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ONAN, DURU; YORULMAZ, AHU; EZGÜ, FATİH SÜHEYL; HAYRAN, KADİR MUTLU; ÇAKMAK, SERAY KÜLCÜ; ARTÜZ, REFIKA FERDA; and YALÇIN, BAŞAK (2019) "The role of IFIH1 gene rs1990760 and rs2111485 single-nucleotide polymorphisms in generalized vitiligo predisposition," Turkish Journal of Medical Sciences: Vol. 49: No. 1, Article 30. https://doi.org/10.3906/sag-1808-63
Available at: https://journals.tubitak.gov.tr/medical/vol49/iss1/30

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This article is available in Turkish Journal of Medical Sciences: https://journals.tubitak.gov.tr/medical/vol49/iss1/30
The role of IFIH1 gene rs1990760 and rs2111485 single-nucleotide polymorphisms in generalized vitiligo predisposition

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Background/aim: Interferon-induced helicase (IFIH1) is a gene locus that has been recently defined as a candidate for susceptibility to generalized vitiligo (GV). The objectives of this study were to assess the association of IFIH1 gene, rs2111485, and rs1990760 single-nucleotide polymorphisms (SNP) with susceptibility to GV and the autoimmune diseases accompanying GV.

Materials and methods: We prospectively studied GV patients and frequency-matched healthy controls by age and sex. The genotypes of the participants were determined for rs1990760 and rs2111485 SNPs of IFIH1. Dominant, recessive, and additive models were evaluated for each SNP adjusted for age and sex.

Results: The patients and their controls were observed to be in the Hardy–Weinberg equilibrium for SNP1 (2q24.2, rs1990760, IFIH1, T/C) and SNP2 (2q24.2, rs2111485, IFIH1, G/A), respectively (all P > 0.7). For SNP1, every T allel addition was significantly associated with 1.53 times protectiveness in terms of vitiligo risk (P = 0.033). As for SNP2, every G allel addition was associated with 1.42 times protectiveness, close to statistical significance (P = 0.100).

Conclusions: We detected that for SNP1, each T allel and for SNP2, each G allel are protective in terms of vitiligo development. Hereby, we confirmed that IFIH1 gene locus has a role in GV susceptibility.

Key words: Vitiligo, single nucleotide polymorphism, genes

1. Introduction
Vitiligo is a common acquired disorder in which depigmented skin results from destruction of melanocytes (1). Generalized vitiligo (GV), the prevalent form of the disease, results from the interaction among multiple genetic and environmental factors that ultimately cause autoimmune destruction of melanocytes in affected regions (2). Vitiligo occurrence in other members of patients' families confirms the role of genetic factors in pathogenesis of the disease (3).

Vitiligo pathogenesis involves innate immune responses triggered by environmental and cell-intrinsic factors that cause melanocyte stress. Damaged melanocytes release damage-associated molecular patterns (DAMPs), such as reactive oxygen species (ROS) and heat shock proteins (HSPs), that function as ligands for innate pattern recognition receptors (PRRs). Activation of PRRs by stress-induced DAMPs initiate inflammation that drives melanocyte destruction (4).

Vitiligo often accompanies other autoimmune diseases, including type 1 diabetes mellitus (T1D), autoimmune thyroid disease, rheumatoid arthritis (RA), alopecia areata, adult-onset autoimmune diabetes mellitus, inflammatory bowel disease, pernicious anemia, autoimmune gastritis, psoriasis, systemic lupus erythematosus (SLE), discoid lupus, Guillain–Barré syndrome, myasthenia gravis, Addison's disease, linear morphea, and Sjögren's syndrome (2,5). This epidemiologic association can be seen also in the close relatives of GV patients, proposing that common genes lie behind predisposition to this group of diseases (6).

IFIH1 (interferon-induced helicase), also called MDA-5 (melanoma-differentiation-associated gene 5), is a locus in the innate immunity viral RNA receptor gene region on chromosome (2q24.3) (Gene ID: 64135) (7). The natural immune system senses viral infection by distinguishing a variety of viral components and thus initiates antiviral responses (8). IFIH1 encodes an RNA helicase that...
mediates the natural immune system’s antiviral interferon response through viral double-stranded (ds)RNA recognition (8,9). By functioning as a PRR, IFIH1 also activates innate immune responses on binding DAMPs that are not associated with pathogens, hereby giving way to autoimmune triggering (10).

