Rs3213758 in the *RPGRIP1L* Gene Associated with Susceptibility to Segmental Vitiligo in a Chinese Han Population

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To the Editor: Vitiligo is an acquired chronic depigmentation disorder of the skin resulting from selective destruction of melanocytes.[1] Although it is not a life-threatening disease, it may lead to disfigurement and is associated with many other autoimmune diseases. According to a recent international consensus conference, vitiligo can be classified into two major forms: nonsegmental vitiligo (NSV) and segmental vitiligo (SV, accounts for 5–16% of all vitiligo cases). NSV typically evolves over time, in both distribution and extension patterns, including acrofacial, generalized, mucosal, and facial vitiligo [Figure 1a and 1b]. SV is relatively rare and is characterized by unilateral or banded distribution along the ganglion segment. SV usually has an early onset and spreads rapidly in the affected dermatomal area, including single SV, double SV, and the multi-SV [Figure 1c and 1d].[2]

There is still controversy about the pathogenesis of vitiligo. At present, there are several major hypotheses: autoimmune mechanisms, cytotoxic mechanisms, intrinsic defect, oxidant-antioxidant mechanisms, and neural mechanisms.[3] In recent years, genetic factors have been found to play an increasingly important role in the occurrence and development of vitiligo. One study has shown that the rs3213758 locus in the retinitis pigmentosa GTPase regulator-interacting protein 1-like (*RPGRIP1L*) gene might increase the risk of vitiligo in a Korean population.[4] In addition, several mutations in the *RPGRIP1L* gene were associated with various clinical phenotypes of nervous system diseases.[5] However, the relationship between *RPGRIP1L* gene polymorphisms and susceptibility to SV in the Chinese Han population has not been reported. Therefore, this case-control study involving 121 Chinese Han patients with SV (SV group), 129 Chinese Han patients with NSV (NSV group), and 133 Chinese Han healthy controls (control group) was conducted to explore the association of single nucleotide polymorphism (SNP) of the *RPGRIP1L* gene with SV susceptibility and clinical features in the Chinese Han population, then providing further theoretical evidence for the neurological theory of SV.

The study was conducted in accordance with the *Declaration of Helsinki* and was approved by the Clinical Ethics Committee of Tongji Hospital. Only Han Chinese subjects were included to avoid genotype and allele frequency variations among ethnic groups. Patients with SV and NSV were diagnosed by more than two experts in Tongji Hospital, and those who had undergone related treatment in the preceding 6 months were excluded. Active vitiligo was defined as the appearance of new lesions or the enlargement of existing lesions in 3 months before the study. The healthy controls were excluded if they had received blood transfusions in the last 6 months or if they had other autoimmune diseases or other depigmentation disorders, such as piebaldism and albinism. A questionnaire was provided for all subjects to obtain basic information. Three groups were matched for age ($P = 0.914$), gender ($P = 0.986$) and ethnicity. The genotype frequency of healthy controls was consistent with Hardy-Weinberg equilibrium ($P = 0.170$).

Genomic DNA was obtained from peripheral venous blood using an OMEGA D3392-02 E.Z.N.A.TM Blood DNA Kit. The purity and concentration of the DNA were measured by an ultramicrospectrophotometer nucleic acid-protein analyzer (MaestroNano, Maestrogen Inc., USA). The *RPGRIP1L* gene polymorphism data were obtained from the literature and human genome databases, and rs3213758 was selected as the SNP locus of this study. Mutation analysis of the allele was performed using a custom-designed TaqMan-MGB SNP genotyping assay and TaqMan-MGB Gene Expression MasterMix (Thermo Fisher Scientific, assay ID: C__25937352_10). After the genotyping was completed, 10% of the samples of each group were randomly selected to repeat the genotyping with polymerase chain reaction. The primer sequences were as follows: forward 5'-CTGAGCAACACTTTCACCCAT-3'; and reverse 5'-CTGCCTTACCAGCCTTCG-3'. The product length was 169 bp, and the genotyping results were completely consistent with the TaqMan-MGB probe genotyping results.

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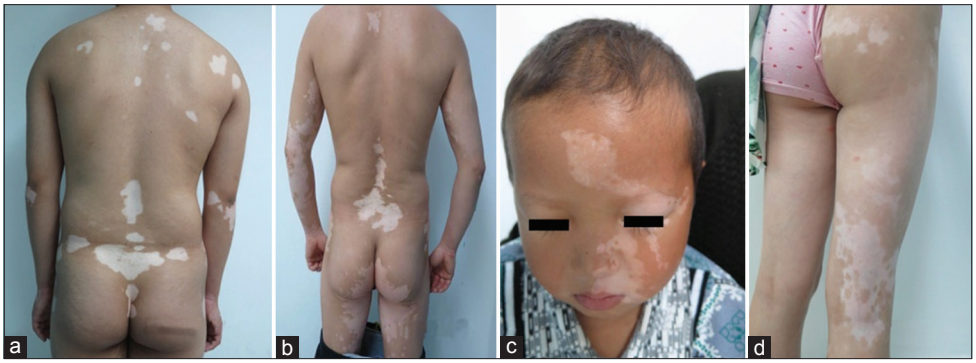

Figure 1: Clinical features of patients with nonsegmental vitiligo (a and b) and segmental vitiligo (c and d).

