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Obesity-related genetic variants, human pigmentation, and risk of melanoma

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Abstract

Previous biological studies showed evidence of a genetic link between obesity and pigmentation in both animal models and humans. Our study investigated the individual and joint associations between obesity-related single nucleotide polymorphisms (SNPs) and both human pigmentation and risk of melanoma. Eight obesity-related SNPs in the *FTO*, *MAP2K5*, *NEGR1*, *FLJ35779*, *ETV5*, *CADM2*, and *NUDT3* genes were nominally significantly associated with hair color among 5,876 individuals of European ancestry. The genetic score combining 35 independent obesity-risk loci was significantly associated with darker hair color (beta-coefficient per ten alleles=0.12, P-value=4 10⁻⁵). However, single SNPs or genetic scores showed non-significant association with tanning ability. We further examined the SNPs at the *FTO* locus for their associations with pigmentation and risk of melanoma. Among the 783 SNPs in the *FTO* gene with imputation R-square quality metric >0.8 using the 1000 genome data set, ten and three independent SNPs were significantly associated with hair color and tanning ability respectively. Moreover, five independent *FTO* SNPs showed nominally significant association with risk of melanoma in 1,804 cases and 1,026 controls. But none of them was associated with obesity or in linkage disequilibrium with obesity-related variants. *FTO* locus may confer variation in human pigmentation and risk of melanoma, which may be independent of its effect on obesity.

Keywords

obesity; pigmentation; melanoma; genetic association; *FTO* gene

Introduction

Obesity results from an interaction between genetic and environmental factors. While excessive energy intake and lack of physical activity cause obesity, genetic factors play an important role in conferring susceptibility to the trait. Pigmentation is a trait with high heritability and substantial variation in the general population (Frisancho et al. 1981; Harrison and Owen 1964). Typical visible human pigmentation traits include tanning ability, hair color, skin color, and eye color, the regulation of which involves a handful of pigmentation genes (Gerstenblith et al. 2010; Nan et al. 2009b).

Pharmacological and genetic studies demonstrated the role of melanocortins in various physiological functions, including energy homeostasis and pigmentation, which suggests possible genetic link between these two phenotypes (Gantz and Fong 2003). Melanocyte-stimulating hormones (MSHs), derived from a precursor molecule known as the pro-opiomelanocortin (POMC) protein, exert their numerous biological effects by binding and activating the melanocortin receptors (MCRs) which belong to the family of G-protein-coupled seven-transmembrane receptors (Gantz and Fong 2003). MC1R is expressed in melanocytes where α -MSH signals through it to determine skin and hair pigmentation (Rees 2003, 2004). MC4R is expressed predominantly in the hypothalamus, and is involved in the regulation of feeding behavior, energy homeostasis, and obesity development (Adan et al. 2003; Getting 2006). To date, five MCRs have been cloned and identified, among which there is a high sequence homology with a shared signaling pathway (Cone 2006).

Evidence of genetic link between obesity and pigmentation can be traced back to very early time when scientists discovered that dominant mutations of the *agouti* gene led to yellow fur, adult-onset obesity, insulin resistance, and increased susceptibility to cancer in mice (Danforth 2012; DICKIES 1962; Heston and Vlahakis 1961, 1968; Yen et al. 1994). Moreover, in 1998, Krude *et al.* demonstrated that early-onset obesity was correlated with red hair color in two patients with *POMC* mutations (Krude et al. 1998). The patients had a mutation which either interferes with the synthesis of α -MSH or abolishes POMC translation. Most recently, PPAR- γ co-activators, key regulators of energy metabolism, have been shown to regulate the transcription of *MITF* and melanin production in response to α -MSH in melanocytes, and the genetic variants in PPAR- γ co-activators are associated with human tanning, which provides a novel link between energy metabolism and pigment formation (Shoag et al. 2012).