IFIH1 has been reported to be associated with several autoimmune diseases such as T1D, Graves’ disease, multiple sclerosis, psoriasis, and possibly lupus (5,7,11–16); it has also been recently defined as a candidate for susceptibility to GV (10,17). No population-based, randomized controlled study has been reported since then to confirm the association between IFIH1 and vitiligo.

The research question of this study is whether IFIH1 gene locus plays a role in GV susceptibility. Accordingly, we aimed to investigate the association of IFIH1 gene polymorphisms with susceptibility of GV and the autoimmune diseases accompanying GV.

2. Materials and methods
This gene analysis study was designed as a case-control study. Institutional Ethics Committee of our research hospital approved the project (757/2014) and informed consent was obtained from the subjects. We prospectively studied GV patients and frequency-matched healthy controls by age and sex in the Turkish population. The subjects were selected consecutively from the patients who were admitted to the Dermatology department of our hospital between January 2014 and August 2015. Patients who did not meet the term of nonsegmental vitiligo in the dermatological examination and controls with any autoimmune disease diagnosis and family history of vitiligo were excluded from the study.

The patient group was questioned for skin phototype, age of vitiligo onset, disease duration and activity, treatment history, and repigmentation history (spontaneous/after treatment). Any other concomitant autoimmune disease, family history of vitiligo, or any other autoimmune disease were also recorded.

2.1. The analysis of rs2111485 and rs1990760 single-nucleotide polymorphisms (SNPs)
2.1.1. DNA isolation
Blood samples were collected from the patients and the controls into EDTA tubes in order to isolate the genomic DNA. The samples were transferred to a molecular analysis laboratory where the DNA isolation was carried out by using InVitrogenprep® isolation system. The quality and the quantity of the isolated DNA was detected with Nanodrop ® spectrophotometer. DNA samples were stored in eppendorf tubes that do not contain DNase and RNase until the time of analysis in a calibrated deep-freezer at −200 °C.

2.1.2. Amplification of the target regions
rs2111485 and rs1990760 SNPs were prepared and ordered from the ABI File Builder Software® system. Genotyping solution was diluted 20X with 1XTE buffer in order to form the study solution which was then stored at −200 °C. The analysis was performed with ABI Fast 7500 HT real-time PCR system by using a plate of 96 wells. For the amplification, 11.25 µL of genomic DNA was mixed with TaqMan® Universal PCR Master Mix (2X), 12.5 µL of No AmpErase UNG, and 1.25 µL of 20X working stock of SNP GenotypingAssay per each well and was amplified in a thermal cycler. Thermal cycler conditions for amplification: enzyme activation at 95 °C for 10 min; 40 cycles of denaturation at 92 °C for 15 s; then 40 cycles of annealing and extension at 60 °C for 1 min.

2.1.3. Allelic discrimination and analysis
After the PCR amplification was completed, the genotypes of the participants in terms of rs2111485 and rs1990760 SNPs of IFIH1 locus were determined by ABI 7500 Fast analysis system.

The results were analyzed by a clinical and biochemical geneticist. A precise result was obtained for at least one of the SNPs in 127 of the patients and 111 of the controls. The missing results were due to amplification failure in PCR, probably resulting from unmatched primers due to a different underlying SNP or low-quality DNA. With the completed number of patients, the study has 80% power to detect an odds ratio of 2.2 for SNP1 and 2.4 for SNP2 with 5% type-I error level as statistically significant.

2.2. Statistical analysis
IBM SPSS version 21 was used for data analysis. Descriptive statistics were presented by frequencies, percentages, and median (min, max) values. The patients and their controls were tested for the Hardy–Weinberg equilibrium for SNP1 (2q24.2, rs1990760, IFIH1, T/C) and SNP2 (2q24.2, rs2111485, IFIH1, G/A), respectively. Dominant (C/C, C/T, T/T; A/A, A/G-G/G), recessive (C/C-C/T, T/T; A/A-A/G, G/G), and additive models were evaluated for each SNP, with adjustment for age and sex using logistic regression. In the additive model, additional protectiveness of each T allele for SNP1 and each G allele for SNP2 were estimated. The association between patient characteristics and each SNP was investigated using Chi-square test. A P-value of less than 0.05 was considered to indicate statistical significance.

