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Two newly identified genetic determinants of pigmentation in Europeans

Patrick Sulem[1], Daniel F Gudbjartsson[1], Simon N Stacey[1], Agnar Helgason[1], Thorunn Rafnar[1], Margret Jakobsdottir[1], Stacy Steinberg[1], Sigurjon A Gudjonsson[1], Arnar Palsson[1], Gudmar Thorleifsson[1], Snæbjörn Pálsson[1], Bardur Sigurgeirsson[2,3], Kristin Thorisdottir[2–4], Rafn Ragnarsson[4], Kristrun R Benediktsdottir[2,3], Katja K Aben[5,6], Sita H Vermeulen[6], Alisa M Goldstein[7], Margaret A Tucker[7], Lambertus A Kiemeney[5,6,8], Jon H Olafsson[2,3], Jeffrey Gulcher[1], Augustine Kong[1], Unnur Thorsteinsdottir[1] & Kari Stefansson[1]

We present results from a genome-wide association study for variants associated with human pigmentation characteristics among 5,130 Icelanders, with follow-up analyses in 2,116 Icelanders and 1,214 Dutch individuals. Two coding variants in *TPCN2* are associated with hair color, and a variant at the *ASIP* locus shows strong association with skin sensitivity to sun, freckling and red hair, phenotypic characteristics similar to those affected by well-known mutations in *MC1R*.

We recently reported a genome-wide study identifying six loci associated with variation in hair, eye and skin pigmentation in Europeans[1]. To search for additional sequence variants correlating with human pigmentation, we expanded the genome-wide scan from 2,986 individuals to 5,130. The findings of this discovery phase were followed up in 2,116 Icelanders and 1,214 Dutch individuals. The Icelandic and Dutch studies were both approved by their respective ethical review boards, and informed consent was obtained from all study participants (**Supplementary Methods** online). We examined the association of sequence variants with pigmentation traits in eight genome-wide association analyses: three analyses for eye color (blue versus green, blue versus brown and blue versus non-blue), two for hair color (red versus nonred and blond versus brown) and three for skin pigmentation traits (skin sensitivity to sun, the presence of freckles and a combination of skin sensitivity to sun and presence of freckles, herein referred to as 'burning and freckling'). These analyses identified 99 distinct SNPs (**Supplementary Table 1** online) with genome-wide significant associations ($P < 1.5 \times 10^{-7}$) in at least one of the eight pigmentation scans, 88 of which are located in areas described in our initial report[1]. The 11 remaining SNPs are located at three loci on chromosomes 9p23, 11q13 and 20q12. The findings of the discovery phase were confirmed in an analysis of an additional 2,116 Icelanders and 1,214 Dutch individuals (see **Supplementary Table 2** online for quality control information).

A total of six SNPs within a region of strong linkage disequilibrium (LD) on 20q11.22 showed association with burning and freckling that reached genome-wide significance (max OR = 1.60, $P = 3.9 \times 10^{-9}$; **Supplementary Table 1**). Multipoint analysis of the area revealed an extended haplotype tagged by a two-SNP haplotype (rs1015362[G], rs4911414[T]) that we will refer to as the *ASIP* haplotype (**Table 1**, **Supplementary Methods** and **Supplementary Fig. 1** online). The *ASIP* haplotype accounts for the association of the other SNPs in the region (**Supplementary Table 3** online) and was significantly associated in both the Icelandic and Dutch replication samples (**Table 1**). In the combined analysis of the discovery and replication samples, the *ASIP* haplotype reached genome-wide significance for red hair color, freckling and skin sensitivity to sun, in addition to burning and freckling (**Table 1**). This pattern of association is very similar to that observed for the mutations associated with red hair color in *MC1R* (encoding melanocortin-1 receptor)[2] (**Supplementary Tables 3**, **4** and **5** online). The region covered by the extended haplotype contains a large number of genes, including *ASIP* (encoding agouti signaling protein), a gene with a well-documented role in pigmentation. In melanocytes, the agouti signaling protein antagonizes the activation of *MC1R* by α-MSH (alpha melanocyte-stimulating hormone), resulting in a switch to the production of red or yellow phaeomelanin. Sequence variants at the agouti locus are responsible for animal coat colors such as yellow and dark color[3,4]. A polymorphism in the 3′ untranslated region of *ASIP*, rs6058017 (8818A>G), has been studied for its association with pigmentation characteristics within populations of European ancestry[5–7] and has also been related to differences in skin pigmentation among populations of mixed African and European ancestry[8]. The *ASIP* haplotype, rs1015362[G] and rs4911414[T], occurs on the background of the major allele of rs6058017, but the correlation between the two is very weak ($D' = 1$; $r^2 = 0.008$). The strength of association of rs6058017 with the pigmentation traits is much less than that of the *ASIP* haplotype, and after adjustment for rs6058017, the *ASIP* haplotype remains highly significant for burning and freckling ($P = 1.3 \times 10^{-46}$). On the other hand, after adjustment for the haplotype, rs6058017 is only marginally associated with pigmentation characteristics ($P = 0.057$ for burning and freckling; **Fig. 1a** and **Supplementary**

