De Novo Occurrence of the Filaggrin Mutation p.R501X with Prevalent Mutation c.3321delA in a Japanese Family with Ichthyosis Vulgaris Complicated by Atopic Dermatitis

Journal of Investigative Dermatology (2008) **128,** 1323–1325; doi:10.1038/sj.jid.5701164; published online 15 November 2007

TO THE EDITOR

The common inherited skin disorder ichthyosis vulgaris (IV; OMIM 146700) results from mutations in the gene encoding filaggrin (*FLG*), the keratin filament aggregating protein. Recently, the homozygous or compound heterozygous loss-of-function mutations p.R501X and c.2282del4 in *FLG* have been identified in moderate or severe IV patients from Ireland, Scotland, and USA (Smith *et al.*, 2006). These variants are carried by ~9% of European people and were also revealed to be an important predisposing factor for atopic dermatitis (AD; Palmer *et al.*, 2006). Meanwhile, c.3321delA and p.S2554X mutations in *FLG* were identified in Japanese IV and AD patients (Nomura *et al.*, 2007). Here, we performed direct sequencing of *FLG* in an additional Japanese family with IV complicated by AD, as well as screening for the above mutations. Analysis revealed compound heterozygous p.R501X and c.3321delA mutations.

The family was Japanese and included three affected individuals from two generations. The most severely affected boy was first referred to us when he was 7 years old. He showed diffuse, fine superficial scaling on the lower abdomen, and extensor surfaces of the extremities (Figure 1a). Later, he developed severe AD (Figure 1b). The 4-year-old younger sister of this patient was also severely affected with IV and had mild AD. The patient and his sister also developed asthma in early childhood. Although the mother showed mild scaling at a younger age, neither parent had atopic diathesis.

All described studies were performed following the medical ethical committee of Kurume University School of Medicine. Written informed consent was obtained from each individuals and the study was conducted according to the Declaration of Helsinki Principles. Genomic DNA from all family members was extracted from peripheral blood samples using standard methods. For *FLG* mutation analysis, PCR fragments were amplified as described previously (Smith *et al.*, 2006; Nomura *et al.*, 2007) and sequenced directly in an ABI310 genetic analyzer (Applied Biosystems, Foster City, CA). Direct sequencing of the most severely affected patient disclosed compound heterozygous nonsense/frameshift mutations: p.R501X and c.3321delA (GenBank NM_002016; Figure 2). The same compound heterozygous mutations were found in his sister. The mother and father were heterozygous for p.R501X and c.3321delA, respectively. The screening for p.R501X, c.2282del4, c.3321delA, and p.S2554X in additional four Japanese IV patients revealed negative results. The heterozygous mutation c.3321delA was found only in one Japanese AD patient but not in 200 ethnically matched controls by sizing of fluorescently labeled PCR fragment on an ABI310 genetic analyzer, as described previously (Nomura *et al.*, 2007). In addition, p.R501X and c.2282del4 were also not detected in 200 Japanese controls by restriction digestion as described previously (Smith *et al.*, 2006).

As this Japanese family had no European ancestry, it was of considerable interest to learn if p.R501X mutation, which is common in Europe, was present on the same chromosomal haplotype in this family (Sandilands *et al.*, 2007). Specifically, in Europeans, p.R501X is carried on one of the two known 11-repeat filaggrin size variant alleles (designated FLG^{8+} carrying a duplicated repeat 8), and this mutant allele also carries a non-synonymous single nucleotide polymorphism in filaggrin repeat 10, p.R3564L (Sandilands *et al.*, 2007). Direct sequencing showed that all family members here were homozygous for the 12-repeat filaggrin allele FLG^{8+10+}, which consists of duplicated repeat 8 and duplicated repeat 10 (Sandilands *et al.*, 2007). Furthermore, all family members were homozygous for a different single nucleotide polymorphism involving the same codon in repeat 10, p.R3564H. Therefore, the Japanese p.R501X mutation identified here is (a) on a different haplotype from that found in Europeans and (b) uncommon in Japan, having been excluded from 200 controls and 4 IV patients here and from 191 Japanese controls and 143 Japanese AD patients previously (Palmer *et al.*, 2006; Nomura *et al.*, 2007). As p.R501X (DNA change CGA to TGA) is consistent with being a CpG mutation hotspot (Cooper and Krawczak, 1993), this may occur as a *de novo* mutation in all populations, as well as being a prevalent, ancestral mutation in Europeans.

