

Published in final edited form as:

Nat Genet. 2013 April ; 45(4): 428–432. doi:10.1038/ng.2571.

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

Mark M Iles¹, Matthew H Law², Simon N Stacey³, Jiali Han^{4,5,6}, Shenying Fang⁷, Ruth Pfeiffer⁸, Mark Harland¹, Stuart MacGregor², John C Taylor¹, Katja K Aben^{9,10}, Lars A Akslen^{11,12}, Marie-Françoise Avril¹³, Esther Azizi¹⁴, Bert Bakker¹⁵, Kristrun R Benediktsdottir^{16,17}, Wilma Bergman¹⁸, Giovanna Bianchi Scarrà¹⁹, Kevin M Brown²⁰, Donato Calista²¹, Valérie Chaudru^{22,23,24}, Maria Concetta Fagnoli²⁵, Anne E Cust²⁶, Florence Demenais^{22,27,24}, Anne C de Waal^{28,10}, Tadeusz Dębniak²⁹, David E Elder³⁰, Eitan Friedman³¹, Pilar Galan³², Paola Ghiorzo¹⁹, Elizabeth M Gillanders³³, Alisa M Goldstein⁸, Nelleke A Gruis¹⁸, Johan Hansson³⁴, Per Helsing³⁵, Marko Hočvar³⁶, Veronica Höiom³⁴, John L Hopper³⁷, Christian Ingvar³⁸, Marjolein Janssen³⁹, Mark A Jenkins³⁷, Peter A Kanetsky^{40,41}, Lambertus A Kiemeny^{9,10,42}, Julie Lang⁴³, G Mark Lathrop^{24,44}, Sancy Leachman⁴⁵, Jeffrey E Lee⁴⁶, Jan Lubiński²⁹, Rona M Mackie⁴⁷, Graham J Mann⁴⁸, Jose I Mayordomo⁴⁹, Anders Molven^{11,12}, Suzanne Mulder³⁹, Eduardo Nagore⁵⁰, Srdjan Novaković⁵¹, Ichiro Okamoto⁵², Jon H Olafsson⁵³, Håkan Olsson⁵⁴, Hubert Pehamberger⁵², Kitty Peris²⁵, Maria Pilar Grasa⁴⁹, Dolores Planelles⁵⁵, Susana Puig^{56,57}, Joan Anton Puig-Butille^{56,57}, Q-MEGA and AMFS investigators⁵⁸, Juliette Randerson-Moor¹, Celia Requena⁵⁰, Licia Rivoltini⁵⁹, Monica Rodolfo⁵⁹, Mario Santinami⁶⁰, Bardur Sigurgeirsson⁵³, Helen Snowden¹, Fengju Song^{4,61}, Patrick Sulem³, Kristin Thorisdottir⁵³, Rainer Tuominen³⁴, Patricia Van Belle⁶², Nienke van der Stoep¹⁵, Michelle M van Rossum²⁸, Qingyi Wei⁶³, Judith Wendt⁵², Diana Zelenika⁴⁴, Mingfeng Zhang⁴, Maria Teresa Landi⁸, Gudmar Thorleifsson³, D Timothy Bishop¹, Christopher I Amos⁷, Nicholas K Hayward², Kari Stefansson^{3,16}, Julia A Newton Bishop¹, and Jennifer H Barrett¹ on behalf of the GenoMEL Consortium⁶⁴

¹Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, Leeds Cancer Research UK Centre, St James's University Hospital, Leeds, UK ²Queensland Institute of Medical

⁵⁸A full list of members is in the Supplementary Note online

⁶⁴A full list of members is in the Supplementary Note online

Author Contributions

MMI led, designed and carried out the statistical analysis and wrote the manuscript.

MHarland was involved in the Leeds replication genotyping design

JCT carried out statistical analysis

HS, JR-M, MJ, SM and NvdS carried out genotyping and contributed to the interpretation of genotyping data

BB contributed to the design of the GWAS and supervised processing of GWAS samples

JANB led the overall consortium and contributed to study design

NAG was deputy lead of the consortium and contributed to study design

DTB and JHB designed and led the overall study

NKH, SMCg and MHL led and carried out statistical analysis of the Australian replication data

KS, SNS, PS and GT led and carried out statistical analysis of the Icelandic, Dutch, Viennese, Milanese, Valencian and Zaragoza replication data

JH carried out statistical analysis of the Harvard replication data

CIA and SF led and carried out statistical analysis of the Houston replication data

