function due to long-term consequences of fragmented dermal collagen micro-environment in photodamaged human skin.

Taihao Quan[1], Emily Little[1], Hehui Quan[1], Zhaoping Qin[1], John J. Voorhees[1] and Gary J. Fisher[1]

[1]*Department of Dermatology, University of Michigan Medical School, Ann Arbor, Michigan, USA*
E-mail: thquan@umich.edu or gjfisher@umich.edu

REFERENCES

Bissell MJ, Hines WC (2011) Why don't we get more cancer? A proposed role of the micro-environment in restraining cancer progression. *Nat Med* 17:320–9

Brenneisen P, Sies H, Scharffetter-Kochanek K (2002) Ultraviolet-B irradiation and matrix metalloproteinases: from induction via signaling to initial events. *Ann N Y Acad Sci* 973:31–43

Egeblad M, Werb Z (2002) New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2:161–74

Fisher GJ, Datta SC, Talwar HS *et al.* (1996) Molecular basis of sun-induced premature skin ageing and retinoid antagonism. *Nature* 379:335–9

Fisher GJ, Quan T, Purohit T *et al.* (2009) Collagen fragmentation promotes oxidative stress and elevates matrix metalloproteinase-1 in fibroblasts in aged human skin. *Am J Pathol* 174:101–14

Fisher GJ, Wang ZQ, Datta SC *et al.* (1997) Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 337:1419–28

Quan T, Qin Z, Xia W *et al.* (2009) Matrix-degrading metalloproteinases in photo-aging. *J Investig Dermatol Symp Proc* 14: 20–4

Spencer VA, Xu R, Bissell MJ (2007) Extracellular matrix, nuclear and chromatin structure, and gene expression in normal tissues and malignant tumors: a work in progress. *Adv Cancer Res* 97:275–94

Varani J, Schuger L, Dame MK *et al.* (2004) Reduced fibroblast interaction with intact collagen as a mechanism for depressed collagen synthesis in photodamaged skin. *J Invest Dermatol* 122:1471–9

Varani J, Spearman D, Perone P *et al.* (2001) Inhibition of type I procollagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen *in vitro*. *Am J Pathol* 158:931–42

Yaar M, Gilchrest BA (2007) Photoageing: mechanism, prevention and therapy. *Br J Dermatol* 157:874–87

Rare Pathogenic Variants in *IL36RN* Underlie a Spectrum of Psoriasis-Associated Pustular Phenotypes

TO THE EDITOR

Mutations of the *IL36RN* gene have been recently identified in patients affected by generalized pustular psoriasis (GPP), a neutrophilic dermatosis that presents as an acute pustular eruption accompanied by features of systemic inflammation (Marrakchi *et al.*, 2011; Onoufriadis *et al.*, 2011). Although GPP is traditionally classified as a variant of psoriasis vulgaris (PV; Griffiths and Barker, 2010), our group has demonstrated that the two conditions are genetically distinct (Onoufriadis *et al.*, 2011).

In this context, the aim of this study was to characterize the spectrum of psoriasis-associated pustular phenotypes that are caused by *IL36RN* alleles. To achieve this objective, we analyzed an extended collection of GPP cases and, in parallel, investigated the possibility that *IL36RN* may contribute to palmar-plantar pustulosis (PPP) and acrodermatitis continua of hallopeau (ACH), two acral forms of pustular psoriasis that have been historically grouped with GPP (Griffiths and Barker, 2010), and which are also thought to be genetically distinct from PV (Asumalahti *et al.*, 2003).

We sequenced the four *IL36RN* coding exons in 84 GPP, 9 ACH, and 139 PPP cases (Supplementary Table S1 online). Owing to the rarity of the examined diseases, we defined as potentially pathogenic any non-synonymous substitution, frameshift mutation, or splicing defect that had a minor allele frequency (MAF) < 0.01 in the relevant ethnic group. We observed homozygous/compound heterozygous alleles meeting the above criteria in 7/84 GPP patients, thus validating previous observations of genetic heterogeneity in this disease (Onoufriadis *et al.*, 2011; Li *et al.*, 2012; Sugiura *et al.*, 2012). Importantly, we also identified recessive *IL36RN* variants in 2/9 ACH and 3/139 PPP patients (Table 1).

