Three New Single Nucleotide Polymorphisms Identified by a Genome-Wide Association Study in Korean Patients with Vitiligo

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Received: 28 August 2012 Accepted: 15 February 2013

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This study was supported by a grant of the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (Grant No.: A103017).

INTRODUCTION

Vitiligo is an acquired depigmentary disorder of the skin. Although the etiology is unclear, the melanocytes involved in vitiligo are genetically susceptible and vulnerable to extracellular insults. Identification of genes involved in susceptibility to vitiligo has been attempted by association studies of candidate genes, which related to pathogenesis of non-segmental vitiligo including autoimmunity (1-8). However, genetics of vitiligo is characterized by incomplete penetrance, multiple susceptibility loci and genetic heterogeneity. Therefore, association studies with a whole genome-based approach instead of single or a few candidate genes may be useful to discover new susceptible genes. With completion of the human genome sequencing, there is a focus on developing new sequencing methodologies considering read length, thorough-put, and cost (9). A technical improvement in whole genome amplification with small amount of genomic DNA has been adopted for single nucleotide polymorphism (SNP) analysis (10). Recently, genome-wide association analysis has been attempted in vitiligo, with identification of genes including X-box binding protein 1 (XBP1) (11), SMOC2 (12), and TYR (13). A study could be conducted with a unique isolated population with elevated prevalence of generalized vitiligo and other autoimmune disease, which may provide a condition for detection of susceptible alleles with smaller sample sizes (12). However, studies have frequently been done with independent patients and controls (11, 13).

There is a consensus that vitiligo should be classified into segmental and non-segmental vitiligo forms based on clinical manifestations (14). The etiology of each type is considered to be distinct, although the association between segmental and non-segmental vitiligo has been reported. Nevertheless, the association between gene polymorphisms and vitiligo has been reported without defining types. In cases in which the type has been clarified, the association has usually been identified in non-segmental form.

A genome-wide association study was conducted in Korean patients with vitiligo, although the whole genome SNP analysis was performed in a very small number of patients. Functional classes of significant SNPs were validated by genotyping patients and independent healthy controls. Association between SNPs and vitiligo types was examined.

MATERIALS AND METHODS

Subjects

Twenty patients with non-segmental vitiligo were examined for whole genome-based SNPs. In total, 163 Korean patients with...
vitiligo were included to validate the 10 selected target SNPs in this study. A total of 113 cases were non-segmental, and 50 were segmental. An additional 97 patients with non-segmental (71 patients) and segmental vitiligo (26 patients) were added, resulting in 184 patients with the non-segmental and 76 with the segmental type to analyze the association between the three significant gene mutations and either type of vitiligo. In the 184 non-segmental and 76 segmental types, 86 and 27 patients were male, and 98 and 49 were female, respectively. Their ages ranged from 5 to 81 yr (mean, 40.1 yr) and 4 to 70 yr (mean, 23.6 yr), respectively. The control group included 192 healthy individuals (82 males and 110 females), with ages between 30 and 80 yr (mean, 50.0 yr).

**Genome-wide genotyping with significant SNP analysis**

Genomic DNA was isolated from peripheral blood leukocytes using the GenEX Genomic kit (Geneall, Seoul, Korea). Whole-genome-based SNPs were examined using 2 μg of the genomic DNA from 20 patients with non-segmental vitiligo. The Affymetrix GeneChip 500K mapping array, which contained 500,568 probes, was used for the genome-wide association analysis. The genotypes were determined using the BRLMM algorithm. Before applying the algorithm, whole-genome-based SNPs from 44 individuals offered by the Affymetrix were put together for the genotype calling to compensate for the small number of patients. The rates of mismatch between the genotyping from 20 patients with non-segmental and 76 segmental types, 86 and 27 patients were male, and 98 and 49 were female, respectively. Their ages ranged from 5 to 81 yr (mean, 40.1 yr) and 4 to 70 yr (mean, 23.6 yr), respectively. The control group included 192 healthy individuals (82 males and 110 females), with ages between 30 and 80 yr (mean, 50.0 yr).