3. Results
There was no difference in terms of mean ages between the vitiligo patient group (40.6 ± 16.5) (range: 13–82) and the control group (43.0 ± 17.0) (range: 10–84) (P = 0.262). Similarly, no difference was detected in terms of sex between the vitiligo patient group [71 females (55.9%); 56 males (44.1%)] and the control group [64 females
etiopathogenesis of vitiligo.

IFIH1 gene polymorphisms possibly play a role in the susceptibility of GV. We detected an association between IFIH1 gene polymorphisms and GV susceptibility. We showed that, in terms of vitiligo development, each T allele is protective for SNP1 and each G allele is protective for SNP2. These findings reveal that IFIH1 gene polymorphisms possibly play a role in the etiopathogenesis of vitiligo.

Although no relationship was found between family history and gene polymorphism in our study, since the reliability of the family history given by the vitiligo patients was not certain and some of the positive family history was implying distant relatives, this result might not be reflecting the actual relationship.

To identify genes involved in susceptibility to GV; candidate gene association studies, genome-wide linkage studies and recently genome-wide association studies (GWASs) have been applied in sequence according to time. So far, GWASs have discovered approximately 50 different genetic loci that contribute to vitiligo risk, some of which are shared with other autoimmune disorders that have epidemiological association with vitiligo (18). One of these gene loci IFIH1, previously proven to be related with several autoimmune diseases, was recently defined as a candidate for susceptibility to GV (1,10,17).

IFIH1 is one of the most significantly associated loci with vitiligo (1). Several common variants in IFIH1, the most associated variant being the rs2111485, showed highly significant association with vitiligo. A missense variant of IFIH1, rs35667974 (I923V), was reported to show significant conditional association independent of rs2111485. All missense variants of IFIH1, i.e. rs1990760 (A946T), rs3747517 (H843R), and rs35667974 (I923V), showed association with vitiligo in the independent replication study and the former two showed associations in all three GWASs (10).

Many vitiligo susceptibility genes that control immunoregulatory and apoptotic functions are in common with those that control other autoimmune disorders. These shared genetic associations are consistent with clinical epidemiological relations of these disorders (17,18). IFIH1, together with nsSNP rs1990760, was defined as the strongest candidate for T1D susceptibility (7). Nejentsev et al. confirmed the association of the two common missense polymorphisms, rs1990760 (T946A) and rs3747517 (R843H), with T1D (19). Sutherland et al. confirmed IFIH1 as a new autoimmunity locus, extending its role in susceptibility of T1D to Graves’ disease and proposed that Ala946Thr IFIH1 polymorphism of SNP rs1990760 is significantly associated with organ-specific autoimmune diseases, including Graves’ disease (16). Gateva et al. confirmed the association of the same missense allele of IFIH1 (rs1990760) with SLE (14). Psoriasis was also reported to be associated with rare missense variants in the IFIH1 gene locus (12).

Vitiligo-associated IFIH1 functional variants, missense SNPs rs1990760 (A946T) and rs3747517 (R843H), were reported to be protective against T1D in European individuals (7,9,19). It was proposed that rare alleles of all associated IFIH1 polymorphisms protect from T1D,
Table 1. Clinical characteristics of the study subjects.

| Clinical characteristics | Vitiligo patients | Controls |
|--------------------------|-------------------|----------|
|                         | n = 127           | n = 111  |
| Age (years) (mean ± SD)  | 40.6 ± 16.5       | 43.0 ± 17.0 |
| Sex (female/male)       | 71/56             | 64/47    |
| Skin phototype          |                   |          |
| II                       | 23                | 18.1     |
| III                      | 46                | 36.2     |
| IV                       | 58                | 45.7     |
| Disease activity        |                   |          |
| stable                   | 35                | 27.6     |
| progressive              | 88                | 69.3     |
| regressive               | 4                 | 3.1      |
| Treatment history (+/-)  | 66/61             | 52.0/48.0|
| Family history of autoimmune disease (+/-) | 38/89 | 29.9/70.1 |
| Family history of vitiligo (+/-) | 43/84 | 33.9/66.1 |

Table 2. Distribution of autoimmune disease types accompanying vitiligo.

