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A variant in *FTO* shows association with melanoma risk not due to BMI

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We report the results of an association study of melanoma that is based on the genome-wide imputation of the genotypes of 1,353 cases and 3,566 controls of European origin conducted by the GenoMEL consortium. This revealed an association between several SNPs in intron 8 of the *FTO* gene, including rs16953002, which replicated using 12,313 cases and 55,667 controls of European ancestry from Europe, the USA and Australia (combined $P = 3.6 \times 10^{-12}$, per-allele odds ratio for allele A = 1.16). In addition to identifying a new melanoma-susceptibility locus, this is to our knowledge the first study to identify and replicate an association with SNPs in *FTO* not related to body mass index (BMI). These SNPs are not in intron 1 (the BMI-related region) and exhibit no association with BMI. This suggests *FTO*'s function may be broader than the existing paradigm that *FTO* variants influence multiple traits only through their associations with BMI and obesity.

Cutaneous melanoma is a disease predominantly of fair-skinned individuals. Established risk factors include a family history of melanoma[1], pigmentation phenotypes such as an inability to tan[2–5] and many melanocytic nevi[6,7]. Established genetic risk factors include rare, highly penetrant variants, at least 11 common variants of lower effect identified by genome-wide association studies (GWAS)[8,9] (many related to pigmentation or nevus count[10,11]) and mutations of intermediate effect in the *MITF* gene identified through a candidate-gene approach in indidviduals affected with melanoma and renal-cell carcinoma[12] and sequencing genomes of multiply affected melanoma families[13].

The *FTO* gene was first found to be associated with obesity in GWAS of type 2 diabetes[14] and obesity[15,16]. Most[14,17–21] but not all[22,23] studies found no association between *FTO* and type 2 diabetes risk after adjustment for BMI. The strongest associations were with variants in intron 1 of *FTO*, but linkage disequilibrium (LD) stretches across introns 1 and 2 and exon 2. No SNP outside intron 1 has been previously associated with any trait, and no SNP in intron 1 has been associated with any trait unrelated to BMI.

The GenoMEL consortium focuses on genetic susceptibility to melanoma and has conducted two melanoma GWAS (Phase 1 and Phase 2) using samples from populations of European or Israeli ancestry[9,11]. Genotypes of the 1,373 cases and 3,571 controls from Phase 1 of the GenoMEL GWAS of melanoma[9] were imputed, giving 2.6 million SNPs, each tested for association with melanoma risk using

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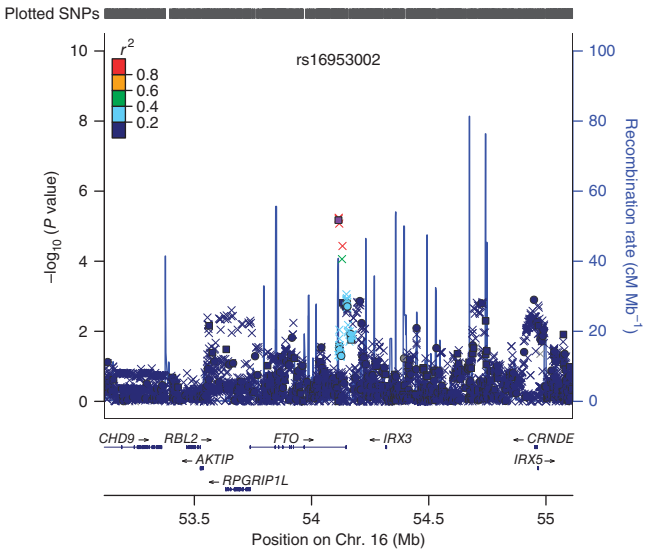

Figure 1 Results of stratified trend tests of imputed data for association with melanoma in region around *FTO* in GenoMEL Phase 1 and 2 data combined. $-\log_{10}$ *P* values for association between SNPs in the region of *FTO* and melanoma case-control status are shown adjusted for geographic region. Color of points indicates extent of LD with rs16953002 (indicated by purple square). SNPs genotyped in all GenoMEL samples are plotted as circles, SNPs imputed in all samples as crosses and SNPs genotyped in some samples and imputed in others (as a result of chip differences) as squares. Positions of genes are given under the graph, and estimated recombination rates given by the blue line along the bottom, with scale on the right. Plot was produced using LocusZoom[39].

geographic region as a covariate (Online Methods). The most significant SNP in a region not previously associated with melanoma was in *FTO*. Three SNPs in intron 8 of *FTO* were significant at $P < 10^{-5}$, the most significant being rs16953002 ($P = 5.59 \times 10^{-6}$, per-allele odds ratio (OR) = 1.33, risk allele A, risk allele frequency = 0.19) and rs12596638 ($P = 4.43 \times 10^{-6}$, per-allele OR = 1.34, risk allele A, risk allele frequency = 0.19; in strong LD, $r^2 = 0.96$). We confirmed imputation quality by subsequent genotyping (Online Methods).

