Common genetic variation near *MC4R* is associated with waist circumference and insulin resistance

John C Chambers[1,6], Paul Elliott[1,6], Delilah Zabaneh[1], Weihua Zhang[1], Yun Li [2], Philippe Froguel[3,4], David Balding[1], James Scott[5] & Jaspal S Kooner[5]

We carried out a genome-wide association study (318,237 SNPs) for insulin resistance and related phenotypes in 2,684 Indian Asians, with further testing in 11,955 individuals of Indian Asian or European ancestry. We found associations of rs12970134 near *MC4R* with waist circumference ($P = 1.7 \times 10^{-9}$) and, independently, with insulin resistance. Homozygotes for the risk allele of rs12970134 have ~2 cm increased waist circumference. Common genetic variation near *MC4R* is associated with risk of adiposity and insulin resistance.

Indian Asians comprise one-quarter of the world's population and are projected to account for ~40% of the global cardiovascular disease burden by 2020 (ref. 1). Increased cardiovascular disease risk in Indian Asians is closely associated with a high prevalence of insulin resistance and related metabolic disturbances (central adiposity, raised triglycerides, low high-density lipoprotein (HDL) cholesterol and type 2 diabetes)[2]. The reasons underlying increased insulin resistance among Indian Asians are not well understood; family studies implicate an important genetic component[3]. We carried out a two-stage genome-wide association study of insulin resistance and related phenotypes in a population cohort of individuals from the UK of Indian Asian or European ancestry.

In stage one, we genotyped 318,237 SNPs in 2,684 Indian Asian men aged 35–75 years from the London Life Sciences Population (LOLIPOP) study using the Illumina Hap300 BeadChip (**Supplementary Methods** and **Supplementary Table 1** online). Indian Asians were selected if all four grandparents originated from the Indian subcontinent. We carried out single SNP marker tests for association with ten insulin resistance and related phenotypes: homeostasis model assessment of insulin resistance (HOMA-IR)[4], waist circumference and waist-hip ratio (as measures of central adiposity), weight, body mass index, diastolic blood pressure, triglycerides, HDL cholesterol, type 2 diabetes and composite metabolic syndrome (defined by Adult Treatment Panel III criteria[5]). We used principal component analysis to control for population structure (**Supplementary Methods**)[6]. We identified 31 SNPs associated with one or more of the above phenotypes at $P < 10^{-5}$ (**Supplementary Tables 2** and **3** online); eight were in well-described loci known to influence HDL cholesterol and triglyceride levels (*CETP, GCKR* and *LPL*) and were not tested further[7,8]. We carried forward the remaining 23 SNPs for further testing in stage two, of which 22 were successfully genotyped in 11,955 men and women (7,394 Indian Asian individuals and 4,561 individuals of European ancestry; **Supplementary Table 1**).

In stage two and the combined analysis, linear regression analyses were done under an additive genetic model with age, sex and ethnicity as covariates; there was no evidence for heterogeneity between the ethnic groups. For each of the 22 genotyped SNPs, we selected the phenotype with the most significant association in stage one for primary testing in stage two data, adopting a conservative threshold of $P < 0.002$ for statistical significance on the basis of Bonferroni correction for 22 SNPs. We confirmed significant association of SNPs rs12970134, rs4450508, rs477181 and rs502933

Table 1 SNPs showing confirmed association with waist circumference in stage two and the combined analysis

SNP	Chr.	Position	Nearest gene	Alleles	Risk allele	Stage one effect size in cm (95% CI)	*P*	Stage two effect size in cm (95% CI)	*P*	Combined effect size in cm (95% CI)	*P*
rs12970134	18	56035730	*MC4R*	AG	A	1.48 (0.84, 2.11)	4.6×10^{-6}	0.74 (0.42, 1.07)	5.5×10^{-6}	0.88 (0.59, 1.17)	1.7×10^{-9}
rs477181	18	56047018	*MC4R*	TG	G	1.23 (0.54, 1.92)	7.0×10^{-6}	0.56 (0.24, 0.87)	2.3×10^{-4}	0.68 (0.40, 0.97)	8.1×10^{-7}
rs502933	18	56047454	*MC4R*	AC	C	1.23 (0.54, 1.93)	6.6×10^{-6}	0.58 (0.26, 0.89)	1.4×10^{-4}	0.70 (0.42, 0.99)	4.4×10^{-7}
rs4450508	18	56064414	*MC4R*	AG	A	1.39 (0.79, 2.00)	6.5×10^{-6}	0.64 (0.34, 0.95)	3.8×10^{-5}	0.79 (0.52, 1.07)	1.8×10^{-8}

Genomic context of the SNPs selected for further testing and their association test results against waist circumference (most strongly associated phenotype) in the stage one genome-wide association study ($n = 2,684$), the stage two study ($n = 11,955$) and the combined analysis of stage one and two data ($n = 14,639$). Effect sizes are shown as change in waist circumference per copy of high-risk allele (95% confidence interval), as calculated by linear regression analysis, assuming an additive genetic model, with adjustment for age in stage one, and for age, gender and ethnic group in stage two and in the combined analysis.