To better understand the relationship between obesity and human pigmentation at the genetic level, we conducted a series of analyses to investigate the association between obesity susceptibility loci identified from genome-wide association studies (GWAS) and human pigmentation phenotypes (hair color and tanning ability). We also evaluated the association between those obesity-related loci and risk of melanoma.

Materials and Methods

Study population

We firstly assessed the association between obesity SNPs and pigimentary phenotypes in five previous case-control studies nested within the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS), including one postmenopausal invasive breast

cancer case-control study, two type 2 diabetes case-control studies, and two coronary heart disease case-control studies. Due to the fact that breast cancer, type 2 diabetes, and coronary heart disease are all associated with obesity, we excluded all the cases and conducted the analysis only among 5,876 controls. The study protocol was approved by the Institutional Review Board of Brigham and Women's Hospital and the Harvard School of Public Health. We then evaluated the association between *FTO* SNPs and the risk of melanoma among 1,804 cases and 1,026 controls in the MD Anderson Cancer Center melanoma case-control study. [See Supplementary Material for more information about study population, laboratory assays, imputation and quality control]

SNP selection and genetic score calculation

We initially selected 57 obesity-related SNPs from published obesity GWAS (He et al. 2010; Kilpeläinen et al. 2011; Loos et al. 2008; Speliotes et al. 2010; Thorleifsson et al. 2008; Willer et al. 2008) (see Supplementary Table 1 for detailed information of all the SNPs), but only the SNPs with imputation R-square quality metric greater than 0.8 in all of the five sets were considered for further analysis. We first calculated R-square between all SNPs to detect possible linkage disequilibrium (LD). Among those correlated SNPs (R-square >0.8, see Supplementary Table 1 for LD), only the ones with the strongest association (smallest *P*-value) with obesity in our dataset were kept for genetic score calculation. Genetic scores were calculated only for individuals who had no missing value in all of the chosen SNPs. For each individual, we summed the dosage of obesity-risk alleles of those independent loci to get the first type of genetic score, which is denoted as “genetic score of independent loci”. The second type of genetic score, “genetic score of independent gene”, was created by choosing only one SNP (the one with the most significant association with obesity in our dataset) from one gene for sensitivity analysis. We did not perform this sensitivity analysis for tanning ability, because none of the SNPs showed significant association with tanning ability. Both “genetic score of independent loci” and “genetic score of independent genes” were calculated using unweighted approach. Weighted genetic scores were also created to confirm our findings.

Statistical analyses

We used linear regression models to test the associations between continuous BMI in 1986 and individual SNPs in both cohorts, adjusting for age at 1986, sex, study set, and top three eigenvectors (EVs). In both NHS and HPFS, hair color was categorized as: red, blonde, light brown, dark brown, and black. Tanning ability was categorized as: practically no tan, light tan, average tan, and deep tan in NHS; and burn/peel, burn then tan, and tan without burn in HPFS. Linear regression models were applied to assess the relationships between hair color (excluding red hair, others coded as 1=blonde, 2=light brown, 3=dark brown, 4=black) and obesity risk alleles as well as the genetic scores, adjusting for study set and top three EVs. We further divided our samples into quintiles according to their “genetic score of independent loci” and coded them as dummy variables in order to compare each of the groups with reference group (lowest genetic score quintile). The same methods were used for evaluating the associations between tanning ability (coded as 1=practically none/light tan, 2=average tan, 3=deep tan in NHS; and 1=pain:burn/peel, 2=burn then tan; 3=tan without burn in HPFS) and single obesity variants as well as the genetic scores. We used logistic regression to assess the effects of individual SNPs and the genetic scores on red hair color phenotype (red hair color vs. non-red hair color). The associations between SNPs in the *FTO* gene and pigimentary phenotypes were evaluated in linear regression models, controlling for study set and top three EVs. We then used a forward selection approach to identify independent SNPs associated with pigmentation in the *FTO* gene. We included the most significant SNP as a predictor variable into the model each time until the associations between all the other SNPs and pigimentary phenotypes became non-significant (*P*-

value>0.05). Analyses described above were all conducted using NHS and HPFS datasets. We also applied logistic regression models to evaluate the associations between candidate *FTO* SNPs and melanoma risk within the MD Anderson case-control study. In all the analyses, we adjusted for top principle components to control for potential population stratification within individuals of European ancestry. The SAS statistical package was used for all the analyses (SAS, version 9.0 for UNIX; SAS Institute Inc, Cary, North Carolina). LD plots were drawn using Haploview. All hypotheses tests were two-sided.