Following this finding, we imputed a region 1 Mb either side of rs16953002 for 1,449 cases and 4,043 controls in GenoMEL melanoma GWAS Phase 2 (ref. 11) and regressed SNP dosage on melanoma case-control status with geographic region as a covariate. In this analysis, we genotyped rs16953002 ($P = 0.015$, OR = 1.16) and imputed rs12596638 ($P = 0.023$, OR = 1.15). Combining all GenoMEL GWAS data gave five SNPs within 18 kb with $P < 10^{-4}$ in intron 8 of *FTO* and over 250 kb from the closest SNP associated with BMI (**Fig. 1**).

We sought replication (mainly using existing GWAS data) using other samples of European ancestry from Europe, Australia and the United States, totaling 10,865 cases and 51,624 controls (**Supplementary Table 1**). All replication samples combined exhibited association between rs16953002 and melanoma with an allelic OR of 1.14, $P = 4.8 \times 10^{-9}$, with all sample sets showing OR estimates in the same direction as the original finding and with no evidence of heterogeneity. When we combined these data with the GenoMEL sample data, we observed strong evidence of association with melanoma: $P = 3.6 \times 10^{-12}$, per-allele OR = 1.16, 95% confidence interval (1.11, 1.20), and no evidence of heterogeneity ($I^2 = 0$; Online Methods, **Fig. 2** and **Table 1**).

BMI has, at best, a weak effect on risk of melanoma[24,25]. Given the clear association between variants in *FTO* and BMI, we investigated whether the melanoma-associated SNPs showed any association with BMI or, conversely, whether the known BMI-associated SNPs showed any association with melanoma.

BMI data were available for 37% of cases and 59% of controls (many of the GenoMEL samples and seven of the replication sets; **Supplementary Table 1**), with additional controls collected in Iceland to give 63,518 samples from Iceland with BMI data and 14,222 from elsewhere with BMI data. Adjusting log(BMI) for age and age-squared, and regressing this on SNP genotype, with case-control status and sex as covariates, there was no significant association between rs16953002 and BMI with a combined *P* value of 0.15 (**Supplementary Fig. 1**). A more powerful data set for assessing BMI-SNP associations is that of the GIANT consortium[26] (http://www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files). In the GIANT consortium data, allele A of rs16953002 was very weakly associated with decreased BMI ($P = 0.0156$ in 123,852 individuals, indicating at most a very small effect size).

In contrast, the genotyped SNP in the *FTO* region that was most strongly associated with BMI in the GenoMEL data was rs8050136 ($P = 8.7 \times 10^{-56}$ in all our data sets combined; **Supplementary Figs. 2** and **3**). In the GIANT data set this association with BMI reached $P = 1 \times 10^{-59}$.

We also found very little LD between the two SNPs ($r^2 = 0.000039$ in 35,583 Icelandic controls and $r^2 < 0.006$ in every other control set). In a recent study in which *FTO* was sequenced, only SNPs in intron 1 were associated with BMI[27]. It could be that the rs16953002-BMI association in the GIANT data is due to a very well-powered data set picking up on slight LD. The great difference between the strength of association between BMI at rs8050136 and at rs16953002 can clearly be seen in a plot of the GIANT results (**Supplementary Fig. 4**).

rs8050136 was not associated with melanoma, having a combined meta-analysis *P* value with GenoMEL of 0.19 (per-allele OR = 1.02; **Supplementary Fig. 5**). Therefore, from our data, the known BMI-related SNPs were associated with BMI but not with melanoma risk, and the melanoma-associated SNPs exhibited no evidence of association with BMI (**Table 1**). We also found no association between melanoma risk and adjusted BMI in the GenoMEL data ($P = 0.96$).