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**Target SNP selection**

The number of significant SNPs was 114. They were divided into eight functional classes, such as non-synonymous, synonymous, mRNA UTR, intron, locus region, in-gene, intergenic, and unknown. The number of non-synonymous SNPs was eight, which were selected as the target SNPs. The SNPs within the linkage disequilibrium (LD) block, which included non-synonymous, synonymous or mRNA UTR SNPs, were also examined and seven significant SNPs were detected. Two (rs17035120 and rs2588400) of the seven SNPs were already included as target SNPs. One SNP (rs3763979) with a function class of synonymous and the other (rs1444216) with a function class of in-gene were added to the list of target SNPs, resulting in a total of 10 target SNPs (Table 1).

**Genotyping for clinical validation of the target SNPs**

The real-time-polymerase chain reaction (PCR) using genomic DNA from patients with vitiligo and healthy controls was performed to examine the association between non-segmental or segmental vitiligo, the 10 target SNPs, and the three significant SNPs. 10 ng of each genomic DNA was mixed with 20 μL of reaction mixture containing 1.0 μL of reaction mix, 2.0 μL of Fast-Start DNA master, and 1.6 μL of MgCl₂. Real-time PCR was done automatically using specific primers and probe included in the LightSNiP kit (TIB MOLBIOL, Berlin, Germany) with a LightCycler 480 Instrument (Roche, Mannheim, Germany). The LightSNiP kit was designed as an individual SNP base by TIB MOLBIOL.

**Statistical analysis**

All statistical tests were two-sided, and the level of significance was 0.05. Substitution of missing values was not performed. Data from both vitiligo and healthy control groups were compared using the chi-square test. One allele association analysis and four genotype association analyses were performed using dominant, recessive, co-dominant, and additive genetic models. Significant SNPs were chosen by the method of significance which was either \( \alpha = 0.001 \) or \( \alpha = 0.01 \) with multiple test correction, a Bonferroni method.

**Results**

The genotype frequencies of the 10 target SNPs were compared
between vitiligo patients and normal healthy controls. The genotype and allele frequencies showed a significant difference in three of the target SNPs, such as DNAH5 (rs2277046), STRN3 (rs2273171), and KIAA1005 (rs3213758) in all four different genetic models (Table 2), regardless of the vitiligo type difference (Table 3), although the STRN3 genotype was not significantly different in the dominant model. In DNAH5 (dynein axonemal heavy chain5), the genotype and allele frequencies of 12658 A > G and STRN3 (striatin calmodulin binding protein 3) 1376 T > C were significantly different from those in healthy controls. The AA genotype frequency was lower, whereas GG was higher in vitiligo patients, suggesting that G allele at the DNAH5 A12658G polymorphism increased susceptibility to vitiligo. The genotype and allele frequencies of STRN3 were also significantly different between the patients and healthy controls. The TT genotype was less frequent, whereas CC was more common in patients than controls, indicating that the C allele at the STRN3 T1376C may increase susceptibility to vitiligo. In KIAA1005, the genotype and allele frequencies of 3854 G > A (1264 Asp > Asn) in vitiligo patients were significantly different compared to healthy controls. The AG frequency was higher, whereas AA frequency was lower in vitiligo, showing an A allele at the KIAA1005 G3854A may increase susceptibility to vitiligo.

The genotype and, particularly, allele frequencies of DNAH5 12658 A > G and STRN3 1376 T > C were not significantly different between the non-segmental and segmental forms in all four models (Table 3), whereas those of KIAA1005 3854 G > A was different (Table 3). Therefore, a direct comparison between non-segmental and segmental vitiligo was performed. In fact, the frequency of allele A in the KIAA1005 gene was significantly (P = 0.025) higher in the segmental compared to that in non-segmental vitiligo, although the genotype frequency suggested only a tendency for statistical significance in recessive (P = 0.058).

