OBJECTIVE—Single nucleotide polymorphisms (SNPs) in the $P2$ promoter region of $HNF4A$ were originally shown to be associated with predisposition for type 2 diabetes in Finnish, Ashkenazi, and, more recently, Scandinavian populations, but they generated conflicting results in additional populations. We aimed to investigate whether data from a large-scale mapping approach would replicate this association in novel Ashkenazi samples and in U.K. populations and whether these data would allow us to refine the association signal.

RESEARCH DESIGN AND METHODS—Using a dense linkage disequilibrium map of 20q, we selected SNPs from a 10-Mb interval centered on $HNF4A$. In a staged approach, we first typed 4,608 SNPs in case-control populations from four U.K. populations and an Ashkenazi population ($n = 2,516$). In phase 2, a subset of 763 SNPs was genotyped in 2,513 additional samples from the same populations.

RESULTS—Combined analysis of both phases demonstrated association between $HNF4A$ $P2$ SNPs (rs1884613 and rs2144908) and type 2 diabetes in the Ashkenazim ($n = 591; P < 1.6 \times 10^{-6}$). Importantly, these associations are significant in a subset of Ashkenazi samples ($n = 531$) not previously tested for association with $P2$ SNPs (odds ratio [OR] ~1.7; $P < 0.002$), thus providing replication within the Ashkenazim. In the U.K. populations, this association was not significant ($n = 4,022; P > 0.5$), and the estimate for the OR was much smaller (OR 1.04; [95%CI 0.91–1.19]).

CONCLUSIONS—These data indicate that the risk conferred by $HNF4A$ $P2$ is significantly different between U.K. and Ashkenazi populations ($P < 0.00007$), suggesting that the underlying causal variant remains unidentified. Interactions with other genetic or environmental factors may also contribute to this difference in risk between populations. Diabetes 2008;57:3161–3165, 2008

The presence of type 2 diabetes susceptibility genes on chromosome 20 has been suggested by linkage scans in several populations. The 20q12–q13 region (Online Mendelian Inheritance in Man [OMIM] 603694) is the best replicated and harbors the gene $HNF4A$, mutations that lead to type 1 maturity-onset diabetes of the young (OMIM 125850). Evidence for association between SNPs in the $P2$ promoter region of $HNF4A$ has been recognized in Finnish (1) and Ashkenazi (2) populations, with data suggesting that the $HNF4A$ $P2$ SNPs (or variants in strong linkage disequilibrium with them) contribute to the linkage signal on chromosome 20q (1,2). Association with $HNF4A$ $P2$ SNPs has been replicated in some (3–7) but not all (8–12) populations tested. In other populations, there was evidence for association with SNPs or haplotypes in the $HNF4A$ region other than the $P2$ SNPs (10,13–15). More recently, the association between $HNF4A$ $P2$ SNPs and type 2 diabetes has been confirmed in Scandinavians but not in a broader meta-analysis with additional populations (16), suggesting that $P2$ SNPs confer varying risk effects in different populations, possibly due to the underlying causal variant not having been identified. We investigated a 10-Mb interval (38.1–48.2 Mb National Center for Biotechnology Information build 35) centered around $HNF4A$, including genotypes from 4,608 nonredundant ($\chi^2 < 1$) SNPs (one SNP per 2 Kb, on average) in five type 2 diabetic case-control populations, to evaluate whether we could confirm and refine the association signal in Ashkenazim and whether this association was also present in U.K. populations. We were also interested in assessing whether there was evidence for additional association signals within this broader interval. We tested an Ashkenazi type 2 diabetes case-control study ($n = 998$), including novel samples ($n = 531$) not previously tested for linkage or association with $HNF4A$ $P2$ SNPs (2); two U.K. population-based case-control studies where linkage and association studies with $HNF4A$ $P2$ had not been carried out ($n = 2,189$); and two additional U.K. case-control collections ($n = 1,842$), with one enriched for earlier-onset type 2 diabetes where linkage studies had not been done but that showed suggestive association with $HNF4A$ $P2$.
SNPs (4) and one that included samples where, despite no evidence of linkage to chromosome 20q, association of HNF4A P2 SNPs with type 2 diabetes risk had previously been suggested (4,17).

