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Genome-Wide Association Analysis Implicates Elastic Microfibrils in the Development of Nonsyndromic Striae Distensae

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TO THE EDITOR

Striae distensae, or stretch marks, are a common skin condition that appear initially as red, and later on as white, lines on the skin. These lines represent scars of the dermis, and are characterized by linear bundles of collagen lying parallel to the surface of the skin, as well as eventual loss of collagen and elastin. Reports differ on the level of fragmentation of elastic fibers (Zheng *et al.,* 1985; Sheu *et al.,* 1991). Estimates of the prevalence of stretch marks range from 50 to 80% (Atwal *et al.,* 2006; Cho *et al.,* 2006). Although stretch marks are only harmful in extreme cases (Dosal *et al.,* 2012), even mild stretch marks can cause distress to the bearer.

The causes of stretch marks are not well understood. Excessive skin distension (such as that which occurs during pregnancy, growth spurts in puberty, or rapid weight gain), prolonged exposure to cortisol (such as in individuals with Cushing syndrome), and genetics may all have a role (Elsaie *et al.,* 2009). A few monogenic connective tissue diseases, including Marfan syndrome and congenital contractural arachnodactyly, are known to be associated with stretch marks. These syndromes are caused by mutations in genes that encode extracellular matrix proteins (fibrillin-1 and fibrillin-2, respectively) that are part of elastic microfibrils present in skin and other tissues. However, to date, no genetic variants are known to be associated with isolated stretch marks that afflict the general population.

To identify variants associated with the development of stretch marks, we conducted a genome-wide association analysis of stretch marks in a discovery cohort of 33,930 unrelated 23andMe customers (Supplementary Table S1 online) of European descent. There were a total of 13,068 cases and 20,862 controls. The 18,650 men in the cohort were much less likely to report stretch marks (25% versus 55% of women), which is consistent with other reports (Elsaie *et al.,* 2009). We further evaluated the associated variants in a cohort consisting of 4,967 female 23andMe customers of European descent (disjoint from the first group) who reported on severity of stretch marks during pregnancy (also known as striae gravidarum, a closely related phenotype). See Supplemental Methods for additional details on phenotyping. The protocol for this study was approved by an independent institutional review board (E&I Review Services) and was conducted according to the Declaration of Helsinki Principles; all study participants provided informed consent online, which was recorded in an electronic database. See Supplementary Methods for details on genotyping and imputation. All analyses used logistic (discovery cohort) or linear (pregnancy cohort) regression against imputed allele dosages, controlling for age, population structure (using five principal components), and (except for the pregnancy cohort) sex. Because the prevalence of stretch marks differs between men and women, we checked for but did not observe differing effects for the single-nucleotide polymorphisms (SNPs) in men and women.

Four regions were significantly ($P < 5e-8$) associated with stretch marks (Table 1, Figure 1, Supplementary Figure S1 online). The most strongly associated SNP in the first region, rs7787362 ($P = 1.8e-23$, odds ratio (OR) = 0.84), lies 40 kb upstream of the *ELN* (elastin) gene. It was also associated with striae gravidarum in the pregnancy cohort ($P = 7e-5$, $\beta = -0.072$, Supplementary Table S2 online). Elastin is the major component of elastic fibers, which provide reversible extensibility to connective tissue. Mutations in elastin that result in a loss of

Abbreviations: BMI, body mass index; SNP, single-nucleotide polymorphism

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Table 1. Index SNPs for regions associated with striae distensae at a significance level of $P<1e{-}6$

						Discovery		Pregnancy	
SNP	Chr (pos)[1]	Gene[2]	All[3]	MAF[4]	r^2 [5]	P[6]	OR (CI)[7]	P[6]	β (CI)[8]
rs7787362*	7 (73392603)	*ELN* (u)	C/T	0.467	0.992	1.8E − 23	0.84 (0.81–0.87)	7e − 5	0.072 (0.053–0.091)
rs35318931	X (38009121)	*SRPX* (i)	G/A	0.079	0.964	1.1E − 13	0.82 (0.77–0.86)	0.026	0.067 (0.033–0.102)
rs10798036	1 (186052962)	*HMCN1* (i)	G/C	0.485	0.989	6.9E − 10	1.11 (1.08–1.15)	0.06	− 0.029 (− 0.048 to − 0.010)
rs7594220	2 (643320)	*TMEM18* (d)	A/G	0.194	0.946	9.8E − 09	0.88 (0.84–0.92)	0.70	− 0.067 (− 0.192 to 0058)
chr6:36311047	6 (36311047)	*PNPLA1* (d)	C/T	0.007	0.988	9.7E − 08	1.80 (1.45–2.23)		
rs3910516	2 (216303053)	*FN1* (u)	G/A	0.262	0.909	2.7E − 07	1.11 (1.07–1.16)		
rs62034322	16 (28535834)	*NPIPL2* (i)	G/A	0.359	0.946	4.7E − 07	1.10 (1.06–1.14)		