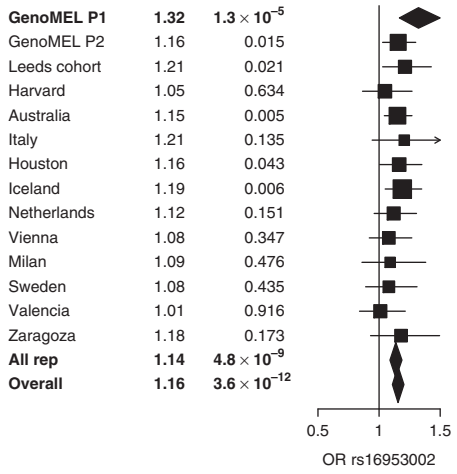

Figure 2 Forest plot of estimated per-allele ORs and *P* values for effect of rs16953002 on melanoma risk. Horizontal bars indicate 95% confidence intervals. Results are shown for GenoMEL Phase 1 discovery data and subsequent replication data with meta-analysis for replication data only (All rep) and all data (Overall).

Table 1 Association between rs16953002 and melanoma, and the BMI-related SNP rs8050136 and melanoma

		rs16953002 and melanoma			rs8050136 and melanoma		
	Minor allele frequency	Number of cases (controls)	OR (95% CI)	*P*	Number of cases (controls)	OR (95% CI)	*P*
GenoMEL Phase 1	0.16	1,353 (3,566)	1.32 (1.17, 1.50)	1.3×10^{-5}	1,353 (3,566)	1.09 (0.99, 1.19)	0.08
All replicates	0.17	12,314 (55,667)	1.14 (1.09, 1.19)	4.8×10^{-9}	11,707 (57,160)	1.01 (0.98, 1.05)	0.45
Overall	0.17	13,667 (59,233)	1.16 (1.11, 1.20)	3.6×10^{-12}	13,060 (60,726)	1.03 (0.97, 1.10)	0.37

The association between rs16953002 and melanoma risk was consistent across geographic regions (**Fig. 2** and **Supplementary Fig. 6**), and we found no significant difference in effect across subsets of the GenoMEL data defined by sex, tumor site, family history, early onset of disease and multiple primary tumors or association with any established melanoma-related trait (nevus count or sun sensitivity; data not shown). The association between rs16953002 and melanoma risk persisted in the subset of samples with BMI recorded even after adjusting for BMI ($P = 0.01$) despite a substantial reduction in sample size (**Supplementary Table 2** and **Supplementary Note**).

We split the GenoMEL data into quartiles defined by adjusted BMI of controls and regressed case/control status on rs16953002 with sex as a covariate in each quartile. The association was stronger for those samples in the first quartile (lowest BMI) than those in the other quartiles (OR = 1.66, $P = 3.00 \times 10^{-5}$ versus maximum OR = 1.03, minimum $P = 0.82$; **Supplementary Fig. 7**), a difference that was significant ($P = 0.0005$). This is consistent with rs16953002 only being associated with melanoma risk in those people with low BMI. When we attempted to replicate the results defining BMI quartiles in each population, samples collected in Australia exhibited a similar effect ($P = 0.003$), but samples collected in other countries outside of the UK gave more equivocal results (**Supplementary Fig. 7**; $P = 0.6$ for all replicate samples and $P = 0.06$ with GenoMEL samples included). However, in the nine replication studies for which BMI data were available, rs16953002 always had the greatest association with melanoma risk for those in quartile 1 or 2.

Although the functional effect(s) of *FTO* is far from understood, evidence points to a variety of possible effects on BMI-related traits. However, a loss-of-function mutation in *FTO* caused gross developmental defects in nine members of a Palestinian family, suggesting a broader function for *FTO*[28].

FTO has been associated with end-stage renal disease[29], acute coronary syndrome[30], myocardial infarction[31], all-cause mortality[32], Alzheimer's disease[33] and osteoarthritis[34]. Even after adjustment for BMI, some BMI-related traits exhibit association with *FTO* variants, but it may be that BMI simply correlates with a weight-related factor that acts more directly on the trait of interest. Given that BMI is a risk factor for many cancers, the BMI-related SNPs in intron 1 of *FTO* have been studied in some of these cancers. A study of lung, kidney and upper aero-digestive cancers revealed no significant effect overall after correction for multiple testing[35]. The largest study of *FTO* and endometrial cancer found an association with a known BMI-associated SNP ($P = 0.01$)[36] that disappears after adjustment for BMI.

