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Short report

Compound heterozygotes for filaggrin gene mutations do not always show severe atopic dermatitis

Running head: Compound heterozygotes for *FLG* mutations

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Conflicts of interest

None of the authors has any conflict of interest to declare.

Abstract

Background Mutations in *FLG*, which encodes profilaggrin, cause ichthyosis vulgaris (IV) and are an important predisposing factor for atopic dermatitis (AD). IV shows autosomal hemidominant (semidominant) inheritance, and patients with bi-allelic *FLG* mutations tend to have severe IV phenotypes. However, the effect of bi-allelic *FLG* mutations on AD incidence and severity remains a subject of controversy.

Objective In the present study, we studied individuals with bi-allelic null *FLG* mutations to elucidate the effect of bi-allelic FLG mutations on AD incidence and severity.

Methods Six individuals with bi-allelic FLG null mutations from three families of IV/AD were investigated. We report the detailed clinical features of the individuals. The phenotype was confirmed by the clinical examinations and the severity of IV and AD was scored using ichthyosis score and EASI (Eczema Area and Severity Index).

Result It was found that five of the six patients had severe IV, and the remaining patient showed moderate IV. Two of the six had moderate AD, and three of the six had mild AD. The remaining patient had no AD.

Conclusion Our results suggest that individuals with bi-allelic *FLG* mutations do not always have severe AD and confirm that not all individuals with bi-allelic *FLG* mutations have AD.

Main text

Introduction

Mutations in *FLG*, the gene encoding profilaggrin/filaggrin, cause ichthyosis vulgaris (IV) and, in addition, are an important predisposing factor for atopic dermatitis (AD)[1]. Furthermore, *FLG* mutations are significantly associated with asthma with AD[2, 3, 4].

The presence of population-specific *FLG* mutations has been reported in both Europeans and Asians[1, 5, 6] and is a serious obstacle to *FLG* mutation screening in each population. Recently, we established a real-time polymerase chain reaction (PCR)-based rapid detection system for the comprehensive screening of ten Japanese population-specific *FLG* mutations[7]. In the present study, using this high-throughput screening system, we found six patients with compound heterozygous *FLG* mutations from three families and we detail the clinical features of these patients with complete filaggrin deficiency.

Materials and Methods

The *FLG* genotypes and clinical features of the patients with bi-allelic *FLG* mutations are summarized in Table 1.

Patients and families

Family A

A 9-year-old Japanese boy (Patient A-II-1) with dry skin, palmar hyperlinearity, lamellar scaling and eczema was diagnosed with AD and IV (Fig. 1a). His 6-year-old younger brother (Patient A-II-2) with AD and IV also had dry skin, palmar hyperlinearity and lamellar scaling (Fig. 1b, c). Their 35-year-old mother (Patient A-I-2)

had IV with dry skin, palmar hyperlinearity and lamellar scaling, but did not have AD (Fig. 1d, e). Their father also had IV, but not AD.

Family B

An 11-year-old Japanese girl (Patient B-II-5) had been suffering from AD from early childhood (Fig. 1f). She had dry skin, palmar hyperlinearity and lamellar scaling, which lead to the diagnosis of IV (Fig. 1f, g). Her 13-year-old brother (Patient B-II-4) was diagnosed with AD and IV with dry skin, palmar hyperlinearity and lamellar scaling (Fig. 1h, i). Her parents had IV and AD.

Family C

A 28-year-old Japanese woman (Patient C-I-1) with dry skin and lamellar scaling had been suffering from AD since early childhood (Fig. 1j, k).

FLG mutation search

As we reported[7], a DNA sample from each patient was screened by real-time PCR-based genotyping of the ten *FLG* null mutations (p.R501X, c.3321delA, p.S1695X, p.G1701X, p.S2554X, p.S2889X, p.S3296X, p.K4022X, p.Q1790X, c.441-442delA) which have been identified in the Japanese population, 9 of which were found by our group[6, 7].

We have already performed sequencing of all the coding regions of *FLG* for more than 40 Japanese families with IV, to comprehensively characterize Japanese-specific *FLG* mutations[7]. We expect that screening for these 10 mutations can detect almost all Japanese *FLG* mutation carriers[7]. This study was approved by the Ethics Review Committee of the Nagoya University Graduate School of Medicine.

Evaluation of clinical features

Each patient with bi-allelic *FLG* null mutations was examined for typical symptoms of AD (eczema, erythema, induration, papulation, excoriation and lichenification) and IV (scaling, rhagades and palmar hyperlinearity). The severity of IV and AD was scored using ichthyosis score (IS)[8] and EASI (Eczema Area and Severity Index)[9], respectively.