[1]Chromosome (chr) and position (pos) are with respect to build 37.

[2]Gene is gene that is the most likely candidate for the association or the association or the closest gene. Whether the single-nucleotide polymorphism (SNP) is upstream (u), downstream (d), or within (i) the gene is indicated in parentheses.

[3]Alleles are major/minor in the context of European ancestry.

[4]MAF is minor allele frequency in the entire 23andMe European research cohort (over 120,000 individuals).

[5]r^2 is the estimated imputation accuracy.

[6]Associations with a P-value $<5e-8$ are genome-wide significant, and those with P-values between $1e-6$ and $5e-8$ are defined as suggestive.

[7]For the discovery set (which included both men and women), the odds ratio (OR) plus confidence interval (CI) is with respect to the minor allele and represents the risk of developing stretch marks.

[8]For the pregnancy set (which included only women), the β plus CI is with respect to the major allele, with positive numbers representing an increase in the severity of stretch marks. These tests were run only for SNPs reaching genome-wide significance in the discovery set. SNPs marked with an asterisk are typed by our genotyping array.

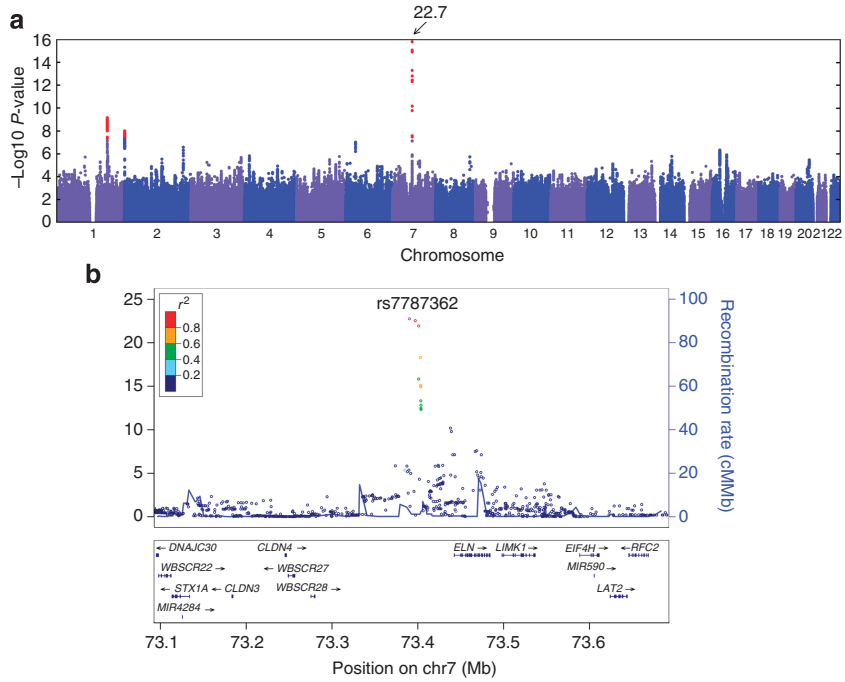

Figure 1. Manhattan plot depicting single-nucleotide polymorphism (SNP) associations with stretch marks. (**a**) Genome-wide view of associations. SNPs shown in red are genome-wide significant ($P<5e-8$). Regions are named with the postulated candidate gene. (**b**) Detailed view of local region around rs7787362. Colors depict the squared correlation (r^2) of each SNP with rs7787362, shown in purple. Gray indicates SNPs for which r^2 information was missing.