MTL and RP led and carried out statistical analysis of the Italian replication data

DZ and GML interpreted and contributed genotype data

AMG PAK EMG FD advised on statistical analysis

KMB DEE contributed to the design of the GWAS

All other authors contributed to the design and sample collection of either the initial GWAS or one of the replication studies

Research, Brisbane, QLD 4029, Australia ³deCODE Genetics, Reykjavik, Iceland ⁴Department of Dermatology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA ⁵Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA ⁶Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts, USA ⁷Department of Genetics, The University of Texas MD Anderson Cancer Center ⁸Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA ⁹Comprehensive Cancer Center The Netherlands, Nijmegen, The Netherlands ¹⁰Department of Epidemiology, Biostatistics and HTA, Radboud University Medical Centre, Nijmegen, The Netherlands ¹¹The Gade Institute, University of Bergen, Bergen, Norway ¹²Department of Pathology, Haukeland University Hospital, Bergen, Norway ¹³Assistance Publique–Hôpitaux de Paris, Hôpital Cochin, Service de Dermatologie, Université Paris Descartes, Paris, France ¹⁴Department of Dermatology and the Oncogenetics Unit, Sheba Medical Center, Tel Hashomer, Sackler Faculty of Medicine, Tel Aviv, Israel ¹⁵Department of Clinical Genetics, Center of Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands ¹⁶Faculty of Medicine, University of Iceland, Reykjavik, Iceland ¹⁷Department of Pathology, Landspítali-University Hospital, Reykjavik, Iceland ¹⁸Department of Dermatology, Leiden University Medical Centre, Leiden, The Netherlands ¹⁹Department of Internal Medicine and Medical Specialties, University of Genoa; Laboratory of Genetics of Rare Hereditary Cancers, San Martino-IST Research Hospital, Genoa ²⁰Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Gaithersburg, Maryland, USA ²¹Dermatology Unit, Maurizio Bufalini Hospital, Cesena, Italy ²²INSERM, UMR-946, Genetic Variation and Human Diseases Unit, Paris, France ²³Université d'Evry Val d'Essonne, Evry, France ²⁴Fondation Jean Dausset, Centre d'Etude du Polymorphisme Humain (CEPH), Paris, France ²⁵Department of Dermatology, University of L'Aquila, L'Aquila, Italy ²⁶Cancer Epidemiology and Services Research, Sydney School of Public Health, The University of Sydney, Australia ²⁷Université Paris Diderot, Sorbonne Paris Cité, Institut Universitaire d'Hématologie, Paris, France ²⁸Department of Dermatology, Radboud University Medical Centre, Nijmegen, The Netherlands ²⁹International Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland ³⁰Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA ³¹Oncogenetics Unit, Sheba Medical Center, Tel Hashomer, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel ³²Research Unit on Nutritional Epidemiology; Université Paris 13, Sorbonne Paris Cité, INSERM (U557), INRA (U1125), CNAM; F-93017, Bobigny, France ³³Inherited Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Baltimore, Maryland, USA ³⁴Department of Oncology-Pathology, Karolinska Institutet, Karolinska University Hospital, Solna, S-171 76 Stockholm, Sweden ³⁵Department of Dermatology, Oslo University Hospital, Rikshospitalet, N-0027 Oslo, Norway ³⁶Department of Surgical Oncology, Institute of Oncology Ljubljana, Zaloška 2, 1000 Ljubljana ³⁷Centre for Molecular, Environmental, Genetic and Analytic (MEGA) Epidemiology, Melbourne School of Population Health, University of Melbourne, Melbourne, Australia ³⁸Department of Surgery, Clinical Sciences, Lund University ³⁹ServiceXS B.V., Leiden, The Netherlands ⁴⁰Centre for Clinical Epidemiology & Biostatistics, University of Pennsylvania, Philadelphia, Pennsylvania, USA ⁴¹Department of Biostatistics & Epidemiology, University of Pennsylvania, Philadelphia, Pennsylvania, USA ⁴²Department of Urology, Radboud University Medical Centre, Nijmegen, The Netherlands ⁴³Department of Medical Genetics, University of Glasgow, UK ⁴⁴Commissariat à l'Énergie Atomique, Institut de Génomique, Centre National de Génotypage, Evry, France ⁴⁵Department of Dermatology, University of Utah School of Medicine, Huntsman Cancer Institute, Salt Lake City, Utah, USA ⁴⁶Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center ⁴⁷Department of Public Health, University of Glasgow, UK ⁴⁸Westmead Institute of Cancer Research, University of Sydney at Westmead Millennium Institute and Melanoma Institute Australia, Sydney, Australia ⁴⁹University of Zaragoza. 50009 Zaragoza, Spain