Previous studies revealed a number of rare and prevalent European mutations with a combined heterozygous carrier frequency of about 9% (Sandilands *et al.*, 2007). These results predict a high incidence of one IV patient in about 300 of the population, which agrees fairly well with the results of a clinical survey of 6,051 English school children, where 1 in 250 had overt IV, presumably due to homozygous or compound heterozygous mutations (Wells and Kerr, 1966). Although a wide survey of the prevalence rate of

Abbreviations: AD, atopic dermatitis; IV, ichthyosis vulgaris

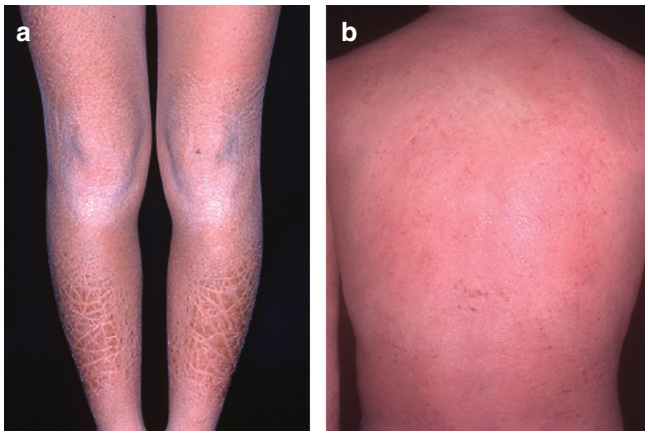

Figure 1. Clinical features of the most severe patient in the present family with IV complicated by AD. (**a**) Fine superficial scaling on the extensor surfaces of the lower legs at the age of 7 years, showing a typical appearance of IV. (**b**) Diffuse patches of pruritic erythema on the back at the age of 17 years, developing features of AD.

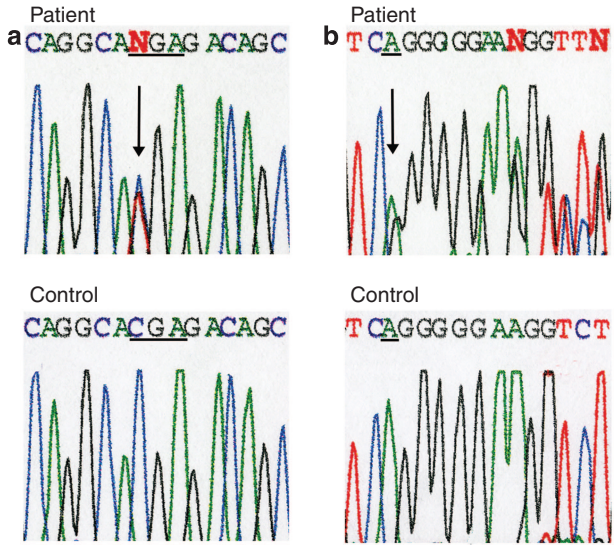

Figure 2. Molecular basis of IV in the present family. Direct sequencing of exon 3 of *FLG* shows (**a**) a heterozygous C>T transition at nucleotide 1501 that converts an arginine residue (CGA) to a stop codon (TGA); the mutation is designated p.R501X. (**b**) A heterozygous 1-bp deletion of A at nucleotide 3321 (c.3321delA); the mutation leads to a frameshift and premature stop codon 41-bp downstream.

Japanese IV has not yet been performed, the disease is considered to be one of the most frequent single-gene disorders in Japan. As the European mutations are not very common in Japan, it is likely that further mutations exist that are specific to this population (Sandilands *et al.*, 2007).

Ichthyosis vulgaris shows a semidominant inheritance pattern: a mild phenotype is heterozygous and a severe phenotype is homozygous or compound heterozygous for *FLG* mutations (Smith *et al.*, 2006). In this study, compound heterozygous mutations p.R501X and c.3321delA were identified in two individuals with a severe phenotype. Interestingly, the mildly affected mother and clinically normal father were a carrier of p.R501X and c.3321delA, respectively. Thus, intrafamilial phenotypic variability of the disease was observed here in this semidominant inherited trait.

Filaggrin plays a key role in terminal differentiation of the epidermis and formation of the skin barrier. Recent genetic evidence suggests that p.R501X and c.2282del4 mutations in *FLG* are important predisposing factors for AD as well as for eczema-associated asthma (Palmer *et al.*, 2006). In several additional cohorts from European population, these common *FLG* variants were also shown to be associated with AD, especially in cases with an early age of onset and a poor prognosis (Weidinger *et al.*, 2006; Barker *et al.*, 2007; Stemmler *et al.*, 2007). Recently, c.3321delA and p.S2554X mutations were shown to be carried by 5.6% of a Japanese AD cohort (Nomura *et al.*, 2007). Here, we have identified *FLG* mutations p.R501X and c.3321delA in a Japanese family with IV who also had AD. Interestingly, this family has a combination of the common *FLG* mutations reported from Europe and Japan, respectively. Further investigations will be necessary to clarify the worldwide population genetics of *FLG* mutations more accurately.