mature elastin can lead to autosomal dominant cutis laxa (a condition characterized by loose, sagging skin, and higher risks of aortic aneurysm) or supravalvular aortic stenosis (a localized narrowing of the ascending aorta caused by thickening of the aortic smooth muscle layer to compensate for the loss of elastin; Milewicz *et al.*, 2000). *ELN* is also one of the genes deleted in Williams–Beuren syndrome, whose symptoms can include lax skin and supravalvular aortic stenosis, among others. Duplication of the elastin gene does not clearly lead to any skin

phenotype (Merla *et al.*, 2010), which suggests that rs7787362 may be associated with a decrease in the expression of functional elastin, although we did not find this SNP in a search of eQTL databases.

The second association, rs35318931 ($P = 1.1e-13$, OR = 0.82), is a missense variant (serine to phenylalanine) in the *SRPX* (sushi-repeat containing protein, X-linked) gene. It is associated with striae gravidarum in the pregnancy cohort ($P = 0.026$, $\beta = -0.067$). Very little is known about the function of this gene. The third association, rs10798036 ($P = 6.91e-10$, OR = 1.11), which did not reach significance in the pregnancy cohort ($P = 0.06$), is located in the *HMCN1* (hemicentin-1) gene. Mutations in *HMCN1* have been associated with age-related macular degeneration (Schultz *et al.*, 2003). As there are no other clear gene candidates for the associations at these loci, it is unclear how these regions might be related to the risk of developing stretch marks.

The final genome-wide significant association, rs7594220 ($P = 9.8e-9$, OR = 0.88), is located 3 kb downstream of *TMEM18* (transmembrane protein 18), which is involved in neural stem cell migration and cancer but has also been associated with obesity and obesity-related traits (Thorleifsson *et al.*, 2009; Elks *et al.*, 2010). This SNP is in linkage disequilibrium with SNPs previously associated with body mass index (BMI), and correcting for BMI weakens this signal, although not the signals for the other three genome-wide significant hits. To further investigate the association between obesity and stretch marks, we looked more closely at 32 SNPs previously associated with BMI. Even after correction for BMI, one SNP was associated with stretch marks, suggesting a potential effect independent of BMI (Supplementary Table S3 online).

Three additional regions show suggestive evidence of association with stretch marks (Table 1). Of these, rs3910516 is of particular interest as it lies 2.2 kb upstream of *FN1*, which encodes fibronectin, an extracellular matrix protein that binds to collagen and integrins. Skin biopsies from individuals with stretch marks demonstrate reduced expression of fibronectin (Lee *et al.*, 1994). The SNP (chr6:36311047) downstream of *PNPLA1* is also interesting, as mutations in *PLPLA1* are associated with autosomal recessive congenital ichthyosis, a rare skin disease (Grall *et al.*, 2012). None of the suggestive associations were found in the smaller pregnancy cohort. Although we did not observe a significant difference in the effect of these SNPs between men and women in the discovery cohort, it is still possible that striae gravidarum may have a different etiology.

Given that loose skin is a symptom of syndromes caused by deletion or loss-of-function mutations in *ELN*, our results support the hypothesis that variations in the elastic fiber component of the skin extracellular matrix contribute to the development of stretch marks. The expression of collagens, elastin, and fibronectin (which was suggestively associated with stretch marks in our analysis) is decreased in striae, which could be linked to the reorganization and overall loss of elastic fibers in skin affected by striae (Lee *et al.*, 1994; Watson *et al.*, 1998). Other variants associated with elastic tissue have been associated with intracranial aneurysm and exfoliation glaucoma (Akagawa *et al.*, 2006; Thorleifsson *et al.*, 2007). The potential effect of genes associated with obesity, both independent of and via changes in BMI, is also an intriguing area for further study. Replicating this work in a more precisely phenotyped population would be a logical next step.

None of the existing treatments for stretch marks are completely effective in removing stretch marks. Interestingly, most popular treatments including topical treatments and laser treatments focus on stimulating collagen production, rather than elastin production, to improve the appearance of stretch marks, although some also increase elastic fibers (Elsaie *et al.*, 2009). These findings may provide further insight into future methods for the prevention and treatment of stretch marks.

CONFLICT OF INTEREST

JYT, AKK, UF, MM, and NE are employees of 23andMe, and JYT, AKK, UF, and NE have stock options in the company. NE holds a patent with 23andMe.