Thus, there is little evidence of variants in *FTO* being associated with any trait unrelated to BMI. It may be that the melanoma-associated SNPs are in LD with functional SNPs outside of *FTO*, but given the low level of LD in the region (**Fig. 1**) this seems unlikely. It should be noted that our most significant SNP, rs16953002, is only 31 kb from exon 9 of *FTO*, over 146 kb from exon 8 of *FTO* and over 202 kb from the nearest other gene, *IRX3*. SNPs overlapping regulatory elements, such as transcription factor–binding sites, can be identified using the recent Encyclopedia of DNA Elements (ENCODE) data as well other data sources[37,38]. In these data for the *FTO* gene, 2,148 SNPs have been identified, only eight of which reach the highest score possible without expression quantitative trait locus (eQTL) data (score 2a: 'likely to affect binding'). Six of these SNPs are in intron 1, the location of most of the BMI-associated SNPs, five of these in a 5.4-kb region less than 1 kb from rs8050136. The other two SNPs are 13 kb apart from one another in intron 8 and, notably, one of these is rs16953002, the melanoma-associated SNP (**Supplementary Note**).

In conclusion, this is the first time to our knowledge that any variant in *FTO* has been shown to have a replicable association with a trait without being associated with BMI. It is also the first time that any variant in *FTO* outside intron 1 has been shown to have any association with any trait. As such, this will be of interest to researchers in the fields of both cancer genetics and obesity research.

URLs. GenoMEL, http://www.genomel.org/; Wellcome Trust Case Control Consortium, http://www.wtccc.org.uk/; RegulomeDB, http://regulomedb.org/; and Epidemiological Study on the Genetics and Environment of Asthma study, https://egeanet.vjf.inserm.fr/.

METHODS

Methods and any associated references are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

M.M.I. led, designed and carried out the statistical analysis and wrote the manuscript. M. Harland was involved in the Leeds replication genotyping design. J.C.T. carried out statistical analysis. H.S., J.R.-M., M.J., S. Mulder and N.v.d.S. carried out genotyping and contributed to the interpretation of genotyping data. B.B. contributed to the design of the GWAS and supervised processing of GWAS samples. J.A.N.B. led the overall consortium and contributed to study design. N.A.G. was deputy lead of the consortium and contributed to study design. D.T.B. and J.H.B. designed and led the overall study. N.K.H., S. MacGregor and M.H.L. led and carried out statistical analysis of the Australian replication data. K.S., S.N.S., P.S. and G.T. led and carried out statistical analysis of the Icelandic, Dutch, Viennese, Milanese, Valencian and Zaragozan replication data. J. Han carried out statistical analysis of the Harvard replication data. C.I.A. and S.F. led and carried out statistical analysis of the Houston replication data. M.T.L. and R.P. led and carried out statistical analysis of the Italian replication data. D.Z. and G.M.L. interpreted and contributed genotype data. A.M.G., P.A.K., E.M.G. and F.D. advised on statistical analysis. F.D. and G.M.L. contributed to the design of the study of the French component of GenoMEL. K.M.B. and D.E.E. contributed to the design of the GWAS. K.K.A., L.A.A., M.-F.A., E.A., K.R.B., W.B., G.B.S., D.C., V.C., M.C.F., A.E.C., A.C.d.W., T.D., E.F., P. Galan, P. Ghiorzo, J. Hansson, P.H., Marko Hočevar, V.H., J.L.H., C.I., M.A.J., L.A.K., J. Lang, S.L., J.E.L., J. Lubiński, R.M.M., G.J.M., N.G.M., J.I.M., A.M., E.N., S.N., I.O., J.H.O., H.O., H.P., K.P., M.P.G., D.P., S.P., J.A.P.-B., C.R., L.R., M.R., M.S., B.S., F.S., K.T., R.T., P.V.B., M.M.v.R., Q.W., J.W. and M.Z. contributed to the design and sample collection of either the initial GWAS or one of the replication studies.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Samples. Approval for these studies was obtained at each recruiting center. Informed consent was obtained from all participants.

Phase 1 of the original GenoMEL GWAS consisted of cases and controls collected from eight centers in six European countries. These were supplemented with controls from the Wellcome Trust Case Control Consortium (WTCCC)[19]. Standard quality-control measures were applied to both samples and SNPs, giving a total of 1,353 cases and 3,571 controls. Phase 2 of the GenoMEL GWAS consisted of cases and controls from ten centers (four not in Phase 1) in eight European countries and in Israel, supplemented again by samples from the WTCCC. In both phases, cases were preferentially selected to have a family history of melanoma, multiple primary tumors or an early age of onset. After quality control, 1,450 cases and 4,047 controls remained (quality control and samples are described in ref. 11). We obtained 680 supplementary UK cases and 1,785 controls from a population-based study of incident melanoma cases diagnosed between September 2000 and December 2006 from a geographically defined area of Yorkshire and the northern region of the UK[9,40,41]. Controls were ascertained by contacting general practitioners to identify eligible individuals. These controls were frequency-matched with cases for age and sex from general practitioners who also had cases as part of their patient register. An additional 220 controls were sex- and age-matched and from the same primary care practice as incident cases of colorectal cancer recruiting from hospitals in Leeds[42].