⁵⁰Department of Dermatology, Instituto Valenciano de Oncología, Valencia, Spain ⁵¹Department of Molecular Diagnostics, Institute of Oncology Ljubljana, Zaloška 2, 1000 Ljubljana ⁵²Department of Dermatology, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria ⁵³Department of Dermatology, Faculty of Medicine, University of Iceland, Reykjavik, Iceland ⁵⁴Departments of Oncology and Cancer Epidemiology, Clinical Sciences, Lund University ⁵⁵Laboratory of Histocompatibility-Molecular Biology, Center for Blood Transfusion, Valencia, Spain ⁵⁶Melanoma Unit, Dermatology Department, Hospital Clinic, Institut de Investigació Biomedicè August Pi Suñe, Universitat de Barcelona, Barcelona, Spain ⁵⁷CIBER de Enfermedades Raras, Instituto de Salud Carlos III, Barcelona, Spain ⁵⁹Immunotherapy of Human Tumors Unit, Department of Experimental Oncology, Fondazione IRCCS Istituto Nazionale Tumori, via Venezian 1, 20133 Milan, Italy ⁶⁰Melanoma and Sarcoma Surgery Unit, Fondazione IRCCS Istituto Nazionale Tumori, via Venezian 1, 20133 Milan, Italy ⁶¹Department of Epidemiology, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China ⁶²University of Pennsylvania Department of Pathology & Laboratory Medicine ⁶³Department of Epidemiology, The University of Texas MD Anderson Cancer Center

Abstract

We report the results of an association study of melanoma 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 a novel association between several single nucleotide polymorphisms (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 OR for A=1.16). As well as identifying a novel melanoma susceptibility locus, this is 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 show 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 melanoma¹, pigmentation phenotypes such as inability to tan²⁻⁵ and number of melanocytic naevi^{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 naevus count^{10,11}) and mutations of intermediate effect in the *MITF* gene identified through sequencing multiple-affected melanoma families^{12,13}.

The *FTO* gene was first found to be associated with obesity in GWAS of type 2 diabetes¹⁴ 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 previously been associated with any trait and no SNP within 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 melanoma⁹ were imputed, giving 2.6M SNPs, each tested for association with melanoma risk using geographic region as a covariate (see Online Methods). The most significant SNP in a region not previously associated with melanoma

was in *FTO*. Three SNPs in intron 8 of the gene *FTO* were significant at $p < 10^{-5}$, the most significant being rs16953002 ($p = 5.59 \times 10^{-6}$, per-allele OR=1.33, risk allele A, risk allele frequency=0.19) and rs12596638 ($p = 4.431 \times 10^{-6}$, per-allele OR=1.34, risk allele A, risk allele frequency=0.19) (in strong LD: $r^2 = 0.96$). Imputation quality was confirmed by subsequent genotyping (see Online Methods).

Following this finding, a region 1Mb either side of rs16953002 was imputed for 1,449 cases and 4,043 controls in GenoMEL melanoma GWAS Phase 2¹¹ and SNP dosage regressed on melanoma case-control status with geographic region as a covariate. In this analysis rs16953002 was genotyped ($p = 0.015$, OR=1.16) and rs12596638 imputed ($p = 0.023$, OR=1.15). Combining all GenoMEL GWAS gave 5 SNPs within 18kb with $p < 10^{-4}$ in intron 8 of *FTO* and over 250 kb from the closest SNP associated with BMI (Fig. 1).

Replication (mainly using existing GWAS data) was sought using other samples of European ancestry from Europe, Australia and the USA, totalling 10,865 cases and 51,624 controls (Supplementary Table 1). Combined, the replication samples showed 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. Combined with the GenoMEL samples, strong evidence of association with melanoma was observed: $p = 3.6 \times 10^{-12}$, per-allele OR=1.16, 95% confidence interval (1.11, 1.20), $I^2 = 0$ (Fig 2).

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 7 replication sets) (Supplementary Table 1), with additional Icelandic controls to give 63,518 Icelandic samples with BMI and 14,222 from elsewhere with BMI. Adjusting log(BMI) for age and age² 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 $p = 0.15$ (Supplementary Fig. 1). A more powerful dataset for assessing BMI-SNP associations is that of the GIANT consortium²⁶ (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 most strongly associated with BMI in the GenoMEL data was rs8050136 ($p = 8.7 \times 10^{-56}$ in all our datasets combined, Supplementary Fig. 2 and Supplementary Fig. 3). In the GIANT dataset this association with BMI reaches $p = 1 \times 10^{-59}$.