RESEARCH DESIGN AND METHODS

Populations

Cambridgeshire case-control study. The population-based Cambridgeshire case-control cohort consisted of randomly selected, unrelated patients with type 2 diabetes (n = 555) and matched, unrelated control subjects (n = 541) (18).

European Prospective Investigation of Cancer-Norfolk. From this population-based cohort study of Norfolk, U.K., 354 type 2 diabetic cases and 739 unrelated control subjects were used in this study (19,20).

Young-onset type 2 diabetes patients from Exeter. From this consecutive-case series of unrelated patients with type 2 diabetes diagnosed before 45 years of age from North and East Devon (21,22) and sex-matched control subjects from parents in the Exeter Family (22,23), we included 414 case and 425 control subjects in this study.

Diabetes U.K. Warren 2 Repository. The Warren 2 Repository index case subjects (all Europid) passing all the stringent genome scan criteria of the Warren 2 Repository, as described (17), totaled 528 probands from 573 families. The 475 control subjects came from the European Collection of Cell Cultures (ECACC) Human Control Resource.

Ashkenazi case-control study sample. The Ashkenazi case-control study comprised of 143 cases from multiplex-affected sibships ascertained for published genome scan (24) and 393 newly ascertained unrelated cases. Control samples are 149 unrelated subjects with no personal or first-degree family history of type 2 diabetes and 313 additional unrelated samples from the Warren 2 Repository (collectively referred to as UK4) and the European Prospective Investigation of Cancer-Norfolk; W2, Warren 2 Repository.

RESULTS

Phase 1 results in UK4 for all SNPs that survived quality control are shown in Fig. 1A. SNPs with nominally significant results (P < 0.05, unadjusted for multiple testing) in the Ashkenazi population are also shown. There was very modest overlap between the nominal significant results in UK4 and Ashkenazim. To avoid both false-positive association claims (type I error) and false-negative claims (type II error), we selected all SNPs with P < 0.15 in the UK4 to test in a further set of independent samples in phase 2 of the study. Additional SNPs with P < 0.01 in Ashkenazim not included in the previous selection were also added. In total, 763 SNPs were tested in phase 2 on an additional 916 type 2 diabetic case and 1,059 control subjects in the U.K. population and 238 case and 298 control subjects in the Ashkenazi population.

In the Ashkenazim, there was significant association between SNPs in the HNF4A P2 region, rs1884613 (P = 6.8 × 10^{-7}) and rs2144908 (P = 1.3 × 10^{-5}), and type 2 diabetes (Fig. 1B); 10 SNPs in the GDAP1L1 gene (P < 5.3 × 10^{-4} [supplementary Table 2]) were also associated with type 2 diabetes. Importantly, the association with SNP rs1884613 (OR 1.70; P = 0.0014 and empirical P = 0.0012) is independently replicated (Table 1) (similarly...
FIG. 1. Single SNP association results. A: Phase 1 results in UK4 (all results) and nominal significant association results in Ashkenazi ($P < 0.05$). B: Joint analysis of phase 1 and 2 results in UK4 (all results) and nominally significant association results in Ashkenazi ($P < 0.05$). $-\log_{10}$ of the unadjusted $P$ value is shown on the $y$-axis, whereas chromosome coordinates are shown on the $x$-axis. Dashed line represents nominal significance ($P = 0.05$). ASH, Ashkenazi data; ASH–HNF4A P2, results for two HNF4A P2 SNPs (rs1884613 and rs2144908) in the Ashkenazim. Genes with most significant results are shown as black bars; location of HNF4A on the chromosome is shown as gray bars.
results were obtained for rs2144908) in the subset of novel Ashkenazi samples (393 case and 138 control subjects). In contrast, of the 16 PREX1 SNPs with significant results in phase 1 ($P < 0.01$) in the joint analysis, none achieved equivalent significance (Fig. 1A and B). There was also no evidence for association with other SNPs (rs2425637, rs2425639, rs2425640, rs6130609, and rs745975; $P > 0.2$) mapping to the coding sequence or PI promoter of HNF4A (data not shown). In contrast with the Ashkenazi data, in UK4, there was no evidence of stronger association based on the joint analysis compared with the results from phase 1 alone (Fig. 1A and B and supplementary Table 3); furthermore, none of the results would survive any kind of multiple-testing correction.

