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A rare variant in CYP27A1 and its association with atopic dermatitis with high serum total IgE

Short title: CYP27A1 association with atopic dermatitis

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Abstract

This study investigated rare variants associated with atopic dermatitis. We performed exome analyses on 37 patients who were diagnosed with atopic dermatitis by board-certified dermatologists and had total serum IgE levels greater than 1,000 IU/ml. The exome analysis identified seven variants with <1% allele frequency in Asian (ASN) population of 1000 Genomes Project phase 1 data and >5% allele frequency in the atopic dermatitis exome samples. We then conducted a replication study using 469 atopic dermatitis patients with total serum IgE $\geq 1,000$ IU/ml and 935 Japanese controls to assess the presence of these 7 candidate variants. The replication study confirmed that *CYP27A1* rs199691576 (A/G) was associated with atopic dermatitis with high serum IgE levels ($P = 0.012$, odds ratio = 2.1). *CYP27A1* is involved in the metabolism of Vitamin D3, which plays important roles in modulating immune function. Previous studies have reported polymorphisms in Vitamin D pathway genes that are associated with allergy-related phenotypes. Our data confirm the importance of genes regulating the Vitamin D pathway in the development of atopic dermatitis.

Introduction

Atopic dermatitis is a common chronic skin disease that is often accompanied by increased serum IgE levels and skin barrier dysfunction (1, 2). The data obtained from both twin and family studies support the genetic basis for atopic dermatitis (3). Additionally, genome-wide association studies (GWASs) have identified several susceptibility genes for atopic dermatitis (4). However, the common single nucleotide polymorphisms (SNPs) identified by GWASs cannot account for much of the heritability of the diseases, which is termed “missing

heritability” (5). One hypothesis is that missing heritability is due to rare variants with large effect sizes. In the genetics of atopic dermatitis, rare variants with loss of function mutations in the filaggrin (*FLG*) gene have been consistently associated with the development of atopic dermatitis in European and Asian populations (6). These rare variants were not captured by commonly used SNP arrays for GWASs. However, the recent advance of next-generation sequencing techniques enabled us to find disease-associated rare variants. The aim of this study is to investigate rare variants associated with atopic dermatitis in the Japanese population by exome analysis.

Patients and Methods

The Medical Ethics Committees of University of Tsukuba and Moriya daiichi hospital approved the study, which was conducted in accordance with the Declaration of Helsinki.

Patients with atopic dermatitis were recruited for exome analyses from the Dermatology Department of the University Hospital of Tsukuba and Dermatology Departments of Moriya daiichi hospital, Japan. A full verbal and written explanation of the study was given to the patients, and informed consents were obtained from the participants.

Atopic dermatitis was diagnosed in subjects according to the criteria described by Hanifin and Rajka. All patients displayed the following symptoms: pruritus, typical appearance of atopic dermatitis, and a tendency of chronic or chronically relapsing dermatitis. The diagnosis of all patients who participated in this study was confirmed by board-certified dermatologists.

Serum IgE levels are associated with the severity of atopic dermatitis and contribute to skin barrier defects (7). Individuals with extreme phenotypes increase the power in rare-variant research (8). Therefore, we increased the efficiency of the exome analysis by selecting the patients with a serum IgE > 1,000 IU/ml. This value is > +2SD of the total serum IgE in the

general Japanese population. The mean age of the 37 patients for exome analysis was 32 ± 11.7 years old; 30% of the patients were female, and their total serum IgE levels were 1,622-106,350 IU/mL (geometric mean 6,573 IU/mL). None of the patients showed characteristic features of hyper IgE syndrome.

Exome sequencing was performed following the protocol described in the SureSelect Library prep kit (post-pool version 4, Agilent Technologies, Inc., Santa Clara, CA, USA) and the sequence was run using SOLiD 5500xl (Life Technologies, Carlsbad, CA, USA). Details of this analysis are described in the supporting information, and our variant selection methods are summarized in Fig S1. We filtered out variants in our in-house references (17 exome samples). Variants with <1% allele frequency in Asian (ASN) population of 1000 Genomes Project phase 1 data including 89 Japanese in Tokyo, Japan (JPT), 97 Han Chinese in Beijing, China (CHB) and 100 Southern Han Chinese (CHS) (1000g2012apr dataset of ANNOVAR website, <http://annovar.openbioinformatics.org/en/latest/user-guide/download/>) or those with <1% allele frequency in the phase I JPT were filtered out. We further selected variants present at >5% allele frequency in the atopic dermatitis exome samples. These criteria were used to find rare variants associated with atopic dermatitis. After this filtering step was applied, 7 variants remained and were further screened using replication samples.