Results

Characteristics of study participants

The information on pigmentation phenotypes and BMI is presented in Table 1. The distributions of hair color, tanning ability, and BMI were similar between men and women.

Associations between individual SNPs and pigmentation

The findings of 57 SNPs are presented in Supplementary Table 1. SNP rs2287019 was excluded due to poor imputation quality (R-square quality metric=0.67). Thirty-five SNPs remained after we removed SNPs which were in high LD ($R^2>0.8$) with the SNP that was most significantly associated with obesity in our data set at each independent locus. Obesity-related SNPs rs7190492 [*RPGRIP1L*, *FTO*], rs2241423 [*MAP2K5*], rs3101336 [*NEGR1*], rs6499640 [*RPGRIP1L*, *FTO*], rs2112347 [*FLJ35779*], rs9816226 [*ETV5*], rs13078807 [*CADM2*], and rs206936 [*NUDT3*] had significant associations with hair color (P -value <0.05, Table 2). However, none of the SNPs was significantly associated with tanning ability or red hair color (P -value>0.05). The associations between pigmentation (hair color and tanning ability) and those 35 selected SNPs are shown in Supplementary Table 2.

Genetic scores and pigmentation

The SNPs included in “genetic score of independent loci” and “genetic score of independent gene” are presented in Supplementary Tables 2 and 3. The “genetic score of independent loci” which ranges from 23.05 to 49.30 and has a mean value of 36.43 was significantly associated with hair color (beta-coefficient per ten obesity-risk alleles = 0.12, P -value = 4×10^{-5}) (Table 3). A higher genetic score (more obesity-risk alleles) was associated with a darker hair color. However, no significant association was found between tanning ability and this 35-SNP composite genetic score. (P -value = 0.41, Supplementary Table 4)

We then divided our study individuals into quintiles according to the value of this score and coded them as dummy variables. The mean genetic score of the five quintiles are 31.29, 34.54, 36.45, 38.34, and 41.57 respectively. The coefficient of the highest quintile group was 0.14 (95%CI: 0.08–0.21, p -value= 2×10^{-5}) compared with the lowest quintile as the reference group. The differences in tanning ability between quintile groups were not significant. (Table 4)

In the sensitivity analysis, “genetic score of independent gene,” which contained 29 SNPs, showed a significant association with hair color (beta-coefficient per ten obesity-risk alleles = 0.10, P -value=0.002) (Table3), but it was not significantly associated with tanning ability, which is consistent with the result obtained from the first type of genetic score. Analyses using weighted genetic scores showed similar results (Supplementary Table 5).

However, the significant association between genetic scores and hair color disappeared after removing SNPs that were nominally and significantly associated (P -value<0.05) with hair color (Table 3). In addition, we repeated all the analyses by adding BMI to the previous models, which did not change the above-mentioned results (Table3). Analysis on the effect

of genetic scores on “red hair vs. non-red hair color” using logistic regression did not show an association.

***FTO* SNPs and pigmentation**

Among the 1,059 imputed SNPs at the *FTO* locus within the NHS and HPFS datasets, 783 with imputation R-square quality metric > 0.8 in all the five sets were retained in further analysis, and we found that 159 and 49 SNPs exhibited nominally significant (P -value < 0.05) associations with hair color and tanning ability, respectively. The association between all 783 *FTO* SNPs and obesity as well as the association between these *FTO* SNPs and pigmentation are displayed in the same figure (Figure 1). The LD plot corresponding to the entire region of *FTO* locus is shown in Figure 2. Although we found that 14 SNPs in the *FTO* gene were nominally significantly associated with both obesity and hair color, these associations were not significant after Bonferroni correction (Supplementary Table 6). There was no strong LD detected between obesity-related SNPs and pigmentation SNPs (all $R^2 < 0.1$).

