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Comment on: Bason-Laubert A, Boehm B, Lang-Muritano M, et al (2005) Association of childhood type 1 diabetes mellitus with a variant of *PAX4*: possible link to beta cell regenerative capacity. Diabetologia 48:900–905

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To the Editor: Type 1 diabetes probably results from the imbalance between selective autoimmune destruction of pancreatic beta cells and their regeneration. *PAX4*, a member of the *PAX* family of transcription factors, is essential to the differentiation and function of beta cells. *Pax4* knock-out mice showed an absence of mature insulin-producing beta cells and a severe diabetic syndrome after birth [1]. In the May 2005 issue of *Diabetologia*, Bason-Laubert and co-workers reported a strong association between type 1 diabetes and the A1168C (P321H) single nucleotide polymorphism (SNP) in exon 9 of *PAX4*, both in a Swiss and in a German population [2]. The authors also showed that the *PAX4* proteins encoded by the two variants have differential binding affinity to the *PAX4* binding site in the insulin promoter [2]. The functional relevance of the SNP and the reported association made it a very attractive candidate mutation; however, these observations need to be confirmed in other populations, because despite their power

in identifying type 1 diabetes and other complex disease genes, associations observed in case/control studies can be caused by mismatch of ethnicity and other factors.

In an attempt to confirm the *PAX4* association with type 1 diabetes, we analysed the A1168C SNP in two large American Caucasian populations. A 398 bp fragment containing the A1168C SNP within the coding region of the *PAX4* gene was amplified using the forward primer hPAX4F (5'-TGTCTCAGCCTCTCTCCCATAGTG-3') and the reverse primer hPAX4R (5'-CTCCAATCAGGTGATGCGC CAG-3'). The amplified products were digested using the restriction enzyme *BsaI* and the digested products were resolved on 2.5% agarose gels. The uncut 1168A allele corresponds to a 398-bp band and the cut 1168C allele yields two fragments (244 and 158 bp).

The study subjects consisted of Caucasians from two US populations (North-Central Florida and Denver, Colorado). The Florida data set included 322 type 1 diabetes patients and 961 control subjects, while the Denver data set included 630 type 1 diabetes patients and 666 control subjects. These patients and control subjects have been used in our previous genetic studies [3–5]. All diabetic patients were diagnosed using the criteria of the National Diabetes Data Group and the control subjects were healthy and had no family history of type 1 diabetes and no islet cell autoantibodies.

The genotypic frequencies for the A1168C SNP are presented in Table 1. The control and patient populations from both Florida and Denver were found to be in Hardy–Weinberg equilibrium (Table 1). The frequencies for the *PAX4* A1168C genotypes were similar in the Florida and Denver populations. More importantly, there was no significant difference between type 1 diabetic patients and control subjects in either population. Our data from two large Caucasian data sets, therefore, do not support the previously reported association between *PAX4* and type 1 diabetes.

It is unlikely that the lack of association is due to the sample size, because our data sets are much larger than those in the previous study. In addition, our sample sets have been used in previous studies, which found significant

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Table 1 Frequency of the A1168C variant of *PAX4* in American Caucasian populations

	Genotypes			HWE <i>p</i> value
Subjects	A/A	A/C	C/C	
Florida population				
Patients (<i>n</i> =322)	16 (5.0%)	103 (32.0%)	203 (63.0%)	0.53
Controls (<i>n</i> =961)	43 (4.5%)	321 (33.4%)	597 (62.1%)	0.98
<i>p</i> value	0.71	0.64	0.77	
Denver population				
Patients (<i>n</i> =630)	27 (4.3%)	221 (35.1%)	382 (60.6%)	0.48
Controls (<i>n</i> =666)	27 (4.0%)	243 (36.5%)	396 (59.5%)	0.17
<i>p</i> value	0.83	0.60	0.67	

HWE, Hardy–Weinberg equilibrium

association with several other genes linked with type 1 diabetes, including *HLA*, *PTPN22*, *INS* and others [3–5]. The discrepancy between our data and the report by Biaisson-Laubert et al. may be explained by genetic heterogeneity for the association between *PAX4* and type 1 diabetes. However, the associations reported there were possibly spurious and may not reflect a causal relation between *PAX4* and type 1 diabetes, since two other studies [6, 7], in addition to this report, were unable to confirm the association. Furthermore, the departure from Hardy–Weinberg equilibrium in both control populations studied in the initial report may indicate major genotyping errors [8]. Therefore, it is reasonable to conclude that *PAX4* is unlikely to be a susceptibility gene for type 1 diabetes.

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