We also find very little LD between the two SNPs ($r^2 = 0.000039$ in 35,583 Icelandic controls and < 0.006 in every other control set). In a recent study that sequenced *FTO*, only SNPs in intron 1 were associated with BMI²⁷. It could be that the rs16953002-BMI association in the GIANT data is due to a very well-powered dataset 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 are associated with BMI, but not with melanoma risk,

and the melanoma-associated SNPs show no evidence of association with BMI. We also found no association between melanoma risk and adjusted BMI in the GenoMEL data ($p=0.96$).

The association between rs16953002 and melanoma risk is 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, tumour site, family history, early onset of disease and multiple primary tumours or association with any established melanoma-related trait (naevus count, sun sensitivity) (data not shown). The association between rs16953002 and melanoma risk persists 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 Methods).

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 max OR=1.03, min $p=0.82$) (Supplementary Fig. 7), a difference that is significant ($p=0.0005$). This is consistent with rs16953002 only being associated with melanoma risk in those people with low BMI. Attempting to replicate the results, defining BMI quartiles within each population, the Australian data showed a similar effect ($p=0.003$), but other non-UK replication sets 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 9 replication studies for which BMI data were available, rs16953002 always had the greatest association with melanoma risk for those in quartile 1 or 2.

While 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 disease²⁹, acute coronary syndrome³⁰, myocardial infarction³¹, all-cause mortality³², Alzheimer's disease³³ and osteoarthritis³⁴. Even after adjustment for BMI, some BMI-related traits show association with *FTO* variants, but it may be BMI simply correlates with a weight-related factor acting 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 a number of cancers. A study of lung, kidney and upper aero-digestive cancers showed no significant effect overall after correction for multiple testing³⁵. 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 31kb from exon 9 of *FTO*, over 146kb from exon 8 of *FTO* and over 202kb from the nearest other gene, *IRX3*. SNPs overlapping regulatory elements, such as transcription factor binding sites can be identified using the recent ENCODE data as well other data sources^{37,38}. Looking up the *FTO* gene, 2,148 SNPs are identified, only 8 of which reach the highest score possible without eQTL data (score 2a - "Likely to affect binding"). Six of these are in intron 1, the location of most of the BMI-associated SNPs, 5 of these in a 5.4kb region less than 1kb from rs8050136. The other two SNPs are 13kb apart from one another in intron 8 and, interestingly, one of these

is rs16953002, the melanoma-associated SNP (see Supplementary material for further details).

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/>

Methods

Samples

Phase 1 of the original GenoMEL GWAS consisted of cases and controls collected from 8 centres across 6 different European countries. These were supplemented with controls from the Wellcome Trust Case Control Consortium (WTCCC)¹⁹. Standard quality control (QC) 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 10 centres (4 not in Phase 1) in 8 different European countries and Israel, supplemented again by samples from the WTCCC. In both phases cases were preferentially selected to have a family history of melanoma, multiple primary tumours or an early age of onset. After QC 1,450 cases and 4,047 controls remained (see ¹¹ for details of QC and samples). 680 supplementary UK cases and 1,785 controls were obtained 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 had also had cases as part of their patient register. A further 220 controls were sex- and age-matched and from the same primary care practice as incident cases of colorectal cancer recruiting from hospitals in Leeds⁴².

The only GenoMEL centre that collected BMI data was Leeds. Within 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 317k 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). 2ul PCR reactions were performed in 384 well plates using 10ng of DNA (dried), using 0.05 ul assay mix and 1ul 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 $MAF < 0.03$, HWE $p\text{-value} < 10^{-4}$ (in controls) and missingness > 0.03 . IMPUTEv2^{43,44} was used and the reference panel consisted of 120 European samples from HapMap release #24 (NCBI build36, Nov 2008). After the initial genome-wide imputation had identified the FTO region as a candidate region, further imputation of this region (1Mb either side of rs16953002, Chr16: 53,114,824–55,114,824) 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 (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 analysed as expected genotype counts based on the posterior probabilities (gene dosage) using SNPTEST⁴⁵ 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 analysed 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 (rising 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 Material 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 Harvard samples.

