A Genome-Wide Association Study of Basal Transepidermal Water Loss Finds that Variants at 9q34.3 Are Associated with Skin Barrier Function

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TO THE EDITOR

Epidermal homeostasis and barrier permeability are very important properties of human skin. Transepidermal water loss (TEWL), the passive diffusion of water from the hydrated layers of the dermis and epidermis toward those layers with a lower water content (Nilsson, 1977), has been widely used to determine epidermal permeability barrier status (Fluhr et al., 2006). For example, TEWL measurement helped to establish that skin barrier function is compromised in skin diseases such as atopic dermatitis (AD) (Elias, 2008). Likewise, it has successfully been used to monitor the effects of different treatments on skin barrier function recovery (Sextius et al., 2010). Although TEWL has been reported to be affected by environmental factors such as temperature, seasonal variation, sun exposure, and smoking (Li et al., 2014; Liu et al., 2010; Xin et al., 2016), the presence of significant ethnic differences in stratum corneum permeability suggests that genetics also plays a role in epidermal barrier function (Kompaore and Tsuruta, 1993). However, to our knowledge, no genomic study has been conducted to explore the genetics of barrier function of healthy human skin. To address this, we performed a genome-wide association study (GWAS) of basal TEWL as a measure of the skin barrier function, with the aim of detecting the potential genetic variants associated with this important skin trait.

We collected 611 samples from healthy Han Chinese in Taizhou, Jiangsu Province, aged between 31 and 87 years. This research was conducted with official approval from the ethics committee of Fudan University, Shanghai, China. All participants provided written informed consent. TEWL measurement was carried out with a DermaMeter Professional 100 (VASEMA GmbH, Vienna, Austria) on the right cheek (see Supplementary Materials and Supplementary Table S1 online for details). Because the obtained TEWL values did not follow the normal distribution (Shapiro-Wilk test, $P < 2.2 \times 10^{-16}$), a logarithmic transformation was performed (see Supplementary Figure S1 online). Principal component analysis found no significant population stratification in our sample (see Supplementary Figure S2 online). Mostly consistent with previous reports, we found TEWL to be significantly correlated with temperature ($r = 0.284$, $P = 9.54 \times 10^{-13}$), sex (two-tailed Student t test, $P = 6.06 \times 10^{-3}$), and skincare habits ($P = 4.74 \times 10^{-3}$). It was not correlated with humidity of the environment ($P = 0.967$), sun exposure ($P = 0.247$), and smoking ($P = 0.089$) (see Supplementary Materials). We then performed a GWAS, adjusting for age, sex, temperature, and skincare habits. Individuals were genotyped on an Illumina (San Diego, CA) Human Omni Zhonghua 8V1.1 chip, and imputation was performed using 1000 Genomes Project data (phase 3) (1000 Genomes Project Consortium et al., 2012; Pickrell et al., 2009; Kent et al., 2002). After quality-control filters, the GWAS was carried out on 795,279 genotyped single-nucleotide polymorphisms (SNPs) and 7,203,134 imputed SNPs (see Supplementary Materials for the details). We found a variant on chromosome band 9q34.3 to be significantly associated with TEWL (rs10858314, $\beta = -0.211 \pm 0.038$, $P = 3.11 \times 10^{-8}$; see Supplementary Figure S3 online). To validate our finding, we performed a second GWAS using the same phenotyping protocol on a replication set including 366 healthy Han Chinese samples from Taixing, Jiangsu Province. There was no genome-wide significant signals in this second GWAS, but the SNP (rs10858314) was replicated with nominal significance ($\beta = -0.167 \pm 0.065$, $P = 9.96 \times 10^{-3}$; see Supplementary Table S2 online).

In a meta-analysis combining the results of the two GWASs, nine SNPs on chromosome band 9q34.3 reached the genome-wide significance level of $P < 5 \times 10^{-8}$ (see Supplementary Table S2), the top signal being at rs11103631 ($\beta = -0.201 \pm 0.033$, $P = 8.16 \times 10^{-10}$; Figure 1a). All nine SNPs are located within the same 3.34-kilobase-pair block of strong linkage disequilibrium (LD) (Figure 1b). We found that subjects with the ancestral allele (G) at rs11103631 have a lower TEWL, with a decrease per copy of approximately 19.5%, suggesting reduced skin barrier permeability compared with carriers of the derived (A) allele (Figure 1c). The frequency of the G allele is higher in Africans than in other populations (Figure 1d). This finding is consistent with a report of a reduced epidermal permeability and more dense stratum corneum in Africans compared with Asians and whites (Kompaore and Tsuruta, 1993). We also performed a scan for signals of natural selection on