| Autoimmune diseases                  | n  | %   |
|--------------------------------------|----|-----|
| Total                                | 36 | 28.3|
| Thyroid disease                      | 23 | 18.1|
| Pernicious anemia                    | 9  | 7.0 |
| Alopecia areata                      | 4  | 3.2 |
| Type 1 diabetes mellitus             | 3  | 2.4 |
| Lupus                                | 2  | 1.6 |
| Bullous pemphigoid                   | 1  | 0.8 |
| Scleroderma                          | 1  | 0.8 |
| Lichen planus                        | 1  | 0.8 |
| Celiac disease                       | 1  | 0.8 |
| Idiopathic thrombocytopenic purpura  | 1  | 0.8 |
| Rheumatoid arthritis                 | 1  | 0.8 |
| Two different autoimmune diseases    | 11 | 8.6 |
whereas common \textit{IFIH1} alleles create predisposition to the disease (19). Missense rs1990760 (A946T) was proposed to be protective against SLE (20), rheumatoid arthritis (21), multiple sclerosis (10), and Graves’ disease (16) in the European and other populations. It was shown that autoimmunity-protective \textit{IFIH1} missense variants decrease function of \textit{IFIH1}, thus reducing the ability to bind DAMPs involved in autoimmune triggering with eventual decreased stimulation of innate immune responses (9,10,12,22). These missense variants might be downregulating the gene response by changing the gene expression.

Genome-wide association studies of vitiligo detected that the association of \textit{IFIH1} functional variants (missense SNPs rs1990760 and rs35667974) was protective (1,10,17). Similar to the results of these GWAS studies which were performed in the European population by Jin et al. (1,10,17), we detected a protective association of the two functional variants of \textit{IFIH1} (rs1990760 and rs2111485) for vitiligo in the Turkish population. In conclusion, although the mechanisms by which \textit{IFIH1} polymorphisms contribute to vitiligo pathogenesis remain to be explored, it is clear that the \textit{IFIH1} gene locus has a role in GV susceptibility.

\textbf{Acknowledgement}
This work was supported in part by the grant of “Scientific Researches Supporting Fund” of Ankara Numune Training and Research Hospital.

\textbf{Table 3.} Estimated risks of vitiligo with the presence of polymorphisms for the two SNPs.

| rs1990760 | Genotype | Case \(n=114\) (%) | Control \(n=108\) (%) | OR\footnote{95\% (CI) lower} \footnote{95\% (CI) upper} | \(P\) |
|-----------|----------|-------------------|---------------------|----------------------|------|
| Additive  | CC (ref.) | 40 (35.1)         | 28 (25.9)           | 1.53\footnote{1.03} \footnote{2.26} | \textbf{0.033} |
|           | CT       | 57 (50.0)         | 54 (50.0)           |                      |      |
|           | TT       | 17 (14.9)         | 26 (24.1)           |                      |      |
| Dominant  | CC (ref.) | 40 (35.1)         | 28 (25.9)           | 1.65\footnote{0.91} \footnote{2.96} | \textbf{0.097} |
|           | CT/TT    | 74 (64.9)         | 80 (74.1)           |                      |      |
| Recessive | CC/CT (ref.) | 97 (85.1) | 82 (75.9)          | 1.89\footnote{0.95} \footnote{3.74} | \textbf{0.070} |
|           | TT       | 17 (14.9)         | 26 (24.1)           |                      |      |
| rs2111485 | Genotype | Case \(n = 98\) (%) | Control \(n = 85\) (%) | OR\footnote{95\% (CI) lower} \footnote{95\% (CI) upper} | \(P\) |
| Additive  | AA (ref.) | 38 (38.8)         | 25 (29.4)           | 1.42\footnote{0.94} \footnote{2.16} | \textbf{0.100} |
|           | AG       | 45 (45.9)         | 41 (48.2)           |                      |      |
|           | GG       | 15 (15.3)         | 19 (22.4)           |                      |      |
| Dominant  | AA (ref.) | 38 (38.8)         | 25 (29.4)           | 1.55\footnote{0.83} \footnote{2.89} | \textbf{0.169} |
|           | GG/AG    | 60 (61.2)         | 60 (70.6)           |                      |      |
| Recessive | AA/AG (ref.) | 83 (84.7) | 66 (77.6)          | 1.67\footnote{0.78} \footnote{3.57} | \textbf{0.183} |
|           | GG       | 15 (15.3)         | 19 (22.4)           |                      |      |

\textit{CI}: confidence interval, \textit{OR}: odds ratios adjusted for age and sex, \textit{SNP}: single-nucleotide polymorphism.

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