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 of Dersimonian and Laird⁴⁷ was used to estimate the between-studies variance, τ^2 . An overall random effects estimate was then calculated using the weights $1/(v_j + \tau^2)$ where v_j is the variance of the estimated effect. $\tau^2 = 0$ for the fixed effects analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Thanks to Mark I. McCarthy and Cecilia M. Lindgren for assistance with the results of the GIANT study.

References

1. Cannon-Albright LA, Bishop DT, Goldgar C, Skolnick MH. Genetic predisposition to cancer. Important Adv Oncol. 1991:39–55. [PubMed: 1869281]

2. Naldi L, et al. Cutaneous malignant melanoma in women. Phenotypic characteristics, sun exposure, and hormonal factors: a case-control study from Italy. *Ann Epidemiol.* 2005; 15:545–550. [PubMed: 16029848]
3. Titus-Ernstoff L, et al. Pigmentary characteristics and moles in relation to melanoma risk. *Int J Cancer.* 2005; 116:144–149. [PubMed: 15761869]
4. Holly EA, Aston DA, Cress RD, Ahn DK, Kristiansen JJ. Cutaneous melanoma in women. I Exposure to sunlight, ability to tan, and other risk factors related to ultraviolet light. *Am J Epidemiol.* 1995; 141:923–933. [PubMed: 7741122]
5. Holly EA, Aston DA, Cress RD, Ahn DK, Kristiansen JJ. Cutaneous melanoma in women. II Phenotypic characteristics and other host-related factors. *Am J Epidemiol.* 1995; 141:934–942. [PubMed: 7741123]
6. Bataille V, et al. Risk of cutaneous melanoma in relation to the numbers, types and sites of naevi: a case-control study. *Br J Cancer.* 1996; 73:1605–1611. [PubMed: 8664138]
7. Chang YM, et al. A pooled analysis of melanocytic nevus phenotype and the risk of cutaneous melanoma at different latitudes. *Int J Cancer.* 2009; 124:420–428. [PubMed: 18792098]
8. Brown KM, et al. Common sequence variants on 20q11.22 confer melanoma susceptibility. *Nat Genet.* 2008; 40:838–840. [PubMed: 18488026]
9. Bishop DT, et al. Genome-wide association study identifies three loci associated with melanoma risk. *Nat Genet.* 2009; 41:920–925. [PubMed: 19578364]
10. Gubjartsson DF, et al. ASIP and TYR pigmentation variants associate with cutaneous melanoma and basal cell carcinoma. *Nat Genet.* 2008; 40:886–891. [PubMed: 18488027]
11. Barrett JH, et al. Genome-wide association study identifies three new melanoma susceptibility loci. *Nat Genet.* 2011; 43:1108–1113. [PubMed: 21983787]
12. Bertolotto C, et al. A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. *Nature.* 2011; 480:94–98. [PubMed: 22012259]
13. Yokoyama S, et al. A novel recurrent mutation in MITF predisposes to familial and sporadic melanoma. *Nature.* 2011; 480:99–103. [PubMed: 22080950]
14. Frayling TM, et al. A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science.* 2007; 316:889–894. [PubMed: 17434869]
15. Dina C, et al. Variation in *FTO* contributes to childhood obesity and severe adult obesity. *Nat Genet.* 2007; 39:724–726. [PubMed: 17496892]
16. Scuteri A, et al. Genome-wide association scan shows genetic variants in the *FTO* gene are associated with obesity-related traits. *PLoS Genet.* 2007; 3:e115. [PubMed: 17658951]
17. Zeggini E, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science.* 2007; 316:1336–1341. [PubMed: 17463249]
18. Scott LJ, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science.* 2007; 316:1341–1345. [PubMed: 17463248]
19. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature.* 2007; 447:661–678. [PubMed: 17554300]
20. Zeggini E, et al. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet.* 2008; 40:638–645. [PubMed: 18372903]
21. Voight BF, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat Genet.* 2010; 42:579–589. [PubMed: 20581827]
22. Hertel JK, et al. *FTO*, type 2 diabetes, and weight gain throughout adult life: a meta-analysis of 41,504 subjects from the Scandinavian HUNT, MDC, and MPP studies. *Diabetes.* 2011; 60:1637–1644. [PubMed: 21398525]
23. Li H, et al. Association of genetic variation in *FTO* with risk of obesity and type 2 diabetes with data from 96,551 East and South Asians. *Diabetologia.* 2012; 55:981–995. [PubMed: 22109280]
24. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet.* 2008; 371:569–578. [PubMed: 18280327]