We used log-likelihood ratio tests to assess whether SNPs rs1884613 and rs2144908 explained all of the observed associations with type 2 diabetes in this region. The correlation between SNPs rs1884613 and rs2144908 is very high ($r^2 = 0.99$); therefore, we cannot separate their effects on type 2 diabetes risk. We consecutively added the other SNPs in this region in a log-additive form to a model containing these SNPs (1 d.f., assuming no dominance at the test locus). This analysis demonstrated that none of the other SNPs in this region of high LD improved the model containing just one of either rs1884613 or rs2144908, and it indicates that no additional genotyped SNP is independently contributing to type 2 diabetes risk (data not shown). The reciprocal analysis showed that addition of either rs1884613 or rs2144908 improved all models containing any of the other SNPs. Repeating this analysis with phase 1 data (higher SNP density, although on a smaller sample size; data not shown) confirms these results and suggests that no other genotyped SNP in this region is independently associated with type 2 diabetes risk.

**DISCUSSION**

This study provides clear evidence for association of HNF4A P2 promoter SNPs with type 2 diabetes in the Ashkenazim. Importantly, these results are replicated in the subset of novel samples (393 case and 138 control subjects) that are independent of those used in the original report (2), with a point estimate of OR 1.70 (74% CI 1.59–1.81) for rs1884613 (Table 1). However, despite the relatively high density of SNPs used to span the 10-Mb candidate interval (1 SNP/2 Kb on average), we were unable to refine the association signal beyond the two originally associated SNPs (rs1884613 and rs2144908) in the Ashkenazim. Thus, further fine-mapping in this region will be required to identify the underlying causal variant. In contrast, in UK4, this association result could not be replicated (OR $1.04$; $P > 0.5$). Furthermore, the CIs for the ORs in UK4 and the Ashkenazim did not overlap (supplementary Table 2), showing that the risk of P2 SNPs on type 2 diabetes in these two groups is significantly different ($P < 0.00007$). Independent analysis of each U.K. population also did not provide evidence for association of HNF4A P2 SNPs and diabetes risk in any of the individual populations (supplementary Table 4).

Within the U.K., the previously reported effect size for nominally associating SNPs (rs4810424 and rs2144908) was small (OR 1.09–1.13) (4), with an MAF of 16%, a 0.05, and a sample size of 4,022, our U.K. populations were underpowered to detect such effects (power 54%), which could underlie our lack of association. Importantly, both the current and previously reported CIs for the effect size in the U.K. (4) do not overlap with those in the Ashkenazim, suggesting population-specific effects. We observed significant heterogeneity of effects at these SNPs ($P < 0.0001$) between U.K. and Ashkenazim populations by comparing logistic regression models, with and without a genotype × study interaction term using a likelihood ratio test.

Allele frequency and Fst values across the region suggested that there was no evidence of significant genetic distance at these loci (supplementary information). Furthermore, LD plots and unit maps across this region (supplementary information) also demonstrated that, overall, the magnitude of LD was not materially different between Ashkenazi and U.K. populations and was unlikely to significantly contribute to divergent results. Thus, our data suggest that if HNF4A P2 SNPs are a risk factor for type 2 diabetes in U.K. European populations, the risk they confer is considerably smaller than that which they impart on the Ashkenazi population, which is consistent with linkage having been observed in Ashkenazim (24) but not in U.K. samples (17) and with other, more recent results (16).

This result provides further support for the involvement of HNF4A P2 SNPs (or others in high LD) in conferring risk of type 2 diabetes in Ashkenazim and has important wider implications because it demonstrates the benefit of performing replication testing in samples that are recruited from the same population sampling frame as those used in the exploratory early stages of an association study. Population-specific effects will have a significant impact in the interpretation of results from replication studies.

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