The most prevalent allele in the European population was the previously characterized p.Ser113Leu substitution (Onoufriadis *et al.*, 2011), which was found in all three patient groups. The most frequent change in the Asian data set was the c.115 + 6T > C variant, which is known to disrupt the splicing of exon 3, leading to the synthesis of a truncated protein (Farooq *et al.*, 2012). As our resource did not include Asian PPP or ACH patients, we could not establish whether the c.115 + 6T > C allele also contributes to these conditions.

An analysis of intragenic SNP haplotypes indicated that both p.Ser113Leu and c.115 + 6T > C are likely to have

Table 1. Summary of patients bearing *IL36RN* alleles with pathogenic potential

Patient ID	Sex	Ethnicity	*IL36RN* variants	Disease	Age of onset (years; disease duration)	PV
ACH-I	F	European (British)	p.Arg102Trp/p.Ser113Leu	ACH	37 (1)	Y
ACH-II	F	European (Swiss)	p.Ser113Leu/p.Ser113Leu	ACH	67 (10)	Y
GLA-I	M	European (British)	p.Lys35Arg/p.Ser113Leu	GPP	29 (13)	N
GLA-II	M	European (British)	p.Ser113Leu/p.Ser113Leu	GPP	26 (10)	N
GLA-III	M	European (British)	p.Ser113Leu[1]	GPP	39 (12)	N
MAL-I	F	Asian (Malay)	c.115+6T>C/ c.115+6T>C	GPP	37 (8)	Y
MAL-II	F	Asian (Chinese)	c.115+6T>C/ c.115+6T>C	GPP	21 (9)	Y
MAL-III	F	Asian (Malay)	c.115+6T>C[1]	GPP	9 (31)	N
MAL-IV	M	Asian (Malay)	c.115+6T>C[1]	GPP	42 (10)	Y
MAL-V	F	Asian (Malay)	c.115+6T>C[1]	GPP	12 (21)	Y
MAL-VI	M	Asian (Chinese)	c.115+6T>C/ c.115+6T>C	GPP	12 (32)	N
MAL-VII	M	Asian (Malay)	c.115+6T>C/p.Ser113Leu	GPP	8 (3)	Y
MAL-VIII	F	Asian (Malay)	c.115+6T>C/ c.115+6T>C	GPP	2 (39)	Y
MAL-IX	F	Asian (Chinese)	c.115+6T>C[1]	GPP	40 (22)	Y
MAL-X	M	Asian (Chinese)	c.115+6T>C[1]	GPP	12 (19)	Y
STJ-I	M	European (British)	p.Ser113Leu/p.Ser113Leu	PPP	5 (41)	Y
STJ-II	F	European (British)	p.Ser113Leu/p.Ser113Leu	PPP	17 (47)	Y
STJ-III	M	European (British)	p.Ser113Leu/p.Ser113Leu	PPP	24 (14)	N
P-GLA-I	F	European (British)	p.Ser113Leu[1]	PPP	77 (13)	N
P-GLA-II	F	European (British)	p.Ser113Leu[1]	PPP	NA	Y
P-MAN-I	M	European (British)	p.Ser113Leu[1]	PPP	NA	NA
P-MAN-II	F	European (British)	p.Ser113Leu[1]	PPP	NA	NA

Abbreviations: ACH, acrodermatitis continua of hallopeau; F, female; GPP, generalized pustular psoriasis; M, male; N, no; NA, the information was not available; PPP, palmarplantar pustulosis; PV, psoriasis vulgaris; Y, yes.
All cases were sporadic and unrelated.
[1]Variant observed in heterozygosity, in the absence of a second pathogenic allele (individuals MAL-III, MAL-IV, and MAL-X also carried a p.Pro76Leu change, but sequencing of cloned PCR products demonstrated that this substitution is always found on the same chromosome as the c.115+6T>C allele).

spread as the result of founder effects (Supplementary Table S2 online).

The other *IL36RN* variants observed in homozygous/compound heterozygous patients were two missense substitutions (p.Lys35Arg, p.Arg102Trp; Table 1) that were classified as damaging by the SIFT (Kumar *et al.*, 2009) and/or PolyPhen (Adzhubei *et al.*, 2010) pathogenicity prediction tools. It is noteworthy that homology modeling of IL36-Ra (the protein encoded by *IL36RN*) indicated that Lys35 maps in proximity to the residues that mediate binding to the protein receptor (Figure 1).

