences of distribution of slow allele of NAT2 between north Indian and south Indian population is due to the different ethnic/geographical environment.

To the best of our knowledge, this is the first study in an Indian population to explore NAT2 genotypes for the vitiligo risk. To evaluate the association study, an adequately large sample size is warranted. In current case–control study, a limited sample size was employed, which underscores the need for further large epidemiological studies to confirm our findings. Another limitation of the present study is that NAT2 has > 59 SNPs and we have examined three SNPs only. Furthermore, it has been warranted to study the other SNPs of NAT2 in large sample size too for exploring of their involvement in the risk of vitiligo.

In conclusion, this study indicates that NAT2 slow acetylator genotype exhibits significant association for the risk of vitiligo, especially in disease predisposition and initiation.

### Financial support and sponsorship
Nil.

### Conflicts of interest
There are no conflicts of interest.

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### Tables 4: Association between gender and NAT2 genotypes

| Sex             | Controls (n=160) | Patients (n=100) | 2-Tailed P | Odds Ratio (95% CI) |
|-----------------|------------------|------------------|------------|---------------------|
| Male            | 125              | 43               |            |                     |
| Fast acetylator | 70 (56.0)        | 13 (30.2)        | (Ref.)     |                     |
| Slow acetylator | 54 (44.0)        | 30 (69.8)        | 0.004      | 2.99 (1.43-6.27)    |
| Female          | 35               | 57               |            |                     |
| Fast acetylator | 19 (54.3)        | 17 (29.8)        | (Ref.)     |                     |
| Slow acetylator | 16 (45.7)        | 40 (70.1)        | 0.027      | 2.79 (1.17-6.69)    |

NAT2: N-acetyltransferase-2, CI: Confidence interval

NAT2*7\(^{[7]}\) have been shown to be associated with slow acetylator genotypes. Previously published literature has shown that NAT2 slow acetylator genotypes have been linked with increased susceptibility of various diseases,\(^{[10-14,22,26]}\) but there is no report from vitiligo.

In present case–control study, higher frequency of NAT2 slow acetylator was observed (70.0%) among the patient group when compared with the controls (45.0%) \( (P < 0.001) \). Our study demonstrated 2.85-fold higher risk for predisposition of vitiligo \( (OR = 2.85; P < 0.001) \) [Table 2] in those persons who possess slow acetylator genotypes of NAT2. Furthermore, in association between BSA and NAT2 slow acetylator genotypes, we observed significant higher risk in low grade of disease \( (BSA = 1%-10%; P = 0.007) \) and its trend in BSA categories was in the order of 1%-10% >11%-30% >30% for predisposition of vitiligo [Table 3].

Our findings are in agreement with previous studies reported in various diseases that showed significant association with slow acetylator genotypes of NAT2 for susceptibility of contact allergic,\(^{[22]}\) atopic dermatitis,\(^{[27]}\) systemic sclerosis and lupus erythematosus,\(^{[21,28]}\) Alzheimer’s, Parkinson’s, diabetes mellitus, rheumatoid arthritis and autoimmune disorders,\(^{[19]}\) sulphonamide-induced toxic epidermal necrolysis and Stevens-Johnson syndrome,\(^{[29]}\) as well as psoriasis and other diseases.\(^{[20]}\) However, studies from north India\(^{[24]}\) and Turky\(^{[30]}\) have shown an increased risk of prostate cancer and allergic contact dermatitis with fast acetylator genotypes of NAT2. The discrimination in association study observed in various populations could be related to ethnic variation. Finding of our study could be suggested that nonacetylated xenobiotics may accumulate in slow acetylator persons and are subsequently metabolized by other enzymes (i.e. CYPs) into reactive intermediates.\(^{[5-7,10,12,14,22]}\) These reactive intermediates may alter self-proteins and affected NAT2 enzyme specificity and activity, which, in turn, initiate pathological and clinical signs of vitiligo.

We have detected significant association with slow acetylator genotypes of NAT2 to the risk of vitiligo in the north Indian population, suggesting that the
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