Results

FLG genotyping

Family A

Analysis for *FLG* mutations showed Patient A-II-1 to be compound heterozygous for p.Q1790X and p.S3296X. Patient A-II-2 was compound heterozygous for p.Q1790X and p.S2889X in *FLG*. Patient A-I-2 was compound heterozygous for p.S2889X and p.S3296X. The father of Patient A-II-1 was heterozygous for p.Q1790X.

Family B

Mutation analysis revealed Patient B-II-5 and Patient B-II-4 to be compound heterozygous for p.Q1701X and p.S2889X in *FLG*. Their mother was heterozygous for p.S2889X. Their father was heterozygous for p.Q1701X.

Family C

Analysis for *FLG* mutations showed Patient C-I-1 to be compound heterozygous for c.3321delA and p.S2889X.

Clinical features

Family A

ISs of Patient A-II-1, Patient A-II-2 and Patient A-I-2 were 26.5, 26.5 and 17, respectively. Concerning the severity of AD, the EASIs of Patient A-II-1, Patient A-II-2

and Patient A-I-2 were 11.6, 4.6 and 0, respectively.

Patient A-II-1 had been also suffering from hay fever (HF), allergic rhinitis (AR) and allergic conjunctivitis (AC). Patient A-II-1 did not have food allergies (FAs), but had a history of FAs to egg and milk in early childhood. Laboratory examinations for Patient A-II-1 revealed elevated total IgE of 2456 U/ml (normal: 0-170) and TARC of 2417 pg/ml (normal: 0-450).

Patient A-II-2 had HF and AR, and also had a history of FAs to egg and milk in early childhood. The results of laboratory examinations for Patient A-II-2 were as follows: IgE of 1641 U/ml and TARC of 1507 pg/ml.

Patient A-I-2 did not have AD, FA, HF or AC.

Family B

EASIs of Patient B-II-5 and Patient B-II-4 were 5.6 and 6.4, respectively. ISs of Patient B-II-5 and Patient B-II-4 were both 27.

Patient B-II-5 had FAs to egg, milk and tuna, and had AS, HF and AR. Patient B-II-4 had also been suffering from FAs to egg and milk, and had AS, HF and AR. Laboratory examinations for Patients B-II-5 and B-II-4 revealed elevated serum IgE of 9633 U/ml and 9485 U/ml, respectively.

Family C

EASI and IS of Patient C-I-1 were 18.4 and 32, respectively.

Patient C-I-1 had FAs to egg, artemisia, rice, soybeans, wheat, buckwheat, shrimp and crab. She also had AS, HY, AR and AC. Laboratory examinations of Patient C-I-1 revealed elevated total serum IgE of 20,136 U/ml and TARC of 1743 pg/ml.

Summary of the clinical features

Five of the six patients showed moderately severe ichthyosis (ISs of 26.5-32). Considering that the IV phenotype is generally very mild ichthyosis, these five patients had significantly severe IV phenotypes (Fig. 1a, b, c, f, g, h, i). IS of the other patient was 17, and she showed moderately severe IV (Fig1d, e). Two patients and three patients out of the six had moderate AD (EASIs of 11.6-18.4)[10] and mild AD (EASIs of 4.6-6.4)[10], respectively (Fig. 1 b, f, h, i, j, k). The other patient had no AD (Fig. 1d, e).

All five AD patients with bi-allelic *FLG* mutations had current FA or a history of FA; the remaining non-AD patient with bi-allelic *FLG* mutations had no current FA and not history of FA.

Three of the AD patients had AS, and all three had current FA.

All five AD patients had HF, AR and AC. One non-AD individual had AR but not HF or AC.

Discussion

It is widely accepted that mono-allelic *FLG* mutations cause IV and bi-allelic *FLG* mutations often result in severe phenotypes of IV[11, 12]. In addition, *FLG* null mutations are well known to be a predisposing factor for AD. However, it is still controversial as to whether individuals with bi-allelic *FLG* mutations have more severe AD and higher incidence of AD than individuals with mono-allelic *FLG* mutations.

As we mentioned above, individuals with bi-allelic *FLG* mutations were described to have severe symptoms of IV[11, 12]. However, it was reported that even if a patient had compound heterozygous *FLG* mutations, sometimes he or she had a mild phenotype of IV[13].