Abbreviations: AD, atopic dermatitis; GWAS, genome-wide association study; LD, linkage disequilibrium; SNP, single-nucleotide polymorphism; TEWL, transepidermal water loss

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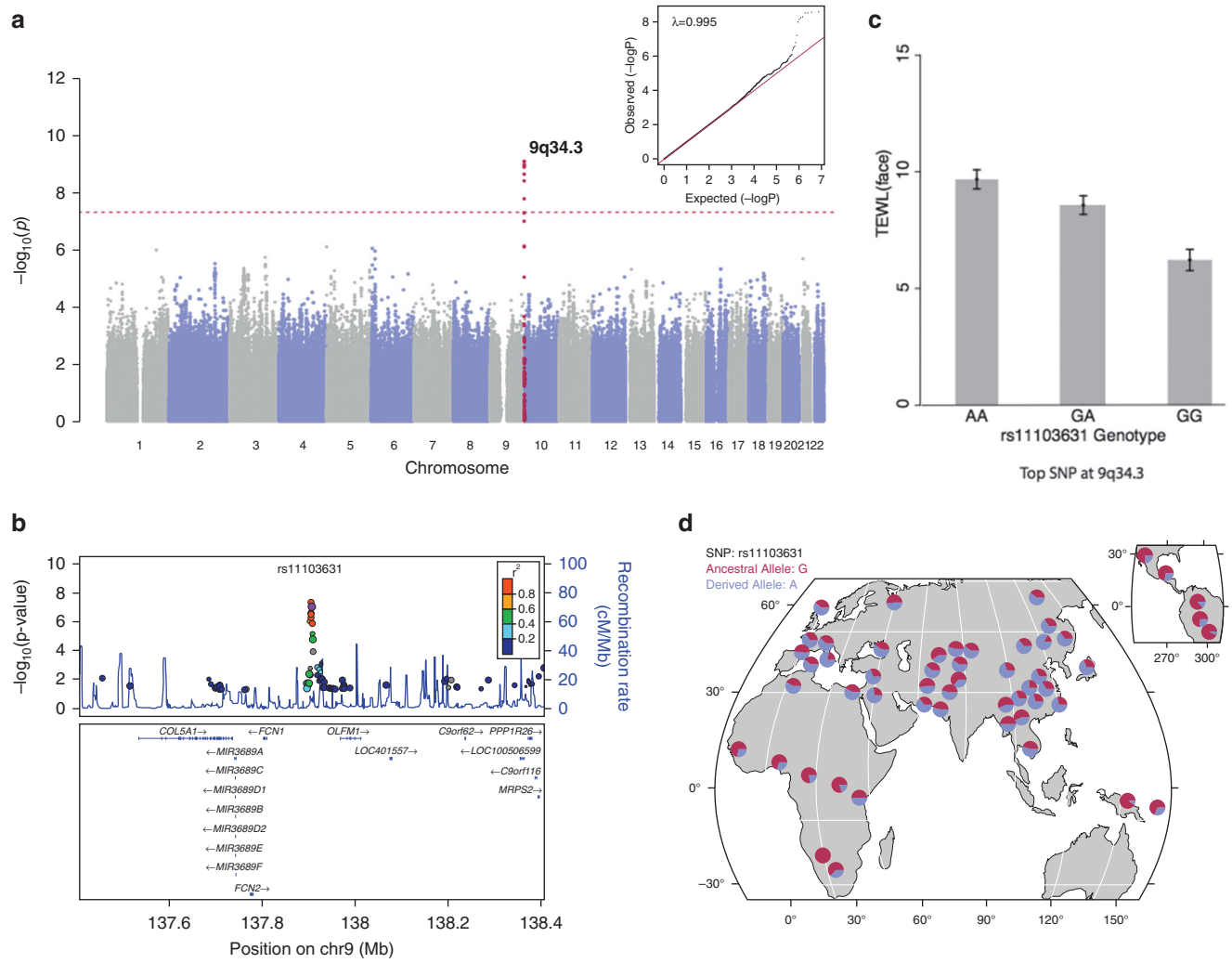