25. Pothiwala S, Qureshi AA, Li Y, Han J. Obesity and the incidence of skin cancer in US Caucasians. *Cancer Causes Control*. 2012; 23:717–726. [PubMed: 22450736]
26. Speliotes EK, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet*. 2010; 42:937–948. [PubMed: 20935630]
27. Sällman Almén M, et al. Determination of the obesity-associated gene variants within the entire *FTO* gene by ultra-deep targeted sequencing in obese and lean children. *Int J Obes (Lond)*. 2012 in press.
28. Boissel S, et al. Loss-of-function mutation in the dioxygenase-encoding *FTO* gene causes severe growth retardation and multiple malformations. *Am J Hum Genet*. 2009; 85:106–111. [PubMed: 19559399]
29. Hubacek JA, et al. The *FTO* gene polymorphism is associated with end-stage renal disease: two large independent case-control studies in a general population. *Nephrol Dial Transplant*. 2012; 27:1030–1035. [PubMed: 21788373]
30. Hubacek JA, et al. A *FTO* variant and risk of acute coronary syndrome. *Clin Chim Acta*. 2010; 411:1069–1072. [PubMed: 20362563]
31. Doney AS, et al. The *FTO* gene is associated with an atherogenic lipid profile and myocardial infarction in patients with type 2 diabetes: a Genetics of Diabetes Audit and Research Study in Tayside Scotland (Go-DARTS) study. *Circ Cardiovasc Genet*. 2009; 2:255–259. [PubMed: 20031593]
32. Zimmermann E, et al. Fatness-associated *FTO* gene variant increases mortality independent of fatness—in cohorts of Danish men. *PLoS One*. 2009; 4:e4428. [PubMed: 19214238]
33. Keller L, et al. The obesity related gene, *FTO*, interacts with *APOE*, and is associated with Alzheimer’s disease risk: a prospective cohort study. *J Alzheimers Dis*. 2011; 23:461–469. [PubMed: 21098976]
34. arcOGEN Consortium and arcOGEN Collaborators. Identification of new susceptibility loci for osteoarthritis (arcOGEN): a genome-wide association study. *Lancet*. 2012; 380:815–823. [PubMed: 22763110]
35. Brennan P, et al. Obesity and cancer: Mendelian randomization approach utilizing the *FTO* genotype. *Int J Epidemiol*. 2009; 38:971–975. [PubMed: 19542184]
36. Lurie G, et al. The obesity-associated polymorphisms *FTO* rs9939609 and *MC4R* rs17782313 and endometrial cancer risk in non-Hispanic white women. *PLoS One*. 2011; 6:e16756. [PubMed: 21347432]
37. Boyle AP, et al. Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res*. 2012; 22:1790–1797. [PubMed: 22955989]
38. Schaub MA, et al. Linking disease associations with regulatory information in the human genome. *Genome Res*. 2012; 22:1748–1759. [PubMed: 22955986]
39. Pruim RJ, et al. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics*. 2010; 26:2336–2337. [PubMed: 20634204]
40. Newton-Bishop JA, et al. Melanocytic nevi, nevus genes, and melanoma risk in a large case-control study in the United Kingdom. *Cancer Epidemiol Biomarkers Prev*. 2010; 19:2043–2054. [PubMed: 20647408]
41. Newton-Bishop JA, et al. Relationship between sun exposure and melanoma risk for tumours in different body sites in a large case-control study in a temperate climate. *Eur J Cancer*. 2011; 47:732–741. [PubMed: 21084183]
42. Barrett JH, et al. Investigation of interaction between N-acetyltransferase 2 and heterocyclic amines as potential risk factors for colorectal cancer. *Carcinogenesis*. 2003; 24:275–282. [PubMed: 12584178]
43. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies via imputation of genotypes. *Nat Genet*. 2007; 39:906–913. [PubMed: 17572673]
44. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet*. 2009; 5:e1000529. [PubMed: 19543373]

45. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies via imputation of genotypes. *Nat Genet.* 2007; 39:906–913. [PubMed: 17572673]
46. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med.* 2002; 21:1539–1558. [PubMed: 12111919]
47. Dersimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986; 7:177–188. [PubMed: 3802833]

Appendix

The GenoMEL study was funded by the European Commission under the 6th Framework Programme (contract no. LSHC-CT-2006-018702), by Cancer Research UK Programme Awards (C588/A4994 and C588/A10589), by a Cancer Research UK Project Grant (C8216/A6129) and by a grant from the Us National Institutes of Health (NIH; CA83115). This research was also supported by the intramural Research Program of the NIH, National Cancer Institute (NCI), Division of Cancer Epidemiology and Genetics.