According to a previous report, 70% of individuals with bi-allelic *FLG* mutations developed AD[14]. Another report showed that all patients with bi-allelic *FLG* mutations had symptoms of IV, but only 5 patients out of 9 (56%) had eczema[15].

Interestingly, our case without AD, Patient A-I-2, had milder IV symptoms than the

other patients with bi-allelic *FLG* mutations. Although we are not sure why she did not have AD, AS or HF, her life history and growth environment might have played a role. For example, a hot, humid environment[16] and proper skin care might have led to the mild IV phenotype, resulting in the prevention of AD in Patient A-I-2. It has been suggested that *FLG* mutations have strong effects for the compound phenotype of AS plus eczema, but are not associated with AS in the absence of eczema[2]. Indeed, Patient A-I-2 without AD had no other allergic diseases, including AS.

As previously reported[17], in the present series of AD patients with bi-allelic *FLG* mutations, all the patients who had severe symptoms of IV developed AD in early childhood and often had other allergic diseases. It was reported that patients with AD and *FLG* mutations tend to have higher serum IgE than AD patients without *FLG* mutations[18]. In our current cases, serum IgE was not particularly high for AD patients. A weakly positive correlation was observed between serum IgE levels and patients' ages, but it was not significant ($p=0.0833$, $r=0.9000$).

All five AD patients with bi-allelic *FLG* mutations in the present study have been treated by experienced dermatologists and their AD symptoms have been improved by topical therapy of steroid and tacrolimus. As a result, their AD symptoms have been well controlled for a while (Fig. 1a, c, g). These patients' courses suggest that appropriate treatments promote persistently favorable outcomes for AD patients with bi-allelic *FLG* null mutations.

To the best of our knowledge, there have been only a few reports describing clinical features of patients with compound heterozygous *FLG* mutations[19, 20]. Our present results suggest that patients with bi-allelic *FLG* mutations do not always have severe AD. Of course, it is necessary to analyse further, large numbers of AD patients and non-AD individuals with bi-allelic *FLG* mutations in order to elucidate their clinical

features and incidence of various allergic disorders including AD, FA, AS, HF, AR and AC.

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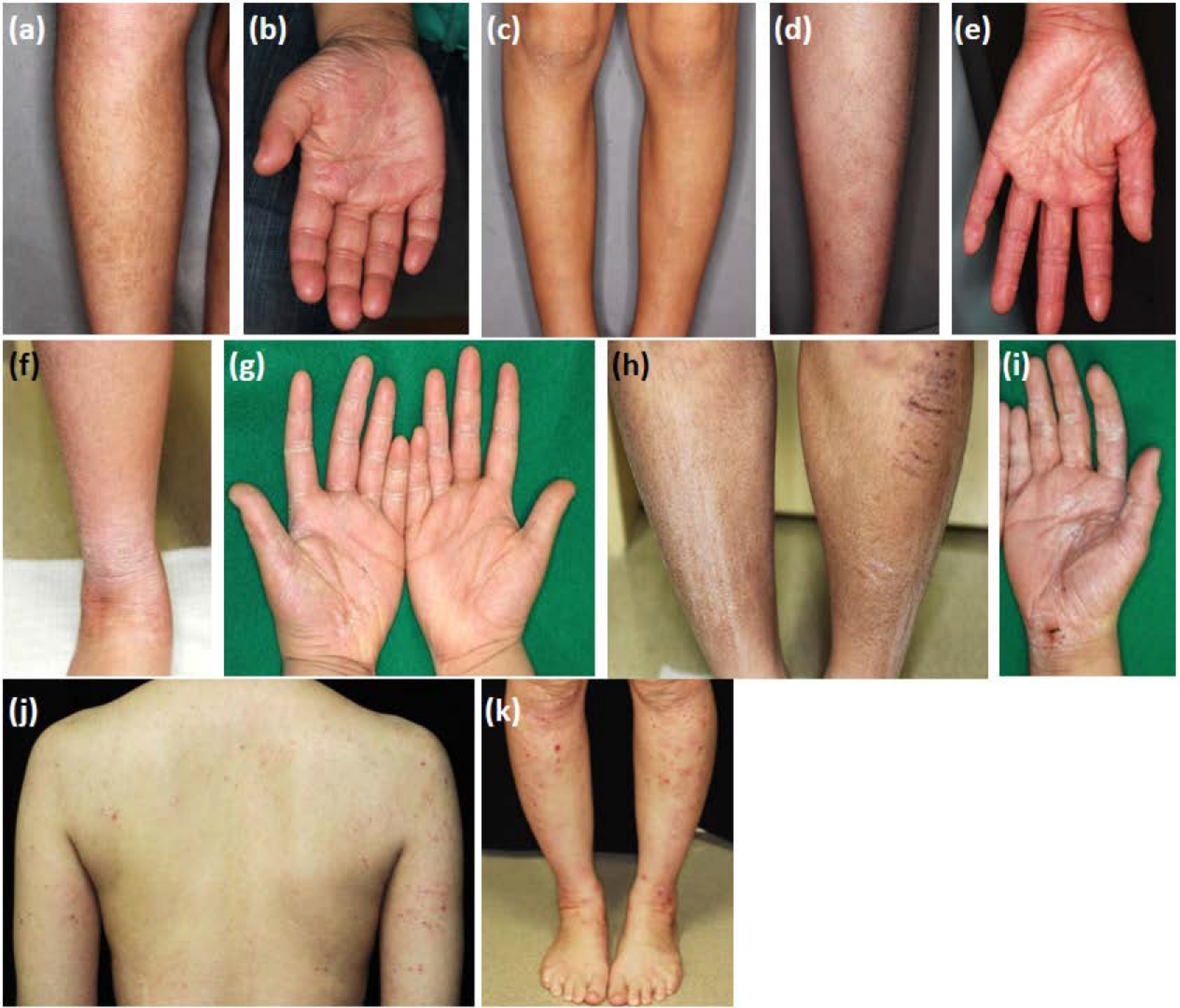
Figure legends