Data were analyzed using SPSS version 24.0 software (SPSS Inc., Chicago, IL,USA). Differences in the distributions of clinical features, alleles and genotypic frequencies of *RPGRIP1L* polymorphisms among three groups were evaluated with the Chi-square test. The odds ratios (*OR*s) and 95% confidence intervals (*CI*s) were calculated using univariate and multivariate logistic regressions to determine the relationship between the gene polymorphism and genetic susceptibility to SV and the relationship between genotype and various factors, such as age and gender. The statistical significance was defined as $P < 0.05$.

The results showed that the CC, CT, and TT genotype frequencies for *RPGRIP1L* rs3213758 were 59.5%, 28.9%, and 11.6% in the SV group; 46.5%, 43.4%, and 10.1% in the NSV group; and 45.9%, 47.4%, and 6.8% in healthy controls, respectively. The C and T allele frequencies for *RPGRIP1L* rs3213758 were 74.0% and 26.0% in the SV group; 68.2% and 31.8% in the NSV group; and 69.5% and 30.5% in healthy controls, respectively. When the CC genotype was used as the reference, these findings showed that the CT genotype could significantly reduce the susceptibility to SV (SV vs. NSV: 28.9% vs. 43.4%, $P = 0.032$, $OR = 0.556$, 95% $CI = 0.324–0.953$; SV vs. HT: 28.9% vs. 47.4%, $P = 0.006$, $OR = 0.471$, 95% $CI = 0.275–0.804$). Meanwhile, the overdominant model also showed that the CT genotype could significantly reduce the susceptibility to SV (SV vs. NSV: $P = 0.017$, $OR = 0.531$, 95% $CI = 0.314–0.897$; SV vs. controls: $P = 0.003$, $OR = 0.452$, 95% $CI = 0.269–0.761$). Although there was no statistical association between the TT genotype and SV genetic susceptibility, the recessive model showed that the TT genotype had a tendency to increase the risk of SV (SV vs. NSV: $OR = 1.168$, 95% $CI = 0.525–2.597$; SV vs. controls: $OR = 1.803$, 95% $CI = 0.750–4.331$). The dominant model showed that the frequency of the combined CT + TT genotype was significantly lowered in the SV group, compared with the NSV group and control group (SV vs. NSV: $P = 0.040$, $OR = 0.592$, 95% $CI = 0.358–0.977$; SV vs. controls: $P = 0.030$, $OR = 0.577$, 95% $CI = 0.350–0.949$). In addition, there was no significant difference in the frequency of the T allele between SV group and NSV group or control group (all $P > 0.05$). And there was no significant difference in the frequency of each genotype and allele between NSV and control groups ($P > 0.05$).

To evaluate the effect of the *RPGRIP1L* gene polymorphism in specific populations, the relationship between the *RPGRIP1L* gene polymorphism and genetic susceptibility to SV in a specific population was analyzed. The results showed that when the CC genotype was used as a reference, compared with the NSV and control groups, the CT genotype frequency of *RPGRIP1L* rs3213758 locus in SV group was significantly different in the following subgroups: male (SV vs. NSV: $P = 0.021$; SV vs. HT: $P = 0.005$) and age <20 years (SV vs. NSV: $P = 0.018$; SV vs. HT: $P < 0.001$); moreover, the CT genotype significantly decreased the susceptibility to SV in males (SV vs. NSV: $OR = 0.418$, 95% $CI = 0.199–0.876$; SV vs. HT: $OR = 0.345$, 95% $CI = 0.164–0.742$) and age <20 years old (SV vs. NSV: $OR = 0.380$, 95% $CI = 0.170–0.846$, SV vs. HT: $OR = 0.217$, 95% $CI = 0.097–0.484$). At the same time, the overdominant model showed that the CT genotype reduced the susceptibility to SV in the following subgroups: male (SV vs. NSV: $P = 0.015$, $OR = 0.410$, 95% $CI = 0.200–0.841$; SV vs. HT: $P = 0.001$, $OR = 0.305$, 95% $CI = 0.148–0.630$) and age <20 years (SV vs. NSV: $P = 0.030$; $OR = 0.427$, 95% $CI = 0.198–0.921$; SV vs. HT: $P = 0.000$, $OR = 0.217$, 95% $CI = 0.100–0.472$). In addition, the dominant model showed that combined CT + TT genotype frequency in male (SV vs. NSV: $P = 0.033$, $OR = 0.472$, 95% $CI = 0.237–0.940$; SV vs. HT: $P = 0.035$, $OR = 0.477$, 95% $CI = 0.239–0.950$) and age <20 years (SV vs. NSV: $P = 0.020$, $OR = 0.423$, 95% $CI = 0.205–0.872$; SV vs. HT $P = 0.001$, $OR = 0.292$, 95% $CI = 0.139–0.611$) also had significant differences between SV group and NSV group or control group. However, there was no significant difference in the frequency of the TT genotype in the recessive model ($P > 0.05$). In population of male and age <20 years, there was no significant difference in the frequency of each genotype and allele between NSV group and control group ($P > 0.05$). For population of female and age ≥20 years, there was no significant difference in the frequency of each genotype among three groups ($P > 0.05$).