Figure 1. Genome-wide scans of TEWL found significant association with chromosome band 9q34.3. (**a**) Manhattan plot and quantile-quantile plot showing the results of a meta-analysis of TEWL GWASs. The meta-analysis was performed in 977 Han Chinese samples (611 from Taizhou, and 366 from Taixing), adjusted for sex, age, temperature, and skincare habits. The quantile-quantile plot shows a degree of genomic inflation ($\lambda = 0.995$), showing no evidence of confounding effects by population stratification or inflation. The red line indicates the threshold for genome-wide statistical significance ($P < 5 \times 10^{-8}$). Red dots represent SNPs that are close (<5 kilobase pairs) to signals of genome-wide significance. Variants on chromosome band 9q34.3 are significantly associated with TEWL, the top signal being at rs11103631. (**b**) Regional association plot for 9q34.3 with SNPs showing significant association with TEWL. The top-signal SNP rs11103631 is shown in purple, and the color of the remaining markers reflects LD (r^2) with the top SNP (increasing red hue associated with increasing LD). The blue spikes show the estimated recombination rate (right-hand y-axis). The data are based on the ASN population from the 1000 Genomes Project (1000 Genomes Project Consortium et al., 2012) . Exons for each gene are represented by vertical bars, based on all isoforms available from the hg19 assembly in the UCSC Genome Browser (Kent et al., 2002). (**c**) Mean value of TEWL as a function of the rs11103631 genotype in Han Chinese. With genotype AA, the mean value of TEWL is 9.681 ± 0.407 g/m^2/h; with GA and GG, the mean value is 8.570 ± 0.397 g/m$^{2/h}$ and 6.220 ± 0.456 g/m^2/h, respectively. Vertical bars correspond to the standard error of the mean. (**d**) Geographical distribution of the allele frequencies at rs11103631. Allele frequency data from 53 world-wide populations are taken from the Human Genome Diversity Project (Pickrell et al., 2009). Ancestral alleles are represented in red, derived alleles in blue. cM, centiMorgan; LD, linkage disequilibrium; Mb, mega base pairs; SNP, single-nucleotide polymorphism; TEWL, transepidermal water loss.

the 9q34.3 region, but we did not find evidence for natural selection in East Asians, Europeans, or Africans (see Supplementary Figure S4 online).

In our samples, 152 individuals reported a personal history for eczema/dermatitis, 22 for AD in childhood, and 86 for asthma/hay fever. In a GWAS controlling for these disease histories, the results remained largely the same (see Supplementary Figure S5 online), suggesting that our findings were not affected by a history of skin disease.

The top signal rs11103631, located in an intergenic region between the *FCN1* and *OLFM1* genes, has been reported to be an expression quantitative trait locus affecting both *FCN1* ($P = 4.1 \times 10^{-5}$) and *OLFM1* ($P = 2.6 \times 10^{-17}$) expression (*cis*-expression quantitative trait locus tested in non-transformed peripheral blood samples) (Westra et al., 2013). The LD block containing the signal exhibits distinct signatures of active enhancers defined by epigenetic marks such as H3K4me1 histone modifications in primary melanocytes and keratinocytes (see Supplementary Figure S6 online). This is in line with a potential regulatory role of this region for *FCN1* and/or *OLFM1* expression. *FCN1* is expressed in basal keratinocytes, and its product has been

postulated to function as a plasma protein with elastin-binding activity. Expression of *FCN1* was recently found to be modulated during barrier recovery in aged populations (Sextius et al., 2015), suggesting that *FCN1* activity may affect TEWL.

Genes such as *FLG* have been reported to cause abnormalities of stratum corneum structure in skin diseases such as AD (Palmer et al., 2006). We therefore investigated the association between TEWL and 266 SNPs in 21 candidate genes related to skin barrier function (see Supplementary Table S3 online), as well as 97 SNPs with known associations with AD, psoriasis, and keloid (see Supplementary Table S4 online). After multiple test correction, none of the SNPs showed significant association with TEWL (see a typical nonsignificant plot in the region of *FLG* in Supplementary Figure S7 online). These results are not entirely surprising. First, most of the included candidate genes have a pathological significance, but our GWAS focuses on a nonpathological normal skin trait. The underlying genes could be different. Second, most of the relevant studies were based on white population samples, whereas our study is based on Chinese populations. In different ethnic groups, the same trait can well be affected by different genes. Lastly, the sample size of this study is relatively small for a GWAS. Shown by a power calculation, our study of approximately 1,000 samples does not have enough power (>80%) to detect SNPs with an effect size of lower than 2.25% of the variance (see Supplementary Materials for details). Future studies with an increased sample size may provide a more complete picture of the genes underlying TEWL.

In summary, our study identified variants on chromosome band 9q34.3 that showed significant association with TEWL, a parameter reflecting epidermal barrier function. Further work is required to assess the functional relevance of these variants at a mechanistic level.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.11.030.

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