Figure 1. Clinical features of the compound heterozygotes for *FLG* mutations, and pedigrees of the two families. (a) Patient A-II-1 shows severe hyperkeratosis and scales as an IV phenotype on the lower leg. The eczema is well controlled (a). (b, c) Patient A-II-2 has moderate palmar hyperkeratosis and severe hyperlinearity (b) and severe hyperkeratosis on the lower legs (c) as an IV phenotype. Eczematous lesions are well controlled on the lower leg (c), although apparent eczema is seen on the palm (b). (d, e) Patient A-I-2 shows very mild hyperkeratosis and scaling without any eczema on the lower leg (d) and mild hyperlinearity and palmar keratosis (e). (f, g) Patient B-II-5 exhibits severe hyperkeratosis and scaling on the lower leg (f) and on the palm (g). Eczematous lesions are apparent on the lower legs (f), but not on the palms (g). Severe hyperlinearity is also seen on the palm (g). (h, i) Patient B-II-4 shows severe hyperkeratosis, scales and severe eczema on the lower legs (h) and severe palmar keratosis with hyperlinearity and eczematous lesions (i). (j, k) Patient C-I-1 has severe hyperkeratosis and scaling as an IV phenotype on the back, the upper arms (j) and the lower legs (k). She also has moderate eczema on the back, the upper arms (j) and the lower legs (k). (l, m) Pedigrees with *FLG* genotypes of Family A (l) and Family B (m).