In the replication study, the ethics committee at the RIKEN Yokohama Institute approved this research project. The diagnostic criteria for patients with atopic dermatitis in the replication samples was consistent with the criteria of Hanifin and Rajka and were described in detail in a previous study (9). This study also included 935 healthy volunteer control subjects from members of the Rotary Club of Osaka-Midosuji District 2660 Rotary International in Japan. We performed genotyping of variants by multiplex-PCR-based

Invader assay (Third Wave Technologies, Madison, WI, USA) to replicate the results of the exome analysis.

Fisher's Exact test was performed to assess whether these 7 variants were associated with susceptibility to atopic dermatitis. We performed a two-stage analysis—screening (exome) and replication (case control study using independent samples)—with the statistical significance of the P value set at 0.05 in the replication step.

Because *FLG* loss-of-function mutations have been repeatedly associated with atopic dermatitis (6), we genotyped the five most common variants (c.3321delA, p.Ser2554X, p.Ser2889X, p.Ser3296X, p.Lys4022X) in exome samples, to determine whether our exome analysis was able to successfully identify them. c.3321delA was determined by size, using a fluorescently-labeled PCR and an Applied Biosystems 3130 Genetic Analyzer (Life Technologies); p.Ser2554X, p.Ser2889X, p.Ser3296X, and p.Lys4022X, using a Taqman Assay-by-Design system for single-nucleotide polymorphism genotyping (Life Technologies).

Results and Discussion

Table 1 shows the variants with <1% allele frequency in the 1000 genomes project phase I ASN population that were present in >5% of the atopic dermatitis exome samples in the present study. All of the identified variants were missense variants. The results of the replication study, which used independent cases and controls, are summarized in Table 2. Of the 7 variants identified in the exome analysis, 1 variant of *CYP27A1* (rs199691576) was confirmed to be associated with atopic dermatitis, with elevated levels of total serum IgE ($P =$

0.012, OR = 2.108 for Fisher's exact test), and the minor A allele is a risk allele for atopic dermatitis. CYP27A1 encodes a member of cytochrome P450 oxidase and is involved in the metabolism of Vitamin D3 (10). In humans, CYP27A1 is the essential enzyme for vitamin D3 25-hydroxylation (10). Vitamin D plays important roles in modulating immune function (11). Previous studies have reported that gene polymorphisms in the vitamin D pathway are associated with asthma and asthma-related phenotypes (9, 12). Wang et al recently reported that the G allele of rs4674343 (G/A) in *CYP27A1* had a protective effect on the development of atopic eczema in the Chinese population (13). In the present study, the minor A allele of rs199691576 (G/A) was shown to be a risk factor for the development of atopic dermatitis with high total serum IgE levels. The A allele of rs199691576 is in complete linkage disequilibrium ($D' = 1$) with the major allele (A allele) of rs4674343, which was the susceptibility allele identified in the study by Wang et al (13). The polymorphism rs199691576 was not genotyped in the previous studies because of the low frequency. Additionally, the risk allele is in linkage disequilibrium with major alleles of common SNPs in *CYP27A1*. As a result, it is difficult to identify this specific polymorphism by genotype evaluation using GWAS data.

Several exome studies have been performed to identify causal variants for atopic dermatitis. Margolis et al performed exome analysis using African atopic dermatitis samples. The authors reported that *FLG2* mutations were associated with more persistent atopic dermatitis (14). In the present study, a common stop gain mutation of *FLG2* (rs12568784) was not associated with atopic dermatitis ($P > 0.1$). *FLG* loss-of-function mutations have been associated with atopic dermatitis in populations of various ethnicities (6). *FLG* is characterized by a repetitive sequence in a large third exon, and our exome analysis successfully identified these mutations (one patient with p.Ser2554X and two with Lys4022X; 3/37 [8.1%]). The *FLG* genotyping results in our exome analysis were concordant

with those by the TaqMan genotyping assay and fluorescently labeled PCR (concordance rate: 100%). None of the in-house references (17 exome samples) carried the *FLG* loss-of-function mutations. The frequency of the mutation carriers in the exome samples was not different from that reported in the general Japanese population (15).