Table 2 Association of SNP rs12970134 (near *MC4R*) with insulin resistance and related phenotypes in stage two participants

	Indian Asian ancestry ($n = 7{,}394$)		European ancestry ($n = 4{,}561$)		All stage two participants ($n = 11{,}955$)		
Phenotype	Effect size or OR (95% CI)	*P*	Effect size or OR (95% CI)	*P*	Effect size or OR (95% CI)	*P*	Heterogeneity *P*
HOMA-IR (%)	5.39 (2.78, 8.07)	4.2×10^{-5}	5.16 (1.18, 9.29)	0.01	5.17 (2.96, 7.42)	3.2×10^{-6}	0.95
Waist-hip ratio	0.004 (0.002, 0.006)	9.8×10^{-4}	0.004 (0.000, 0.007)	0.02	0.004 (0.002, 0.006)	7.7×10^{-5}	0.87
Weight (kg)	0.82 (0.40, 1.24)	1.3×10^{-4}	1.21 (0.48, 1.94)	0.001	0.93 (0.56, 1.31)	1.0×10^{-6}	0.37
Body mass index (kg/m^2)	0.25 (0.10, 0.39)	6.8×10^{-4}	0.28 (0.05, 0.51)	0.02	0.25 (0.13, 0.38)	6.4×10^{-5}	0.86
HDL cholesterol (%)	–0.76 (–1.45, –0.06)	0.03	–1.34 (–2.40, –0.27)	0.01	–0.92 (–1.50, –0.33)	0.002	0.38
Triglycerides (%)	1.74 (–0.0, 3.53)	0.05	1.20 (–1.3, 3.78)	0.35	1.46 (0.01, 2.93)	0.05	0.78
Diastolic blood pressure (mm Hg)	–0.06 (–0.39, 0.26)	0.71	0.11 (–0.36, 0.58)	0.64	–0.01 (–0.28, 0.25)	0.92	0.54
Type 2 diabetes	1.07 (0.98, 1.17)	0.15	1.26 (1.07, 1.48)	0.006	1.11 (1.02, 1.20)	0.01	0.09
Metabolic syndrome	1.13 (1.05, 1.21)	0.002	1.13 (1.00, 1.27)	0.01	1.12 (1.06, 1.20)	2.3×10^{-4}	0.93

Effect sizes are shown as unit or percentage change per copy of high-risk allele (A) for continuous traits and as odds ratio (OR) for categorical traits, as calculated by linear regression analysis, assuming an additive genetic model, with adjustment for age in stage one, and for age, gender and ethnic group in stage two and in the combined analysis. HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein. Metabolic syndrome is as defined by Adult Treatment Panel III criteria[5].

near *MC4R* with waist circumference in stage two (**Table 1**); the most strongly associated SNP was rs12970134. In the combined analysis of stage one and two data, the association of three of these four SNPs with waist circumference also reached genome-wide significance ($P < 5 \times 10^{-7}$, **Table 1**)[7,8]. There were no significant associations of the remaining 18 SNPs with any of the phenotypes studied, either in stage two, or in the combined analysis (**Supplementary Table 2**).

For participants included in stage two, we then examined the association of the four SNPs near *MC4R* with the remaining nine phenotypes (**Table 2** and **Supplementary Table 4** online), adopting $P < 0.006$ to indicate statistical significance after Bonferroni correction for the nine phenotypes tested. We found significant associations of all four SNPs with HOMA-IR, waist-hip ratio, weight and metabolic syndrome (**Table 2** and **Supplementary Table 4**). The association of rs12970134 with HOMA-IR persisted after adjustment for waist, waist-hip ratio, or body mass index, and was also present in non-obese and non-overweight individuals (body mass index <25 kg/m^2) (**Supplementary Table 5** online), suggesting that the relationship of rs12970134 with insulin resistance is in part independent of adiposity. Results were also independent of treatment effects (**Supplementary Table 6** online).