The only GenoMEL center that collected BMI data was Leeds. In Leeds, two studies were used: a family-based study that did not collect BMI and a case-control study that did collect BMI (see **Supplementary Table 1**).

For details of replication samples, see **Supplementary Note**.

Genotyping. Most GenoMEL Phase 1 samples were genotyped on the Illumina HumanHap300 BeadChip version 2 duo array (with 317,000 tagging SNPs), with the exception of the French cases, which were genotyped on the Illumina HumanCNV370k array. The GenoMEL Phase 2 samples were genotyped on the Illumina 610k array.

In the genotyping of the UK case-control samples, rs16953002 and rs12596638 were genotyped using the Taqman assays C__34511379_10 and C__11776446_10, respectively (Applied Biosystems). We performed 2-µl PCR in 384-well plates using 10 ng of DNA (dried), using 0.05 µl assay mix and 1 µl Universal Master Mix (Applied Biosystems) according to the manufacturer's instructions. End-point reading of the genotypes was performed using an ABI 7900HT Real-time PCR system (Applied Biosystems).

Imputation. Imputation was conducted genome-wide on the GenoMEL Phase 1 samples, excluding SNPs with minor allele frequency (MAF) < 0.03, Hardy-Weinberg equilibrium (HWE) $P < 10^{-4}$ (in controls) and missingness > 0.03. IMPUTEv2 (refs. 43,44) was used and the reference panel consisted of 120 European samples from HapMap release #24 (NCBI build36, November 2008). After the initial genome-wide imputation had identified the *FTO* region as a candidate region, additional imputation of this region (1 Mb either side of rs16953002, chromosome 16: 53114824–55114824) was conducted based on the 1000 Genomes Phase 1 integrated variant set (March 2012 release, excluding SNPs with MAF <0.001 in the CEU European samples). The number of well-imputed SNPs (Impute quality metric (info) score > 0.8) in the region increased from 1,245 to 4,874, although the most significant three SNPs remained the same. The first *P* values quoted for rs16953002 and rs12596638 ($P = 5.59 \times 10^{-6}$ and $P = 4.43 \times 10^{-6}$, respectively) were from the genome-wide imputation, but all subsequent analyses are based on the *FTO* regional imputation.

Imputed genotypes were analyzed as expected genotype counts based on the posterior probabilities (gene dosage) using SNPTEST2 (ref. 45) assuming an additive model with geographic region as a covariate. Only those with an 'info' score > 0.8 are considered to be of sufficient quality. The *FTO* region was imputed and analyzed in the GenoMEL Phase 2 data in the same way.

Imputation quality was confirmed by genotyping 3,694 of the previously imputed samples from GenoMEL Phase 1 at rs16953002. The imputed genotype with the highest posterior probability was correct in 97% of cases (increasing to 98% if we only consider those genotypes where the maximum posterior probability is > 0.8). Given this strong confirmation of the quality of the imputation, unless otherwise stated we present the result using the imputed Phase 1 results, rather than interleaving imputed and genotyped data indiscriminately. In the **Supplementary Note** and **Supplementary Table 2** results are presented using only genotyped data for comparison with the imputed results.

In the replication samples, rs16953002 and rs8050136 were genotyped, with the exception of rs8050136 being imputed in the samples collected at Harvard.

Meta-analysis. Meta-analyses assume fixed effects unless otherwise stated. In all cases, heterogeneity between studies is measured with the I^2 metric; it has been suggested that values below 31% are of "little concern" and those above 56% should induce "considerable caution"[46]. Where I^2 is >31%, a random-effects meta-analysis is applied. Here the method published in ref. 47 was used to estimate the between-studies variance, $\hat{\tau}^2$. An overall random effects estimate was then calculated using the weights $\hat{\tau}1/(v_i+\hat{\tau}^2)$, where v_i is the variance of the estimated effect. $\hat{\tau}^2 = 0$ for the fixed-effects analyses.

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