In conclusion, we demonstrated the rare missense variant of *CYP27A1* is associated with atopic dermatitis with elevated levels of total serum IgE. The rare variants are often population-specific and are difficult to be tagged by common SNPs. Although functional studies are needed to elucidate the effect of variants on the development of disease, exome analyses and whole genome sequencing are powerful tools that can identify rare population-specific variants associated with common diseases.

Author contributions

JF and HE are responsible for collection of data. HS, YM, MN, TH, MT and EN performed the experiments and data analyses and all authors contributed substantially to interpretation of data. The paper was written by HS and EN. All authors approved the final version of the manuscript. Conflicts of interest

The authors declare that they have no conflicts of interest.

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Figure Legend

Figure S1. Exome analysis workflow (top) and filtering steps (bottom) for identification of variants associated with atopic dermatitis with high total serum IgE

Table 1. Variants associated with atopic dermatitis with total serum IgE greater than 1000 IU/ml.

SNP ID	Gene	Chromosome (bp)	Allele (minor allele)	Case			1000G_ASN MAF
				Wild	Variant	MAF	
rs145862664	APOB	2p24-p23 (21251380)	C/G (G)	70	4	0.05	0.0035
rs199691576	CYP27A1	2q35 (219677340)	G/A (A)	70	4	0.05	0.0017
rs193146105	C3orf15	3q13.33 (119466698)	G/A (A)	70	4	0.05	0.0017
rs142107211	GAK	4p16 (843498)	G/A (A)	70	4	0.05	0.0035
rs200230703	VNN2	6q23-q24 (133073776)	T/A (A)	69	5	0.06	0.0017
rs200193128	USP35	11q14.1 (77920690)	T/A (A)	70	4	0.05	0.0017
rs76428401	ZNF749	19q13.43 (57956642)	C/T (T)	70	4	0.05	0.0035

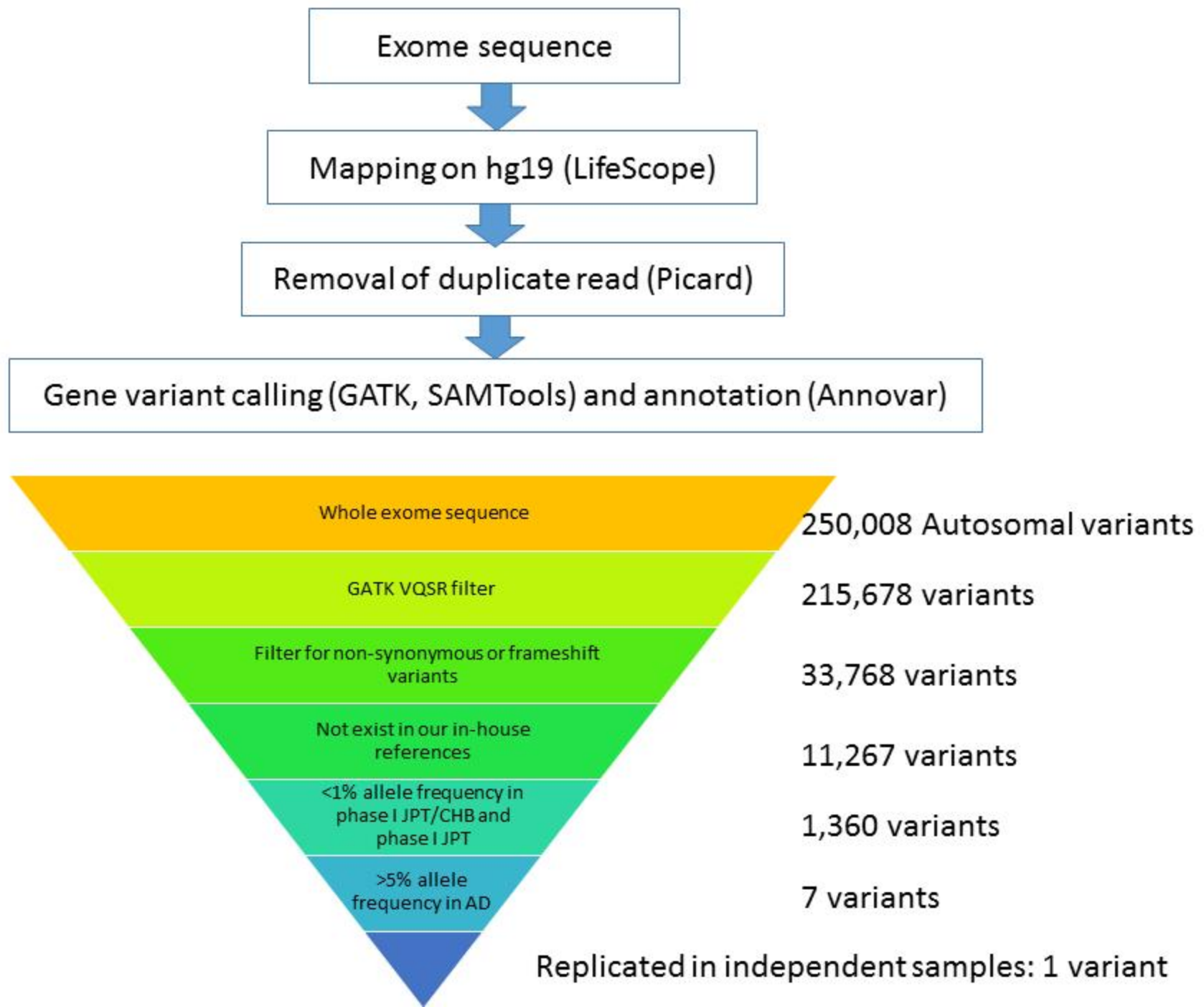
1000G_ASN MAF, minor allele frequency in Asian (ASN) population of 1000 Genomes Project phase 1 data