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SUPPLEMENTARY MATERIAL

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

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Six Mutations in AAGAB Confirm its Pathogenic Role in Chinese Punctate Palmoplantar Keratoderma Patients

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TO THE EDITOR

Palmoplantar keratodermas (PPKs) are a group of rare keratinization disorders characterized by the excessive formation of keratin on the palms and soles. Four clinical types of PPK can be classified by the pattern of lesions: diffuse, punctuate, focal, and striate (Stevens *et al.*, 1996). Punctate palmoplantar keratoderma type I (PPKP1, MIM number 148,600) is an autosomal dominant inherited subtype of PPK characterized by multiple tiny punctate keratoses on the palms and soles, which can gradually increase in diameter with age and even coalesce (Figure 1a and b). The incidence of this disease was reported as 1.17 per 100,000 population in Croatia and 3.3 per 100,000 population in Slovenia (Guo *et al.*, 2012). Two linkage loci for PPKP1 (8q24.13–8q24.21, 15q22.2–15q24.1) have been reported previously (Martinez-Mir *et al.*, 2003; Zhang *et al.*, 2004; Gao *et al.*, 2005; El Amri *et al.*, 2010; Mamai *et al.*, 2012).

In recent years, the success of exome sequencing has been well established in the identification of novel causal mutations for Mendelian diseases (Ku *et al.*, 2012). Here, we subjected two DNA sequences (III8 and III9) from the Chinese PPKP1 family linked to 15q22 (Family 22) to exome sequencing. Informed consent was obtained from all the sequenced participants. This study was authorized by the Ethics Committee of Anhui Medical University and was conducted in accordance with the Declaration of Helsinki Principles. Exome capture and enrichment were performed using the Agilent SureSelect Human All Exon Kit (in solution; Santa Clara, CA) according to the manufacturer's protocols. Exome sequencing was then performed on a HiSeq 2000 platform (Illumina, San Diego, CA), and sequence data were processed to raw sequence reads. These reads were aligned to a human reference genome (NCBI build 37.3, hg19), and the analytical pipeline was then followed by SOAPsnp (Li *et al.*, 2009). Finally, the variants were annotated to obtain information such as genomic position and functional effect.

On average, two sequences were obtained from each individual at ~50-fold coverage depth. We obtained 4.51 billion bases of sequence data as paired-end, 90-bp reads. After discarding reads that had a duplicated start site, 2.69 billion bases of mappable targeted exome were defined by RefSeq genes. An average of 89.2% of the exome was covered at least ~10-fold, and 51,819 variants were identified per individual.

We primarily focused on coding variants, including non-synonymous variants, splice-site variants, and insertions/deletions, of which there were an average of 11,362. We filtered the variants against dbSNP129, eight HapMap individuals, the 1,000 Genomes Project, the YH database, and unaffected individual, and reduced the number of candidate variants to 527. We then used SIFT to predict the functional impact and determine the 261 variants that were the most likely to be damaging. Finally, we found that five mutations in five genes (DAPK2, IGDCC4, RPL4, TPM1, and AAGAB) were located on 15q22.2–15q24.1. Among the five genes, mutations in AAGAB have recently been identified in PPKP1 in two publications (Giehl *et al.*, 2012; Pohler *et al.*, 2012). We tested all the samples and identified the c.552_554TAG>AT mutation in exon 6 of AAGAB as the causative mutation in Family 22 (Figure 1c and d). This mutation resulted in a stop codon at amino acid position 188 (p.Phe184 Leufs* 6).

We then sequenced all the exons and exon–intron boundaries of AAGAB in 59 individuals from an additional seven families (Families 23–29) and 28 sporadic patients (Sporadic patients 1–28) with PPKP1 (Supplementary Figure S1 online). Finally, four truncated mutations in four respective families and a splice-site mutation in one sporadic case were identified (Supplementary Figure S2 online). The *AAGAB* gene mutations were absent in three other PPKP1 families (Families 27–29) and 27 sporadic patients (Sporadic patients 2–28). In addition, we identified the c.481C>T mutation in exon 5 in one patient with cancer from Family 24,

Abbreviation: PPK, palmoplantar keratoderma

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