It is interesting to note that we uncovered heterozygous changes in 4 PPP and 6 GPP cases, who carried the previously mentioned c.115+6T>C and p.Ser113Leu alleles. As all reported

IL36RN mutations have been found in a recessive state (Marrakchi *et al.*, 2011; Onoufriadis *et al.*, 2011; Sugiura *et al.*, 2012), we searched for a second disease allele in these patients. We performed a long-range PCR of the genomic region that encompasses *IL36RN* coding exons and intervening introns. We consistently observed a single amplification product, excluding the possibility that these individuals may carry intragenic deletions or duplications (Supplementary Figure S1 online). We also sequenced a 1007-bp putative promoter fragment (Supplementary Table S3 online), but did not uncover any variants with an MAF<0.01.

To assess the likelihood of repeatedly observing a rare *IL36RN* allele by chance, we examined the prevalence of the p.Ser113Leu substitution in the

Caucasian data set analyzed by the NHLBI GO Exome Sequencing Project. We found that the frequency of this variant among the European patients who did not carry recessive *IL36RN* changes significantly exceeded that observed in 8,600 control chromosomes (1.7 vs. 0.3%; $P=0.004$). This suggests that the p.Ser113Leu allele may have pathogenic potential even in the heterozygous state.

We carefully reviewed the clinical records of the 22 individuals carrying *IL36RN* variants. We observed no significant dissimilarities in age at onset, prevalence of PV, or clinical presentation between the cases bearing two *IL36RN* alleles and the rest of the study cohort. Similarly, the severity of the disease did not differ significantly in patients bearing single heterozygous
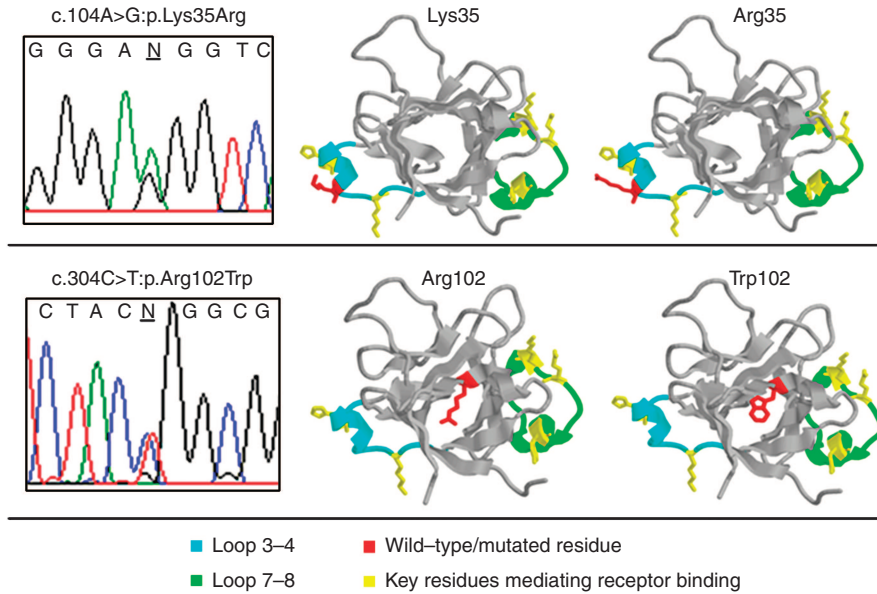
Figure 1. Characterization of the *IL36RN* variants uncovered in this study. Sequence chromatograms for each substitution are shown on the left, whereas the right end panels show the position of the relevant amino acids within the three-dimensional structure of the IL-36Ra protein. Key regions (loop 3–4, residues 30–41 and loop 7–8, residues 82–98) and amino acids (His32, Lys38, Tyr89, Glu94, Lys96) mediating the interaction of IL-36ra with its cognate receptor are highlighted. The pSer113Leu variant is not shown, as this substitution has been previously characterized (Onoufriadis *et al.*, 2011).

changes, compared with those harboring homozygous/compound heterozygous alleles.