SNPs rs12970134, rs4450508, rs477181 and rs502933 are in high linkage disequilibrium (LD; $r^2 > 0.5$) on chromosome 18, $\sim$150 kb from *MC4R* (**Supplementary Fig. 1** and **Supplementary Table 7** online). *MC4R* is a plausible biological candidate for the association of these four common SNPs with adiposity and insulin resistance. In humans, multiple rare mutations conferring loss of function in *MC4R* are associated with hyperphagia, severe childhood obesity and hyperinsulinaemia[9], a phenotype closely resembling that seen in the *Mc4r*$^{-/-}$ mouse[10]. Experimental studies show that *MC4R* is a key regulator of energy balance, influencing food intake and energy expenditure through functionally divergent central melanocortin neuronal pathways[11]. Alterations in MC4R signaling affect glucose utilization and insulin sensitivity[12]. *MC4R* and *PMAIP1* (involved in p53-dependent apoptosis), are the only protein-coding genes within 500 kb of the four SNPs. Although we cannot exclude a role for *PMAIP1*, on the basis of current knowledge of this region and strong biological plausibility, we hypothesize that rs12970134, rs4450508, rs477181 and rs502933 may be markers of causal genetic variants in a regulatory region of *MC4R*. This is consistent with observations that regulatory regions can be several hundred kilobases away from the affected gene[13].

We used imputation to further map SNP associations in this region (**Supplementary Methods**). We identified a cluster of 133 SNPs (including the four genotyped SNPs) in one LD block ($r^2 > 0.2$) showing significant association with waist circumference and HOMA-IR (**Supplementary Fig. 2** online), but no one signal predominated. We also investigated potential regulatory features in the region of association and identified a predicted small-nuclear RNA gene encoding a putative U4 spliceosomal RNA, and predicted DNAse I hypersensitive sites that might be involved in gene silencing by histone methylation[14]. We did not find a relationship between the four SNPs and *MC4R* (or *PMAIP1*) expression in peripheral blood mononuclear cells ($P > 0.24$)[15], which may reflect the predominant expression of *MC4R* in the hypothalamus and other central nervous system (CNS) nuclei.

Homozygotes for the most strongly associated SNP, rs12970134, have a $\sim$2 cm greater waist circumference and $\sim$10% higher HOMA-IR compared with wild type. Risk allele frequencies of rs12970134 are higher among individuals of Indian Asian ancestry than those of European ancestry (36% vs. 27%, $P = 10^{-50}$), as are those of the other three genotyped SNPs (**Supplementary Table 7**). Our finding of higher risk-allele frequencies among Indian Asians in SNPs near *MC4R* suggests a possible genetic mechanism contributing to the increased burden of central adiposity and insulin resistance in Indian Asians.

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

ACKNOWLEDGMENTS

At Ealing Hospital and Imperial College, we thank J. Wrigley, S. Trainor and the team of research nurses for subject characterization and sample handling; and C. Hoggart, R. Walters, X. Li and P. Thomason (supported by Wellcome Trust grant 066786) for assistance with data analysis. The work was supported by the British Heart Foundation Grant SP/04/002.

AUTHOR CONTRIBUTIONS

J.C.C., P.E., D.Z., D.B., J.S., P.F. and J.S.K. designed the study. J.C.C., P.E. and J.S.K. supervised recruitment of study subjects. D.Z., J.C.C., P.E., J.S. and J.S.K. supervised the experiments. J.C.C., D.Z., W.Z., Y.L. and D.B. performed data analysis. J.C.C., P.E., J.S. and J.S.K. wrote the manuscript. All authors commented on and approved the manuscript.

Published online at http://www.nature.com/naturegenetics
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions

1. Lopez, A.D. & Murray, C.C. *Nat. Med.* **4**, 1241–1243 (1998).
2. McKeigue, P.M., Shah, B. & Marmot, M.G. *Lancet* **337**, 382–386 (1991).

3. Kooner, J.S. *et al. Arterioscler. Thromb. Vasc. Biol.* **18**, 1021–1026 (1998).
4. Wallace, T.M., Levy, J.C. & Matthews, D.R. *Diabetes Care* **27**, 1487–1495 (2004).
5. Eckel, R.H., Grundy, S.M. & Zimmet, P.Z. *Lancet* **365**, 1415–1428 (2005).
6. Price, A.L. *et al. Nat. Genet.* **38**, 904–909 (2006).
7. Kooner, J.S. *et al. Nat. Genet.* **40**, 149–151 (2008).
8. Willer, C.J. *et al. Nat. Genet.* **40**, 161–169 (2008).
9. Farooqi, I.S. *et al. N. Engl. J. Med.* **348**, 1085–1095 (2003).
10. Trevaskis, J.L. *et al. Obesity (Silver Spring)* **15**, 2664–2672 (2007).
11. Balthasar, N. *et al. Cell* **123**, 493–505 (2005).
12. Nogueiras, R. *et al. J. Clin. Invest.* **117**, 3475–3488 (2007).
13. Kleinjan, D.A. & van Heyningen, V. *Am. J. Hum. Genet.* **76**, 8–32 (2005).
14. Schones, D.E. & Zhao, K. *Nat. Rev. Genet.* **9**, 179–191 (2008).
15. Dixon, A.L. *et al. Nat. Genet.* **39**, 1202–1207 (2007).