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### Table 2. Genotype and allele frequencies of each gene polymorphism in the vitiligo patients and the healthy controls

| SNP            | Genotype/Allele   | Vitiligo (n = 260) | Normal (n = 192) | Model | Odd ratio (95% CI) | P*  |
|----------------|-------------------|-------------------|-----------------|-------|-------------------|-----|
| DNAH5 rs2277046 | AA                | 59 (22.69)        | 67 (34.90)      | D     | 1.83 (1.21-2.77)  | 0.005 |
|                | AG                | 119 (45.77)       | 89 (46.35)      | R     | 2.00 (1.28-3.12)  | 0.002 |
|                | GG                | 82 (31.54)        | 36 (18.75)      | C     | 2.59 (1.53-4.37)  | 0.000 |
|                | A                 | 237 (45.58)       | 223 (58.07)     | Ad    | 1.60 (1.23-2.08)  | 0.000 |
|                | G                 | 283 (54.42)       | 161 (41.93)     | A     | 1.65 (1.27-2.16)  | 0.000 |
| STRN3 rs2273171 | TT                | 61 (23.46)        | 59 (30.73)      | D     | 1.45 (0.95-2.20)  | 0.084 |
|                | TC                | 130 (50.00)       | 103 (53.66)     | R     | 1.35 (1.21-1.34)  | 0.006 |
|                | CC                | 60 (23.54)        | 30 (15.63)      | C     | 2.22 (1.27-3.89)  | 0.000 |
|                | A                 | 252 (48.46)       | 221 (57.55)     | Ad    | 1.47 (1.11-1.93)  | 0.006 |
|                | C                 | 268 (51.54)       | 163 (42.45)     | A     | 1.44 (1.11-1.88)  | 0.007 |
| KIAA1005 rs3213758 | GG               | 84 (32.31)        | 101 (52.60)     | D     | 2.33 (1.58-3.42)  | 1.69 × 10⁻³ |
|                | AG                | 77 (29.62)        | 81 (42.19)      | R     | 11.19 (6.65-22.18) | 4.48 × 10⁻³ |
|                | AA                | 99 (38.08)        | 103 (53.66)     | C     | 11.90 (6.84-24.25) | 9.06 × 10⁻³ |
|                | G                 | 245 (47.12)       | 283 (73.70)     | Ad    | 2.54 (1.94-3.32)  | 8.88 × 10⁻³ |
|                | A                 | 275 (52.88)       | 101 (26.30)     | A     | 3.15 (2.37-4.18)  | 3.31 × 10⁻³ |

*P value derived from a chi-square test. D, dominant model; R, recessive model; C, co-dominant model; Ad, additive model; A, Allele analysis.

### Table 3. Genotype and allele frequencies of each gene polymorphism in the vitiligo patients with non-segmental (NSV) or segmental (SV) type and the healthy controls (N)