Our data indicate that *IL36RN* alleles are associated with a phenotypic spectrum that encompasses ACH and PPP, as well as GPP. Importantly, we observed both homozygous, compound heterozygous, and single heterozygous *IL36RN* substitutions. Although it is theoretically possible that the heterozygous individuals may harbor a second disease allele (e.g., a large genomic duplication which could not have been detected by our long-PCR assay), the most likely interpretation of our findings is that some GPP/PPP patients present with a single *IL36RN* mutation. This is reminiscent of the genetic makeup of familial Mediterranean fever (FMF), an autoinflammatory condition that is associated with homozygous and heterozygous mutations of the *MEFV* gene (Kastner *et al.*, 2010). The possibility that modifying alleles at other loci may contribute to FMF has been invoked to explain disease occurrence in individuals bearing single *MEFV* mutations (Kastner *et al.*, 2010). Thus, it is possible that the

GPP and PPP cases harboring a single *IL36RN* substitution may represent cases of tri-allelic disease inheritance. The screening of further clinical resources should identify sufficient numbers of heterozygous patients to allow an assessment of this hypothesis by means of exome sequencing.

We only observed *IL36RN* mutations in a minority of patients, indicating the likely involvement of other gene loci and suggesting that pustular forms of psoriasis may be classified on the basis of their genetic etiology in the future. In this context, it is noteworthy that individuals with *IL36RN* mutations upregulate IL-1 production in response to IL-36 stimulation (Onoufriadis *et al.*, 2011). This suggests that the symptoms of these particular patients might be caused by excessive IL-1 production and may therefore be treated by IL-1 blockade. Further investigations will obviously be required to validate the role of IL-1 as a driver of *IL36RN*-associated pustular disease. Such experiments hold the promise of translating the results of genetic studies into significant improvement in patient care.

Niovi Setta-Kaffetzi[1,8],
Alexander A. Navarini[1,8],
Varsha M. Patel[1], **Venu Pullabhatla**[1],
Andrew E. Pink[1], **Siew-Eng Choon**[2],
Michael A. Allen[1], **A. David Burden**[3],
Christopher E.M. Griffiths[4],
Marieke M.B. Seyger[5], **Brian Kirby**[6],
Richard C. Trembath[1,7],
Michael A. Simpson[1], **Catherine H. Smith**[1],
Francesca Capon[1,8] and
Jonathan N. Barker[1,8]

[1]Division of Genetics and Molecular Medicine, King's College London School of Medicine, Guy's Hospital, London, UK; [2]Department of Dermatology, Hospital Sultanah Aminah, Johor Bahru, Malaysia; [3]Department of Dermatology, University of Glasgow, Glasgow, UK; [4]Department of Dermatology, University of Manchester, Manchester, UK; [5]Department of Dermatology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; [6]Department of Dermatology, St Vincent University Hospital, Dublin, Ireland and [7]Queen Mary, University of London, Barts and The London School of Medicine and Dentistry, London, UK
E-mail: francesca.capon@kcl.ac.uk or jonathan.barker@kcl.ac.uk
[8]These authors contributed equally to this work.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Adzhubei IA, Schmidt S, Peshkin L *et al.* (2010) A method and server for predicting damaging missense mutations. *Nat Methods* 7:248–9

Asumalahti K, Ameen M, Suomela S *et al.* (2003) Genetic analysis of PSORS1 distinguishes guttate psoriasis and palmoplantar pustulosis. *J Invest Dermatol* 120:627–32

Farooq M, Nakai H, Fujimoto A *et al.* (2012) Mutation analysis of the IL36RN gene in 14 Japanese patients with generalized pustular psoriasis. *Hum Mutat* (doi:10.1002/huwu.22203)

Griffiths CEM, Barker JNWN (2010) Psoriasis. In: Burns T, Breathnach S, Cox N, Griffiths CEM (eds) *Rook's Textbook of Dermatology* Chichester, England: Wiley-Blackwell, 20.1–20.60

Kastner DL, Aksentijevich I, Goldbach-Mansky R (2010) Autoinflammatory disease reloaded: a clinical perspective. *Cell* 140:784–90

Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 4:1073–81

Li M, Lu Z, Cheng R *et al.* (2012) IL36RN gene mutations are not associated with sporadic generalized pustular psoriasis in Chinese Patients. *Br J Dermatol* (doi:10.1111/j.1365-2133.2012.11195.x)

Marrakchi S, Guigue P, Renshaw BR *et al.* (2011) Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis. *N Engl J Med* 365:620–8

Onoufriadis A, Simpson MA, Pink AE *et al.* (2011) Mutations in IL36RN/IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis. *Am J Hum Genet* 89:432–7

