HIEG clamp and SlisOGTT, with less than 6% disagreement (Fig. 1B).

As has been shown before in an obese and overweight, postmenopausal, non-diabetic population [1], the SlisOGTT-derived index is well correlated with the current gold standard measure of insulin sensitivity in a healthy population, which may also render it suitable for general population screening. However, studies in populations that are glucose-intolerant or diabetic are needed to further validate its utility as an insulin-resistance diagnostic tool.

Conflict of interest

None of the authors has a conflict of interest to declare.

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References


We recently performed the first genome-wide fluorescence-based microsatellite (n = 6000) association screen in diabetic nephropathy (DN). Persons of Irish descent with type 1 diabetes and DN (cases; n = 200) were compared to individuals with type 1 diabetes but without DN (controls; n = 200) [1]. A DNA pooling strategy, based on the genetic analysis of multiple sclerosis in Europeans (GAMES) methodology [2], was employed. The top ranked markers (n = 50) were assessed by individual genotyping using the same DNA samples comprising the pools. Two markers on chromosome 10 (D10S558 and D10S1435) were significantly associated with DN even after correction for multiple testing; in addition, a number of markers with suggestive evidence of association warranted further investigation. In the present report, we have performed replication studies for the D10S558 and D10S1435 markers, together with the next 10 markers most significantly associated with DN from our initial study [1], in a total of 570 cases and 611 controls derived from the British Isles. The clinical definition of cases and controls was as previously reported [3]. The majority of these cases and controls were derived from the UK Genetics of Kidneys in Diabetes (GoKinD) collection [3]. Ethical approval was obtained from the appropriate Research Ethics Committees and written informed consent obtained from participants prior to conducting the study.

Fluorescence-based microsatellite genotyping was performed for all markers in cases and controls [1] using Qiagen Multiplex PCR kits (Qiagen, Crawley, UK). Amplification products were resolved by capillary electrophoresis and alleles scored, using an ABI 3730 Genetic Analyser (Applied Biosystems, Warrington, UK). Clinical characteristics of cases and
controls were compared by the independent samples t test. Genotyping data was analysed using the T2 statistic in the CLUMP program as previously described [1].

Unsurprisingly, there were a greater proportion of males in cases compared to controls (59.3% vs. 43.7%; P<0.001). Mean age at onset of type 1 diabetes was similar between cases and controls (14.5±7.7 years vs 15.3±7.9 years). The mean durations of diabetes were 30.8±31.0 years versus 125.4±7.7 years vs 14.5±7.7 years versus 15.3±7.9 years and mean HbA1c values were 7.2±4.7 versus 7.4±1.5% in cases and controls respectively. Despite antihypertensive treatment, the mean systolic (144.5±21.3 mmHg vs 125.4±15.1 mmHg) and diastolic (80.8±11.6 mmHg vs 75.2±8.3 mmHg) blood pressures were significantly higher in cases compared to controls (P<0.001). No significant differences were observed in allele frequencies between case and control groups for 11 markers (D11S1760 was excluded due to problems in allele-calling) (Table 1). The microsatellite marker D2S2289 was significantly associated with DN before (P=0.013), but not after correction for multiple testing (Table 1).

The failure of our much larger study to replicate the previous positive associations with the two microsatellite markers on chromosome 10 and other high ranked markers, could be due to a number of reasons. Firstly, our original findings were false positives. Type 1 error offers one possible explanation but others are entirely plausible and a recent report has demonstrated the shortcomings of using DNA pooling strategies involving microsatellites in genetic association studies [4]. Our findings in the present study could be false negatives. This is less likely as the sample sizes used were significantly larger than in the initial study, the phenotypic criteria for selection of cases and controls were similar in both studies in that they were Caucasian from the British Isles and the groups had similar clinical characteristics. It is also unlikely that technical errors can explain the discrepancies between studies, since we validated each marker by DNA sequencing in a selection of samples and alleles were independently scored by two individuals.

In conclusion, our results would suggest that initial findings of our previous low-resolution microsatellite association screen [1] should be interpreted with caution. The GAMES strategy has recognised limitations [4] and our failure to replicate positive or interesting findings from our original study most likely reflect these methodological difficulties.

Conflicts of interest
None to declare.

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