CONFLICT OF INTEREST

Irwin McLean has filed patents relating to genetic testing and therapy aimed at the filaggrin gene.

ACKNOWLEDGMENTS

We thank the patients for their participation. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by Health Science Grants for Research on Scientific Disease from the Ministry of Health, Labor and Welfare of Japan, and by an Open Research Center Project of the Ministry of Education, Culture, Sports, Science and Technology of Japan. Filaggrin research in the McLean laboratory is supported by grants from the British Skin Foundation, National Eczema Association, and donations from families in the Tayside Region of Scotland.

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See related commentary on pg 1064

Polymorphisms in the IL-12β and IL-23R Genes Are Associated with Psoriasis of Early Onset in a UK Cohort

Journal of Investigative Dermatology (2008) **128,** 1325–1327; doi:10.1038/sj.jid.5701140; published online 22 November 2007

TO THE EDITOR

A recent genome-wide association study (GWAS) conducted by Cargill *et al.* (2007) reported an association between psoriasis and the *IL-12*β and *IL-23R* genes. Their study focused on the analysis of gene-centric single nucleotide polymorphisms (SNPs) identifying four non-HLA SNPs associated with psoriasis in a North American population. Our study confirms these findings in a single-point association study genotyping these four SNPs (two in the *IL-12*β gene and two in the *IL-23R* gene) in 597 UK psoriasis patients with onset of disease at $\leqslant$40 years of age (type I psoriasis; 53.6% male; 46.4% female; mean age of onset 19.8 years; 58.8% patients HLA-Cw6-positive). Recruitment of type I psoriasis patients for the case cohort was coordinated through the Dermatology Centre, Hope Hospital, The University of Manchester, Manchester, UK. All patients gave written informed consent and the study was approved by the Salford and Trafford Local Research Ethics Committee and conducted according to the Declaration of Helsinki Principles. Control data were available from the Wellcome Trust Case Control Consortium (rs6887695, rs11209026, and rs7530511) and 1958 British Birth Cohort study (rs3212227) (Power and Elliott, 2006). The SNP rs3212227, located in the *IL-12*β gene, which encodes the IL-12β-p40 subunit of the IL-12 and IL-23, was previously associated with the disease in a study of 143 Japanese psoriasis patients and 100 unaffected controls ($P=0.035$) (Tsunemi *et al.*, 2002) and also a recent independent GWAS of UK psoriasis patients ($P=0.036$) (Capon *et al.*, 2007). Our findings replicate the associations found in the US ($P=7.85\times10^{-10}$; Cargill *et al.*, 2007), Japanese, and UK cohorts with association to the same allele ($P=0.003$; Table 1) where risk was conferred by carriage of two copies of the major allele (genotype AA) with an odds ratio of 1.38 (95% CI 1.14–1.68; $P=0.0004$).

The GWAS found association to a second SNP rs6887695 in *IL-12*β (Cargill *et al.*, 2007) and reported a haplotypic association. We also detected association to rs6887695, confirming this single-point genotypic association ($P=0.001$; Table 1) on the basis of a dominant model of inheritance for the major allele (genotype GG) with an odds ratio of 1.72 (95% CI 1.18–2.56; $P=0.0016$); however, we reported low linkage disequilibrium correlation with $r^2=0.21$ (calculated using the genetic analysis software HelixTree; Golden Helix Inc., Bozeman, Montana) between these SNPs in the patient cohort and thus did not conduct any haplotype analysis.

Cargill *et al.* (2007) also investigated the IL-23 cytokine-related proteins (as *IL-23R* shares the IL-12β-p40 protein subunit) and identified two further SNPs in the IL-23 receptor gene (*IL-23R*) associated with psoriasis. SNP rs7530511 was detected in the single-point marker analysis of the GWAS with a *P*-value of 0.006, whereas SNP rs11209026 showed significant association to the disease in combination with this marker in an estimated haplotype analysis ($P=3.3\times10^{-6}$) (Cargill *et al.*, 2007), a finding replicated in the GWAS of UK psoriasis patients ($P=0.00014$) (Capon *et al.*, 2007). Our study also confirms genotypic association to these markers with $P<0.001$ and $P=0.001$ for rs11209026 and rs7530511 SNPs, respectively (Table 1), with a dominant model of inheritance for the major allele in rs11209026 (genotype GG) with odds ratio of 1.67 (95% CI 1.20–2.37; $P=0.0008$) and risk con-

Abbreviations: GWAS, genome-wide association study; IL-23R, *IL-23-receptor; SNP, single nucleotide polymorphism.*