In a SV group-only study, we found that there was no significant difference in the staging of the disease, the age of disease onset, and the area of skin lesions in SV patients with different genotypes. However, for the duration of the disease, when the 0–6-month duration was used as the reference, the rate of patients with the CT genotype at 6–12 months duration was 4.343-time higher than that of the patients with the CC genotype. There was no significant difference in the more than 12-month duration among SV patients with any genotype.

Although the pathogenesis of vitiligo is still unclear, genetic factors are now recognized as an important cause of vitiligo. A study showed that the A allele at the *RPGRIP1L* rs3213758 locus might increase the susceptibility to vitiligo in the Korean population. The *RPGRIP1L* gene is located on chromosome 16q12.2 and plays an important role in promoting normal development of the cerebellum and maintaining normal renal function. Several mutations in the *RPGRIP1L* gene are associated with various clinical phenotypes of nervous system diseases, including developmental delays, ataxia, and abnormal eye movement. These mutations could also lead to kidney disease, Joubert syndrome. The localization of *RPGRIP1L* to the ciliary axoneme, basal bodies, and centrosome or cytoplasm suggested

a shuttling of *RPGRIP1L* between these different subcellular compartments. A dynamic localization has been observed in other nephronophthisis-associated proteins and might reflect a variability of function with the cell cycle. In addition, *RPGRIP1L* was present diffusely through the cytoplasm during cell division.[6] The primary cilium is essential for skin morphogenesis through regulating the Notch, Wnt, and hedgehog signaling pathways disrupting the *RPGRIL1* gene in mice that resulted in reduced proliferation and differentiation of follicular keratinocytes.[7] Several possible mechanisms related to *RPGRIP1L* gene mutation could help to understand correlation between such mutation and vitiligo. One fact that *RPGRIP1L* regulating proteasomal activity indicated that altered proteasomal components, immunosubunits for example, might influence the degradation process of soluble proteins and the generation of antigenic peptides thus resulting in disordered autoimmune, which might play a potential role in vitiligo. In addition, *RPGRIP1L* gene is important for the proliferation and differentiation of keratinocytes while keratinocytes are necessary for maintenance of melanocytes through producing growth factors and cytokines. Hence, the mutation affects melanocytes function in an indirect way. The diffuse cellular localization of *RPGRIP1L* gene suggested that it is engaged into multifunction in different subcellular compartments indicating that such mutation might act in various cellular functions including depigmenting disorder.[8] The D1264N (rs3213758) mutation involved a change from aspartic acid (Asp1264) to asparagine (Asn1264). This study demonstrated that the CT genotype of the *RPGRIP1L* gene rs3213758 locus could significantly reduce the susceptibility to SV in a Chinese Han population. Although there was no significant difference in the frequency of the TT genotype among the three groups, there was an increased trend of susceptibility to SV. However, the combined CT + TT genotype was significantly lowered in the SV group, compared with the NSV and control groups. This finding might be because the CT genotype had a stronger ability to reduce susceptibility to SV than the TT genotype. There was no significant difference in the frequency of each genotype and allele between NSV and control groups, which further proved that the CT genotype was only the protective genotype of SV. This study also demonstrated that the CT genotype of the *RPGRIP1L* gene rs3213758 locus could significantly reduce the susceptibility to SV in males and subjects aged <20 years. These data indicated that the CT genotype of *RPGRIP1L* was protective against SV in the Chinese Han population, especially males and subjects aged <20 years. Since some mutations in the *RPGRIP1L* gene were associated with multiple clinical phenotypes of neurological diseases, we could infer that neural factors are related to the susceptibility to SV in the Chinese Han population. This study provided further evidence for the neural hypothesis of the pathogenesis of SV in the Chinese Han population. There was no significant difference in the staging of the disease, onset age, and the area of lesions among SV patients with the three genotypes. However, in terms of the 6–12 months duration of disease, the rate of patients with the CT genotype was 4.343-time greater than that of patients with the CC genotype. Therefore, CT might reduce the susceptibility to SV, but it could also prolong the duration. In addition, the frequency of the T allele in the SV group was higher than that in the NSV group or the control group, indicating that the T allele might increase the risk of vitiligo in a Chinese Han population. A study with larger sample size is needed to further elucidate the role of the *RPGRIP1L* gene polymorphism and neural factors in the susceptibility to SV in the future.

Declaration of patient consent

The authors certify that they have obtained patient consent form. In the form, the parents have given their consent for the patient's images and other clinical information to be reported in the journal. They understand that the patient's name and initials will not be published and due efforts will be made to conceal her identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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