This study makes use of data generated by the Wellcome Trust Case Control Consortium. A full list of the investigators who contributed to the generation of the data is available from their website (see URLs). Funding for the project was provided by the Wellcome Trust under award 076113

The authors thank the Epidemiological Study on the Genetics and Environment of Asthma (EGEA) cooperative group for giving access to data of the EGEA study. We acknowledge that the biological specimens of the French Familial Melanoma Study Group were obtained from the Institut Gustave Roussy and Fondation Jean Dausset–CEPH Biobanks.

Funding specific to particular centres is given below:

Stockholm: Swedish Cancer Society, Karolinska Institutet's research funds

Lund: Funding to be acknowledged; Swedish Cancer Society, Gunnar Nilsson Foundation.

Genoa: Italian Ministry of Education, University and Research PRIN 2008, IMI and Mara Naum foundation.

Paris: Grants from Institut National du Cancer (INCa-PL016) and Ligue Nationale Contre Le Cancer (PRE05/FD and PRE 09/FD) to F. Demenais, Programme Hospitalier de Recherche Clinique (AOM-07-195) to M.-F. Avril and F. Demenais.

Leiden: Grant provided by European Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) Netherlands hub (CO18).

Spain: The research at the Melanoma Unit in Barcelona is partially funded by Grants from Fondo de Investigaciones Sanitarias P.I. 09/01393, Spain; by the CIBER de Enfermedades Raras of the Instituto de Salud Carlos III, Spain; by the AGAUR 2009 SGR 1337 of the Catalan Government, Spain.

Norway: Grants from the Comprehensive Cancer Center, Oslo University Hospital (SE0728) and the Norwegian Cancer Society (71512-PR-2006-0356).

Vienna: Supported by the *Jubiläumsfonds* of the *Österreichische Nationalbank* (project numbers 12161 and 13036) and the *Hans und Blanca Moser Stiftung*.

Italy replication: The Italian study was partially supported by an RO1 grant CA65558-02 (to M. T. Landi) from the National Institutes of Health (NIH), Department of Health and Human Services (DHHS) and by the Intramural Research Program of NIH, National Cancer Institute, Division of Cancer Epidemiology and Genetics.

Houston (M.D. Anderson): Support by the NIH/NCI (2P50CA093459), and by the Marit Peterson Fund for Melanoma Research.