Using the forward selection procedure, we identified ten independent SNPs for hair color and three independent SNPs for tanning ability. It was shown that further controlling for the 10 SNPs (rs1075440, rs10852522, rs56897002, rs2540784, rs12447135, rs12444954, rs7199716, rs16952450, rs8055834, and rs4310844) eliminated the associations between hair color and all the other SNPs in the *FTO* gene (Supplementary Figure 1), whereas controlling three SNPs (rs12448173, rs2388406, and rs16952581) eliminated the associations between tanning ability and all the other SNPs in the *FTO* gene. (Supplementary Figure 2)

***FTO* SNPs and melanoma**

With data from MD Anderson melanoma case-control study, we also tested the associations between *FTO* SNPs and the risk of melanoma. Only 535 SNPs were available for this analysis within the region ± 50 kb surrounding the *FTO* gene, of which we found that 24 SNPs were in nominally significant association with the risk of melanoma (Supplementary Table 7). The positions and associations with the risk of melanoma of all the 535 SNPs are also shown in Figure 1. All the 24 melanoma-associated SNPs are located outside the small region that harbors most of the obesity-related SNPs (from position 52326794 to 52475757). We also found quite a few melanoma-associated SNPs gathered at the end of the *FTO* locus, where there was a small peak for hair color-related SNPs and two highly significant tanning-related SNPs.

We identified five independent melanoma-risk SNPs (rs12933928, rs12932428, rs1125338, rs12599672, and rs12600192) out of the 24 significant ones in the *FTO* gene (Table 5). The magnitude of association did not change materially after additionally adjusting for BMI, tanning ability, and hair color.

To check the correlation between melanoma-related SNPs, pigmentation SNPs (hair color and tanning ability), and obesity-related SNPs in *FTO* gene, we created a LD plot that contained all the SNPs showing significant associations with at least one of the four phenotypes (Supplementary Figure 3). As displayed in this figure, melanoma SNPs and obesity-related SNPs are independent of each other ($R^2 < 0.1$).

Testing for potential effect modification

For significant SNPs and genetic scores detected in our analyses, interaction terms were added to the corresponding models in order to test whether the associations between obesity-related SNPs and pigmentation/risk of melanoma were modified by other covariates, such as

study set, gender, age, and BMI. Results are shown in Supplementary Materials and Supplementary Tables 8 and 9.

Discussion

In the present study, we examined both individual and joint effects of previously reported obesity-risk loci on hair color and tanning ability. Our results indicated that eight SNPs were nominally significantly associated with hair color, but none with tanning ability. We found that the accumulation of obesity-risk alleles was significantly associated with dark hair color independently of BMI. In addition, there are non-obesity-related SNPs in the *FTO* gene associated with pigmentary phenotypes and melanoma risk.

Although we identified eight significant obesity-related SNPs that were associated with hair color, their associations became non-significant after the Bonferroni correction. Considering the Bonferroni correction may be a bit conservative for our analysis, we also applied the Benjamini-Hochberg approach to control false discovery rate (FDR) which is defined as the expectation value of the proportion of false positive among the identified SNPs. We found that twenty-five out of 35 SNPs were significantly associated with hair color at the FDR level of 0.5, indicating half of the identified SNPs could be expected to be correct (Supplementary Table 2). Given our results, the associations between the eight SNPs and pigmentation should be interpreted cautiously and further replication studies are needed to confirm our findings.