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Table 2. Results of replication study of 7 candidate variants using 469 atopic dermatitis patients.

Gene	AD with IgE $\geq$ 1000 IU/ml				Control				<i>P</i> value*	OR (95%CI)
	AA	Aa	aa	MAF	AA	Aa	aa	MAF		
APOB	445	23	0	0.025	892	41	1	0.023	0.793	1.069 (0.611-1.827)
CYP27A1	445	24	0	0.026	912	23	0	0.012	0.012	2.108 (1.133-3.932)
C3orf15	465	3	0	0.003	926	7	0	0.004	1.000	0.854 (0.142-3.752)
GAK	441	22	0	0.024	889	43	0	0.023	0.895	1.031 (0.583-1.774)
VNN2	458	10	0	0.011	914	18	0	0.010	0.841	1.107 (0.455-2.542)
USP35	461	8	0	0.009	910	23	0	0.012	0.446	0.689 (0.266-1.604)
ZNF749	438	31	0	0.033	880	53	2	0.030	0.731	1.087 (0.673-1.726)

AD, atopic dermatitis; AA, wild type homozygote; Aa, heterozygote; aa, minor allele homozygote; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval
P values were calculated using Fisher's exact test



SUPPORTING INFORMATION

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Short title: CYP27A1 association with atopic dermatitis

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Supplementary Methods

Exome analysis

Exome sequencing was performed following the protocol described in the SureSelect Library prep kit (post-pool version 4, Agilent Technologies, Inc., Santa Clara, CA, USA). The DNA library was subjected to emulsion PCR (SOLiD™ EZ Bead™ Emulsifier kit, Life Technologies, Carlsbad, CA, USA) to generate clonal DNA fragments on beads. We then performed bead enrichment (SOLiD™ EZ Bead™ Enrichment kit, Life Technologies). The enriched template beads were sequenced on a SOLiD 5500xl sequencer as single-end 60-bp reads (Life Technologies). The SOLiD 5500xl output reads were aligned against the human genome reference sequence (hg19) by using LifeScope version 2.5.1 (Life Technologies) to generate BAM files. The gene variant calling was performed following the best practices specified in the Genome Analysis Toolkit (1) (GATK, version 2.7.4), Picard (<http://picard.sourceforge.net>), and SAMtools (2); variants which passed the Variant Quality Score Recalibration (VQSR) (1) filter were selected. Our variant selection methods are summarized in Fig S1. Only the reads that mapped to a unique position in the reference genome were used. We then used the ANNOVAR software to filter out synonymous variants and intronic variants because they are less likely to be pathogenic (3).

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