Sugiura K, Takeichi T, Kono M *et al.* (2012) A novel IL36RN/IL1F5 homozygous nonsense mutation, p.Arg10X, in a Japanese patient with adult-onset generalized pustular psoriasis. *Br J Dermatol* 167:699–701

Association of Generalized Vitiligo with MHC Class II Loci in Patients from the Indian Subcontinent

TO THE EDITOR

Generalized vitiligo is a disease in which patches of depigmented skin and overlying hair result from autoimmune destruction of melanocytes in involved regions (Spritz, 2012). Clinic-based studies cite high prevalence of vitiligo in India, up to 8.8% (e.g., Handa and Kaur, 1999), although population-based surveys report much lower prevalence, 0.46% in Calcutta (Das et al., 1985) and 1.79% in South Gujarat (Mehta et al., 1973).

Vitiligo is a distressing cosmetic problem in individuals of dark skin phototypes, owing to striking contrast between lesions and unaffected skin. This may explain the reported high prevalence of vitiligo in India and the negative impact on perceived quality of life in this population (Parsad et al., 2003). Indeed, vitiligo has long been recognized in India (Singh et al., 1974), the specific use of UV light treatment was pioneered in India (Menon, 1945), and some of the earliest genetic studies of vitiligo were carried out there: of ABO blood groups, α1-antitrypsin, and haptoglobin, and subsequent candidate gene studies, including *GCH1, ACE, CAT, CTLA4, GPX1, IL4, MBL2,* and *PTPN22,* most yielding negative or conflicting results. Recently, Singh et al. (2012) tested the genetic association of vitiligo in Indian patients with *HLA-A, -B, -C* in the major histocompatibility complex (MHC) class I region

and *HLA-DRB1* in the class II region, identifying primary genetic association with *HLA-DRB1*07:01.*

Here, we describe a more comprehensive genetic association study of generalized vitiligo on the Indian subcontinent, using the Immunochip (Cortes and Brown, 2011) to screen 196,524 single-nucleotide polymorphisms (SNPs) in 128 loci previously implicated in autoimmune and inflammatory diseases, including 9,441 SNPs spanning the extended MHC on chromosome 6p. Our results suggest that there are at least two independent association signals in the MHC class II region, one located upstream of *HLA-DRA* and the other located between *HLA-DRB1* and *HLA-DQA1,* generally similar to what we previously found in a genome-wide association study of vitiligo in European-derived whites (EUR) (Jin et al., 2010).

Our initial study group consisted of 255 patients with generalized vitiligo and 377 unrelated non-vitiligo controls of Indian subcontinent (Pakistan, India, Sri Lanka, and Bangladesh) derivation. After quality control procedures, data for 120,724 remaining SNPs from 251 remaining cases were compared with those from 349 remaining controls. Suggestive association signals were considered as clusters of nearby SNPs with trend *P*-values $<10^{-5}$. The International Immunochip Consortium has agreed on a genome-wide significance criterion of $P < 5 \times 10^{-8}$ for studies

utilizing the Immunochip (Cortes and Brown, 2011).

As shown in Figure 1a and Supplementary Table S1 online, the only highly suggestive association signals were in the MHC class II gene region (Figure 1b), from rs3134942 (chr6:32168770) to rs2856674 (chr6:32659644), spanning the upstream part of *NOTCH4* through *HLA-DQB1.* The principal region of association encompassed *c6orf10--BTNL2–HLA-DRA–HLA-DRB5–HLA-DRB1–HLA-DQA1* (Figure 1b), with extensive linkage disequilibrium (LD) through this region in this population (Figure 1c). One SNP, rs482044, located toward the centromeric end of the region, between *HLA-DRB1* and *HLA-DQA1,* achieved genome-wide significance (G allele; $P = 1.94 \times 10^{-8}$, odds ratio (OR) = 1.93; Table 1), remaining significant ($P = 4.86 \times 10^{-8}$) even after correction for the observed genomic inflation factor $\lambda = 1.06$.

To determine which SNPs in the MHC class II region represent primary association with vitiligo versus are signals secondary to LD, we applied a backward regression procedure, comparing a model including the seven most significant MHC class II SNPs to alternative models in which each SNP was removed one by one. This analysis suggested that this region contains two independent associated loci, one represented by rs482044-G (located between *HLA-DRB1* and *HLA-DQA1*) and the