There was some biological evidence of a genetic link between obesity and pigmentation in both animal models and humans, but most of them focused on the effect of mutation in single gene. As mentioned in the Introduction, obesity is correlated with red hair color in *POMC*-deficient patients; a genetic variant of PPAR- γ co-activator was associated with an increase in tanning ability. These studies demonstrate that the connection between obesity and pigmentation is attributable to multiple genetic variants. In the current study, the genetic risk score summarizing the information provided by the 35 obesity-related SNPs showed that people with more obesity-risk alleles were more likely to have darker hair color. However, we did not find any significant association between genetic scores and tanning ability in our analysis, which indicates that the cross link is not for transient pigmentation process. Neither the genetic score nor individual SNPs were associated with red hair color in the present study, which is consistent with the fact that the red hair phenotype is determined mainly by *MC1R* variants (Nan et al. 2009a).

In the analyses, we created a genetic score with only one SNP from one gene. This type of genetic score produced similar but less significant results than the first genetic score. On one hand, it confirmed the significant association between obesity genetic score and hair color. On the other hand, it highlighted the important role of the *FTO* gene, because this score did not contain rs7190492 and rs6499640 (significantly associated with hair color) which are located in the *FTO* gene. In addition, the effect of genetic scores was no longer significant after removing those SNPs significantly associated with hair color. Therefore, we concluded that the overall significant association is likely to be driven by those top SNPs, thus providing more insights into the roles of *FTO*, *MAP2K5*, *NEGR1*, *FLJ35779*, *ETV5*, *CADM2*, and *NUDT3* in hair color determination.

Among the eight significant SNPs, two of them which are the most (rs7190492) and fourth (rs6499640) significant loci found in this study are localized in the *FTO* gene. Hence, we further examined the associations between all high-quality imputed SNPs in the *FTO* gene and risk of melanoma. The *FTO* gene, which maps on chromosome 16 and covers a length of 410.51 kb, is widely expressed in a variety of human tissues. It contains 18 distinct

introns and its transcription produces seven alternatively spliced mRNAs. In 2007, the association between *FTO* and BMI was identified in diverse populations (Frayling et al. 2007; Scott et al. 2007; Scuteri et al. 2007), which has inspired further research aimed at elucidating its functional properties. Sequence analysis showed that *FTO* shares features with Fe(II) and 2-oxoglutarate (2OG) oxygenases (Gerken et al. 2007; Sanchez-Pulido and Andrade-Navarro 2007). Within this super family, *FTO* is most similar to the *Escherichia coli* enzyme AlkB and its eukaryotic homologs, which can repair DNA methylation damage (Trewick et al. 2005). *In vitro* studies showed that murine Fto is indeed a 2OG oxygenase that can catalyze nucleic acid demethylation (Gerken et al. 2007). These studies suggest that FTO might act as a demethylase that regulates the expression of genes involved in multiple molecular pathways. It had been shown that *Pomc* mRNA repression was exaggerated in fasted *Fto*^{-/-} mice (Fawcett and Barroso 2010). Moreover, *Fto* expression was found to be related to central leptin-melanocortin pathway disruption in several murine models (Larder et al. 2010). These results are in support of our finding that the *FTO* gene may be involved in pigmentary phenotype. Alternatively, the *FTO* SNPs that we found to be associated with pigmentation may be in LD with other nearby loci.

Previous knowledge and our plot (Figure 1) both showed that obesity-related SNPs are located within a 47-kilobase (kb) LD block encompassing parts of the first two introns as well as exon 2 of *FTO*. This region is included within the small region (position 52326794 to 52475757) that contains the peak for obesity and hair color phenotypes. However, the SNPs associated with each of the phenotypes are not in LD (Supplementary Figure 3).

We found that five independent SNPs were nominally significantly associated with melanoma risk. Due to the fact that pigmentation is highly related to the risk of melanoma, most of those melanoma SNPs are associated with pigmentary phenotypes or in LD with pigmentary SNPs as expected; however, none of which was associated with obesity or was in LD with obesity-related SNPs (Supplementary Table 7; Supplementary Figure 3).