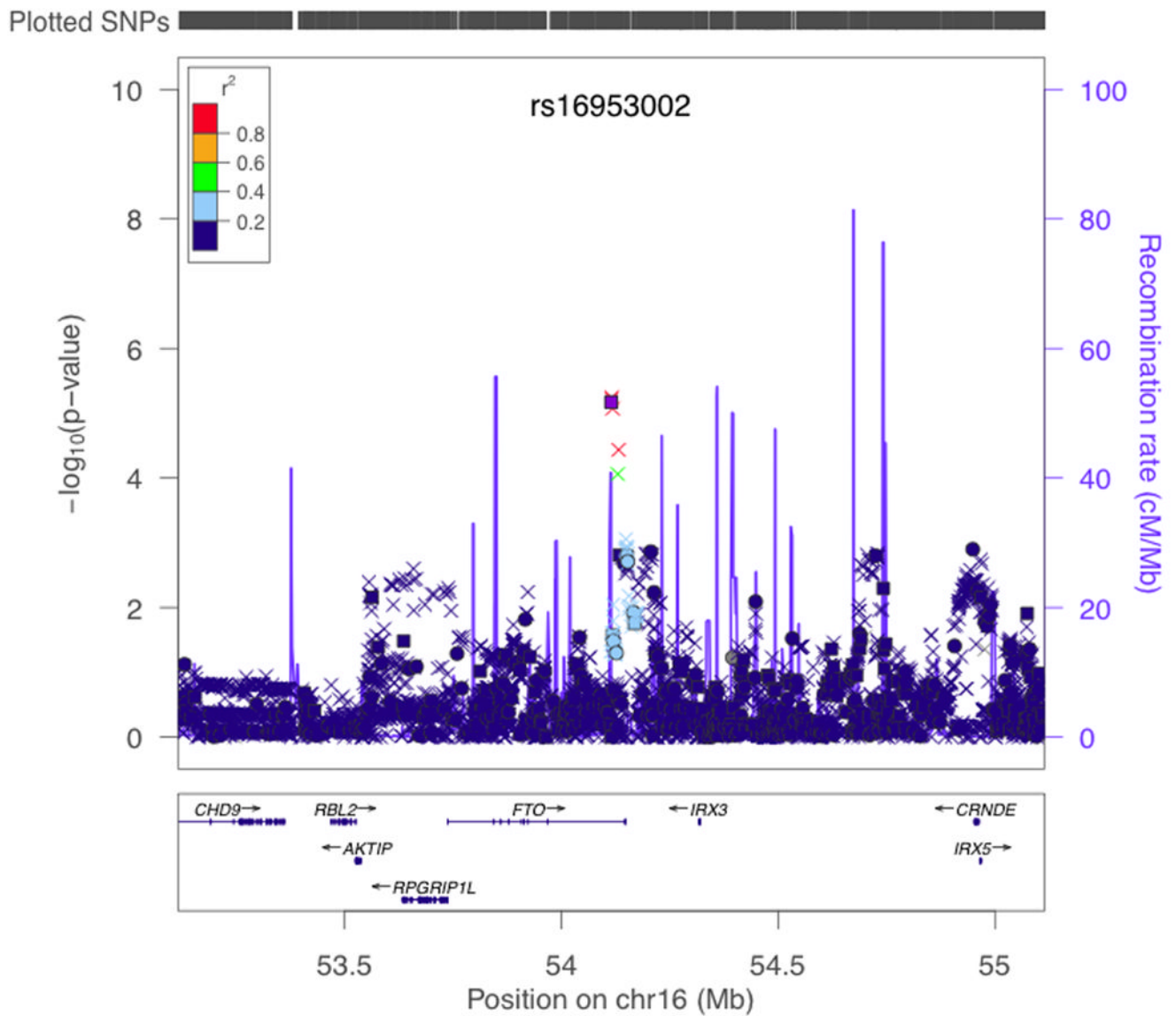


Figure 1.

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. Colour of points indicates degree 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 underneath the graph and estimated recombination rates also given by the blue line along the bottom, with scale on the right hand axis. Plot produced using LocusZoom³⁹.

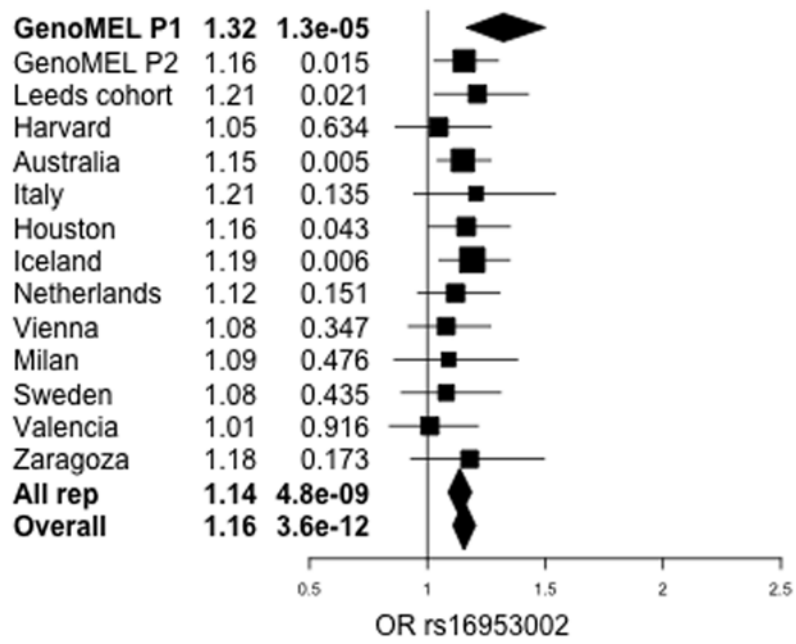


Figure 2. Forest plot of estimated per-allele ORs and p-values for effect of rs16953002 on melanoma risk. Horizontal bars indicate 95% confidence intervals. Results shown for GenoMEL Phase 1 discovery data and subsequent replication data with meta-analysis for replication data only and all data.

Table 1

Results of association between rs16953002 and melanoma and rs8050136 and melanoma

	MAF	rs16953002 and melanoma			rs8050136 and melanoma		
		No of cases/controls	OR (95% CI)	P	No of cases/controls	OR (95% CI)	P
GenoMEL Phase I	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