[1]deCODE genetics, Sturlugata 8, 101 Reykjavik, Iceland. [2]Department of Dermatology, [3]Department of Pathology and [4]Department of Plastic Surgery, Landspitali-University Hospital, 101 Reykjavik, Iceland. [5]Comprehensive Cancer Center, 6501 BG Nijmegen, The Netherlands. [6]Department of Epidemiology and Biostatistics, Radboud University, Nijmegen Medical Center, 6500 HB Nijmegen, The Netherlands. [7]Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland 20892, USA. [8]Department of Urology, Radboud University Nijmegen Medical Center, 6500 HB Nijmegen, The Netherlands. Correspondence should be addressed to D.F.G. (daniel.gudbjartsson@decode.is) or K.S. (kstefans@decode.is).

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Table 1 Association of previously unreported SNPs with pigmentation characteristics in Icelandic and Dutch individuals

Locus and phenotype	OR (95% CI) Icelandic discovery ($n = 5,130$)	Icelandic replication ($n = 2,116$)	Dutch replication ($n = 1,214$)	P
ASIP AH (rs1015362[G], rs4911414[T]) (freq. 8%)				
Burn and freckle[a]	2.56 (2.06, 3.18)	2.90 (2.11, 3.98)	2.27 (1.58, 3.26)	5.8×10^{-37}
Skin sensitivity to sun[b]	1.76 (1.49, 2.08)	1.82 (1.43, 2.32)	1.75 (1.32, 2.32)	1.9×10^{-24}
Freckle[c]	1.95 (1.65, 2.32)	2.13 (1.66, 2.72)	1.56 (1.17, 2.07)	8.2×10^{-29}
Red vs. not-red hair	1.76 (1.34, 2.31)	2.02 (1.38, 2.96)	2.03 (0.93, 4.46)	2.7×10^{-9}
Blond vs. brown hair	1.46 (1.08, 1.96)	1.62 (1.08, 2.43)	1.75 (1.15, 2.66)	1.5×10^{-5}
TPCN2 rs35264875[T] (freq. 22%)[d]				
Blond vs. brown hair	2.49 (1.96, 3.15)	2.13 (1.38, 3.30)	2.03 (1.47, 2.80)	3.6×10^{-30}
TPCN2 rs3829241[A] (freq. 44%)[d]				
Blond vs. brown hair	1.60 (1.35, 1.89)	1.54 (1.12, 2.11)	1.38 (1.07, 1.77)	6.2×10^{-16}
TYRP1 rs1408799[C] (freq. 75%)				
Blue vs. green or brown eyes	1.40 (1.25, 1.57)	1.32 (1.11, 1.58)	1.22 (1.01, 1.47)	5.9×10^{-17}
Blond vs. brown hair	1.29 (1.09, 1.53)	1.10 (0.85, 1.42)	1.10 (0.86, 1.42)	8.3×10^{-5}

Odds ratios (OR) and their 95% confidence intervals (95% CI) are given for each sample. See **Supplementary Tables 5–7** for association with other pigmentation traits.
[a]Compared to those who tan and do not freckle. [b]Compared to those who are not sensitive to sun. [c]Compared to those who do not freckle. [d]The effects of the two *TPCN2* SNPs were estimated jointly.

Table 3). We sequenced the exons and promoter of *ASIP* in 368 individuals without detecting any sequence variant likely to account for the observed association. A stronger association of the ASIP haplotype with skin sensitivity to sun was observed for males than females ($P = 0.0033$; **Supplementary Table 4**), but the difference was not significant after correction for the number of variants tested for sex-specific differences.

Four SNPs on 11q13.2 showed association with blond versus brown hair color in the Icelandic discovery sample that reached genome-wide significance (**Supplementary Table 1**). These SNPs are located within a single LD block that overlaps with only one gene, *TPCN2* (encoding two-pore segment channel 2). We identified three common nonsynonymous variants in exons of *TPCN2* (rs3829241, rs35264875, rs3750965) that, on the basis of the HapMap data, correlate with the four SNPs on the 300K chip giving significant association. These SNPs were typed in the Icelandic discovery samples as well as the two replication samples. The replication samples were also typed with rs1011176, which showed the strongest association in the initial discovery scan. All of the observed association with blond versus brown hair could be explained by two of the coding SNPs: rs35264875 (encoding M484L) and rs3829241 (encoding G734E) (**Supplementary Table 3**), which replicated with similar effects (**Table 1** and **Fig. 1b**).

We did not observe strong association of these two variants with the other pigmentation traits (**Supplementary Tables 5–7** online), similar to what had been observed for the *KITLG* (encoding the ligand for KIT receptor tyrosine kinase) variant that also associates with blond versus brown hair. A link between *TPCN2* and pigmentation has not been previously suspected. The protein encoded by *TPCN2* participates in calcium transport, similar to the proteins encoded by known pigmentation genes *SLC24A4* (ref. 1) and *SLC24A5* (ref. 9).