To our knowledge, this is the first study investigating the associations among obesity, pigmentation, and risk of melanoma using comprehensive SNP data, which may contribute to our understanding of the genetic links among these human traits. The association between genetic score and hair color in current analysis is likely to be driven by a few genes including *FTO*, *MAP2K5*, *NEGR1*, *FLJ35779*, *ETV5*, *CADM2* and *NUDT3*. The *FTO* gene may be important in pigmentation and melanoma development. According to our results, obesity-related SNPs are independent of both pigmentary and melanoma-related variants in the *FTO* gene. It is possible that the *FTO* gene influences multiple phenotypes, which may be independent of its effect on obesity. More studies are needed to further confirm our findings. Studies on these genes and related pathways will provide insight into the shared molecular mechanisms between obesity, hair color, and risk of melanoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Regional plot of association between *FTO* SNPs and pigmentation

This figure shows the region ± 50 kb surrounding the *FTO* gene. Results of associations between *FTO* SNPs and obesity (BMI at year 1986), and associations between *FTO* SNPs and pigmentation (hair color and tanning ability) were available for 783 SNPs. The associations between individual SNPs within this region and the risk of melanoma were assessed in the MD Anderson Cancer Center melanoma case-control study. Due to the difference between datasets, only 535 SNPs were available for melanoma risk assessment. The horizontal dash line shows the significance level of 0.05, and the two vertical lines are the boundaries of the “small region” we mentioned in the text.

Figure 2. LD plot corresponding to the entire region of *FTO* locus

Four hundred and twenty one out of the 783 SNPs are shown on this LD plot which covers the same region as shown in Figure 1. Region between points A and B (1, 2, and 3 together) represents the “small region” we mentioned in the text, where most obesity-related SNPs and hair color-related SNPs are located. Most obesity-related SNPs fall into block 2, and most hair color related SNPs are in block 3. The 24 melanoma-related SNPs are not all presented in this figure due to difference between datasets. For those in this plot, melanoma-risk SNPs are located near region C and the region between D and E.

Table 1Characteristics of the participants in this study ¹

NHS (women)		HPFS (men)	
Tanning ability, n (%)		Tanning ability, n (%)	
practically none/light tan	1016 (30.9)	pain: burn/peel	487 (23.8)
average tan	1534 (46.7)	burn then tan	947 (46.2)
deep tan	737 (22.4)	tan without burn	615 (30.0)
Hair color, n (%)		Hair color, n (%)	
red	148 (4.5)	red	60 (3.0)
blonde	413 (12.4)	blonde	234 (11.9)
light brown	1287 (38.8)	light brown	660 (33.4)
dark brown	1395 (42.0)	dark brown	839 (42.5)
black	78 (2.3)	black	182 (9.2)
BMI, n (%)		BMI, n (%)	
<20 kg/m ²	268 (7.8)	<20 kg/m ²	36 (1.7)
[20,25) kg/m ²	1797 (52.2)	[20,25) kg/m ²	1068 (50.2)
[25,30) kg/m ²	969 (28.2)	[25,30) kg/m ²	893 (41.9)
≥30 kg/m ²	407 (11.8)	≥30 kg/m ²	131 (6.2)

Abbreviations: NHS, Nurses' Health Study; HPFS, Health Professionals Follow-up Study; BMI, body mass index.

¹ Our study participants only included controls who had no missing values in SNPs and BMI.

Table 2SNPs nominally significantly associated with hair color ¹

SNPs	Nearest gene	P-value	Beta coefficient	StdErr
rs7190492	<i>RPGRIP1L, FTO</i>	0.003	0.05	0.02
rs2241423	<i>MAP2K5</i>	0.006	0.05	0.02
rs3101336	<i>NEGR1</i>	0.007	0.04	0.02
rs6499640	<i>RPGRIP1L, FTO</i>	0.01	0.04	0.02
rs2112347	<i>FLJ35779</i>	0.02	0.04	0.02
rs9816226	<i>ETV5</i>	0.02	-0.05	0.02
rs13078807	<i>CADM2</i>	0.03	0.04	0.02
rs206936	<i>NUDT3</i>	0.04	0.04	0.02

¹Linear regression models were used to assess the relationships between hair color (coded as blonde=1, light brown=2, dark brown=3, and black=4) and obesity-risk alleles, adjusting for study set and top three eigenvectors.