Table 1. Summary of *FLG* genotypes and clinical features of the patients

	Patient A-II-1	Patient A-II-2	Patient A-I-2	Patient B-II-5	Patient B-II-4	Patient C-I-1
FLG mutations	p.Q1790X	p.Q1790X	p.S2889X	p.Q1701X	p.Q1701X	c.3321delA
	p.S3296X	p.S2889X	p.S3296X	p.S2889X	p.S2889X	p.S2889X
age/sex	9M	6M	35F	11F	13M	28F
ichthyosis vulgaris (IV)	+	+	+	+	+	+
ichthyosis score	26.5	26.5	17	27	27	32
palmar hyperlinearity	+	+	+	++	++	+
rhagades	−	+	−	+	+	+
lamellar scaling	+	+	+	+	+	+
atopic dermatitis (AD)	+	+	−	+	+	+
onset of AD	< 2y/o	< 2y/o		< 2y/o	< 2y/o	< 2y/o
EASI	11.6	4.6	0	5.6	6.4	18.4
dry skin	+	+	−	+	+	+
food allergy (FA)						
current	−	−	−	+	+	+
history	+	+	−	+	+	+
egg allergy	+	+	−	+	+	+
milk allergy	+	+	−	−	−	−
other allergy	−	−	−	+ (tuna)	−	+
asthma (AS)	−	−	−	+	+	+
hay fever (HF)	+	+	−	+	+	+
allergic rhinitis (AR)	+	+	+	+	+	+
allergic conjunctivitis (AC)	+	−	−	−	−	+
IgE	2456	1641		9633	9485	20136
TARC	2417	1507				1743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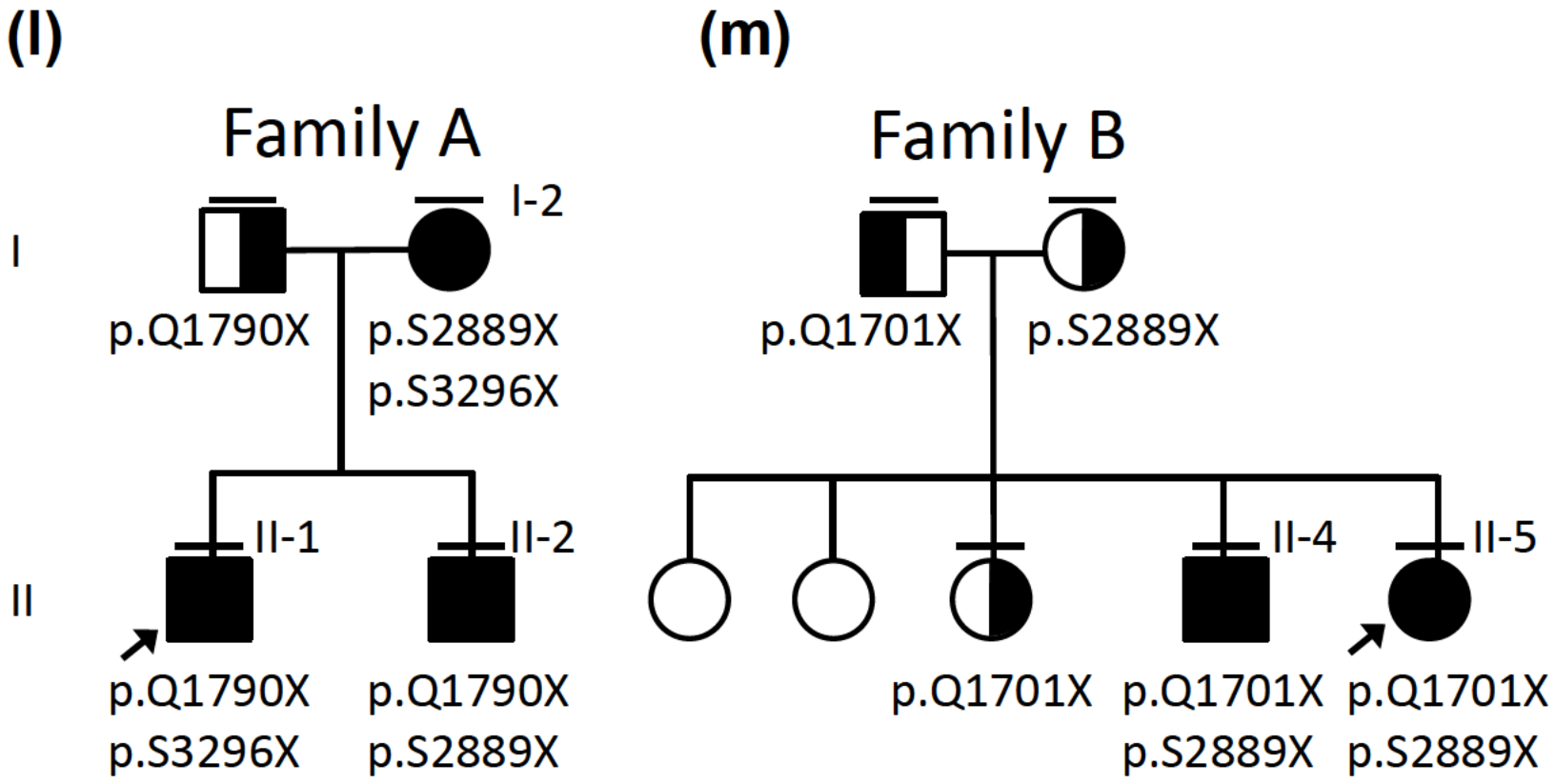
(l)
Family A
I
I-2
p.Q1790X
p.S2889X
p.S3296X
II
II-1
II-2
p.Q1790X
p.S3296X
p.Q1790X
p.S2889X
(m)
Family B
p.Q1701X
p.S2889X
II-4
II-5
p.Q1701X
p.Q1701X
p.S2889X
p.Q1701X
p.S2889X