| SNP            | Genotype/Allele   | NSV (n = 184) | SV (n = 76) | Odd ratio (95% CI) | P*  |
|----------------|-------------------|---------------|-------------|-------------------|-----|
| DNAH5 rs2277046 | AA                | 44 (23.91)    | 15 (19.74)  | 1.71 (1.09-2.68)  | 2.18 (1.15-4.13) | 0.020 |
|                | AG                | 88 (47.83)    | 31 (40.79)  | 1.71 (1.05-2.77)  | 2.83 (1.57-5.08) | 0.030 |
|                | GG                | 52 (28.26)    | 30 (39.47)  | 2.20 (1.24-3.89)  | 3.72 (1.78-7.80) | 0.007 |
|                | A                 | 176 (90.73)   | 61 (40.13)  | 1.48 (1.12-1.97)  | 1.71 (1.35-2.16) | 0.000 |
|                | G                 | 192 (62.17)   | 91 (59.67)  | 1.51 (1.13-2.02)  | 2.07 (1.41-3.03) | 0.005 |
| STRN3 rs2273171 | TT                | 44 (23.91)    | 17 (22.37)  | 1.41 (0.89-2.23)  | 1.54 (0.83-2.86) | 0.139 |
|                | TC                | 92 (50.00)    | 38 (50.00)  | 1.91 (1.14-3.17)  | 2.06 (1.09-3.89) | 0.013 |
|                | CC                | 48 (26.09)    | 21 (27.63)  | 2.15 (1.18-3.91)  | 2.43 (1.12-5.28) | 0.013 |
|                | T                 | 180 (48.91)   | 72 (47.37)  | 1.44 (1.07-1.94)  | 1.55 (1.05-2.31) | 0.016 |
|                | C                 | 188 (51.09)   | 80 (52.63)  | 1.42 (1.06-1.89)  | 1.51 (1.03-2.02) | 0.018 |
| KIAA1005 rs3213758 | GG              | 66 (35.87)    | 18 (23.68)  | 1.98 (1.31-3.00)  | 3.58 (1.96-6.52) | 0.001 |
|                | AG                | 53 (28.00)    | 24 (31.58)  | 9.94 (4.91-20.11) | 14.73 (6.75-32.17) | 1.6 × 10⁻³ |
|                | AA                | 65 (35.33)    | 34 (44.74)  | 9.95 (4.77-20.73) | 19.08 (8.03-45.32) | 8.8 × 10⁻³ |
|                | G                 | 185 (50.27)   | 60 (39.47)  | 2.34 (1.75-3.13)  | 3.97 (2.60-6.60)  | 8.4 × 10⁻³ |
|                | A                 | 183 (49.73)   | 92 (50.53)  | 2.77 (2.04-3.76)  | 4.30 (2.89-6.39)  | 6.2 × 10⁻³ |

*P value derived from a chi-square test. D, dominant model; R, recessive model; C, co-dominant model; Ad, additive model; A, Allele analysis.
co-dominant ($P = 0.055$), and additive ($P = 0.060$) models (Table 4).

**DISCUSSION**

This genome-wide association study in Korean patients with vitiligo identified that three new SNPs, such as DNAH5 (rs2277046), STRN3 (rs2273171), and KIAA1005 (rs3213758), were associated with increased susceptibility to both non-segmental and segmental types of vitiligo (Table 2, 3), although the number of patients was too small to identify the actual number of significant SNPs.

Although an association with other variants in DNAH5, STRN3, and KIAA1005 with particular diseases has been reported (15-21), a clinical association of the SNPs, such as DNAH5 rs2277046, STRN3 rs2273171, and KIAA1005 rs3213758, has not been revealed. The DNAH5 gene is located in the coding exon of chromosome 5p15.2. The A12658G (rs2277046) variant had an amino acid residue (Ser387). Striatin is an intracellular protein characterized by four protein-protein interaction domains, a caveolin-binding domain, and a WD repeat domain (19). Results including the defective calcium uptake in both keratinocytes and melanocytes (20) and the activated nitric oxide synthases by increased levels of intracellular Ca$^{2+}$ via calmodulin (21), suggest an association of striatin with vitiligo development.

In general, segmental vitiligo has been classified separately from other forms of vitiligo including non-segmental vitiligo. An association between gene polymorphisms and vitiligo has been reported for non-segmental vitiligo (1-4). In this study, the association was separately analyzed both in non-segmental and in segmental forms. The association did not differ in either type of vitiligo (Table 3), although a stronger association between KIAA1005 (rs3213758) and segmental vitiligo was suggested (Tables 3 and 4). Neural dysfunction in the pathogenesis of segmental vitiligo and KIAA genes for organization and function in the brain (22) may support this association. However, increasing clinical and etiological evidence suggests an association between segmental vitiligo and non-segmental vitiligo.

Collectively, our genome-wide association study in Korean patients with vitiligo resulted in three new vitiligo-related SNPs, such as DNAH5 (rs2277046), STRN3 (rs2273171), and KIAA1005 (rs3213758), although a stronger association between KIAA1005 (rs3213758) and segmental compared to the non-segmental type of vitiligo was suggested. Future functional studies of these SNPs may contribute to the understanding of vitiligo pathogenesis.
ACKNOWLEDGMENTS

We would like to thank to Dr. Chi-Yeon Lim for a statistical assistance.

DISCLOSURE

The authors have no conflicts of interest to disclose.

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