Table 3
The associations between unweighted genetic scores (per 10 BMI-risk alleles) and hair color.

	Not adjusted for BMI ⁵			Adjusted for BMI ⁶		
	Beta coefficient ⁷	StdErr	p-value	Beta coefficient ⁷	StdErr	p-value
Genetic score of independent loci ¹	0.12	0.03	0.00004	0.12	0.03	0.00003
Genetic score of independent genes ²	0.10	0.03	0.002	0.10	0.03	0.002
Reduced genetic score of independent loci ³	0.06	0.03	0.10	0.06	0.03	0.07
Reduced genetic score of independent genes ⁴	0.05	0.04	0.16	0.05	0.04	0.15

¹Generated by summing up dosage of obesity-risk alleles of SNPs in supplementary table 2. Mean = 36.43; Range = (23.05, 49.30).

²Generated by summing up dosage of obesity-risk alleles of SNPs in supplementary table 3. Mean = 29.19; Range = (18.05, 40.85).

³Generated by removing significant SNPs from the first score. Mean = 29.75; Range = (18.15, 40.90).

⁴Generated by removing significant SNPs from the second score. Mean = 25.01; Range = (13.15, 34.80).

⁵Linear regression model was used to assess the relationship between hair color (coded as blonde=1, light brown=2, dark brown=3, and black=4) and increase in 10 BMI-risk alleles, adjusting for study set and top three eigenvectors.

⁶Additionally adjusted for BMI.

⁷Beta coefficient > 0 means that higher genetic score is associated with darker hair color.

Table 4The quintiles of genetic score of independent loci and pigmentary phenotypes ¹

	Quintile1	2	3	4	5
No. of subjects	1118	1113	1120	1118	1114
Mean genetic score	31.29	34.54	36.45	38.34	41.57
Sd. of genetic score	1.74	0.6	0.49	0.6	1.74
Hair color	Ref	0.069 (0.003, 0.134)	0.105 (0.040, 0.170)	0.104 (0.038, 0.169)	0.143 (0.077, 0.208)
Beta coefficient ² (95% CI)					
P-value	Ref	0.04	0.002	0.002	0.00002
Tanning ability	Ref	-0.031 (-0.099, 0.038)	0.047 (-0.021, 0.115)	-0.010 (-0.078, 0.058)	0.037 (-0.032, 0.105)
Beta coefficient ² (95% CI)					
P-value	Ref	0.38	0.18	0.78	0.29

¹ All the analyses were conducted using linear regression models. Hair color was coded as blonde=1, light brown=2, dark brown=3, and black=4. Tanning ability was coded as practically none/light tan = 1, average tan=2, deep tan=3 in NHS; and pain:burn/peel=1, burn then tan=2; tan without burn=3 in HPFS.

² Beta coefficient > 0 means higher genetic score is associated with darker hair color or deeper tan.

Table 5

The associations between 5 independent SNPs in the *FTO* gene and the risk of melanoma [/]

SNP	Reference allele	Test allele	OR(95% CI)	P
rs12933928	A	G	0.85 (0.74–0.97)	0.01
rs12932428	C	T	0.88 (0.77–0.98)	0.02
rs1125338	T	C	0.89 (0.80–1.00)	0.04
rs12599672	T	A	1.17 (1.01–1.36)	0.03
rs12600192	C	G	1.19 (1.06–1.33)	0.004

[/]These analyses were conducted using logistic regression models among 2,830 samples in the MD Anderson melanoma case-control study, adjusting for top two eigenvectors.