A single SNP, rs1408799, on 9p23 showed genome-wide significant association with blue versus nonblue eyes (OR = 1.41, $P = 1.5 \times 10^{-9}$). This association was confirmed in both the Icelandic and Dutch replication samples with a similar effect (**Table 1**). A suggestive association with blond versus brown hair was also observed for this SNP. The SNP belongs to an LD block that encompasses only one gene, *TYRP1* (encoding the tyrosinase-related protein 1)[10]. *TYRP1* encodes a melanosomal enzyme with a role in the eumelanin pathway. In humans, rare mutations in *TYRP1* are responsible for oculocutaneous albinism type 3 (ref. 11). Previous studies on the genetics of eye color in Europeans have associated polymorphisms at *TYRP1* with eye color[12]. The SNP reported here, rs1408799, is in strong LD with one of the previously reported SNPs, rs2733832, in HAPMAP CEU[13] ($D' = 0.96$; $r^2 = 0.67$).

The larger sample size studied here allows us to make some additional claims for the loci we reported previously. The *TYR* (encoding tyrosinase) coding variant rs1126809 (encoding R402Q)

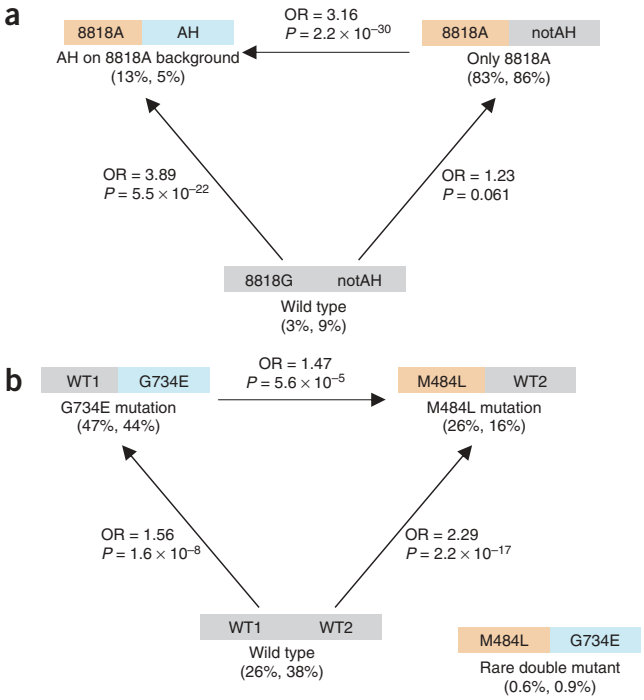

Figure 1 Estimates of odds ratio for haplotypes at *ASIP* and *TPCN2*. (**a**) At *ASIP*, the previously reported variant 8818A is compared to the *ASIP* haplotype (AH) in individuals who burn and freckle and those who tan and do not freckle. Chromosomes not carrying the *ASIP* haplotype are denoted by notAH. (**b**) At *TPCN2*, the two variants encoding G734E and M484L are compared to the wild-type haplotype and to each other. Frequencies in the two pigmentation groups are displayed in brackets (fairer pigmentation, darker pigmentation). Estimated ORs and *P* values, from the pair-wise comparison of the haplotype at the end of arrow versus haplotype at the beginning of the arrow adjusted for all other haplotypes, are shown beside each arrow.

reaches genome-wide significance for skin sensitivity to sun in addition to its previously reported association with eye color (**Supplementary Tables 5–7**). Compound heterozygosity for a mutant allele of *TYR* and the R402Q polymorphism can result in ocular albinism[14].

This report adds sequence variants at two loci to the ever growing list of variants affecting normal variation in pigmentation among Europeans. The strength of the association of the newly identified *ASIP* haplotype described here is close to that of variants in *MC1R* and much stronger than that of the previously reported variants near *ASIP*. This haplotype thus seems to tag more effectively the actual causal variant(s). It is of note that genes encoding calcium ion transporters are emerging as a family of pigmentation genes, as three have now been associated with pigmentation: *SLC24A4*, *SLC24A5* and now *TPCN2*.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

P.S., D.F.G., S.N.S. and K.S. wrote the first draft of the paper. S.N.S., B.S., K.T., R.R., K.R.B. and J.H.O. collected the Icelandic samples and phenotypes. S.N.S., M.J. and U.T. performed the genotyping. K.K.A., S.H.V. and L.A.K. collected the Dutch samples and phenotypes. P.S., D.F.G., A.H., S.S., S.A.G., A.P., G.T., and S.P. analyzed the data. P.S., D.F.G., S.N.S., T.R., A.M.G., M.A.T., L.A.K., J.H.O., J.G., A.K., U.T., K.S. planned, coordinated and supervised the work. All authors contributed to the final version of the paper.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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