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Beta cell regeneration meets autoimmunity: *PAX4* variants in type 1 diabetes

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Type 1 diabetes may be considered as a disease that results from an imbalance between selective autoimmune destruction of pancreatic beta cells and beta cell regeneration. This gives rise to an intriguing question: is impaired beta cell regeneration in itself an important determinant of susceptibility to type 1 diabetes [1, 2]? Two lines of investigation have recently—and more or less independently—investigated the mechanisms that underlie beta cell development and regeneration, and the mechanisms involved in beta cell destruction by immune cells and proinflammatory cytokines. In this issue of *Diabetologia*, Bion-Laubert and coworkers provide evidence that a genomic variant of *PAX4* is linked to the development of type 1 diabetes [3].

PAX4 is a member of the Pax family of homeodomain transcription factors that are operative during the development of the endocrine pancreas and have been shown to be essential for beta cell development and beta cell function [4]. In mice, this transcription factor is expressed on or around Day 9 in cells of the dorsal pancreatic rudiment, with peak expression occurring between Day 13 and 15, followed by a decline until birth. *PAX4* has been identified as a downstream target of neurogenin 3, another important transcription factor for the development of the endocrine pancreas [4]. Studies on *pax4* knock-out mice showed a profound reduction in beta cell mass and a severe diabetic syndrome after birth. Atypically clustered alpha cells and ghrelin-positive cells were detected in the pancreas, and it was concluded that *PAX4* suppresses the development of these cell types [4].

The pivotal role played by *PAX4* in beta cell development and function has prompted genetic linkage analyses of this gene in cohorts of diabetic patients, with particular focus on type 2 diabetes. These studies yielded conflicting

results. Screening of French MODY families showed no linkage between type 2 diabetes and markers of *PAX4* [5]. Although the *P321H* missense mutation was not associated with MODY diabetes, it does seem to be linked to susceptibility to type 1 diabetes, as discussed below. A recent study reported a linkage between a homozygous missense mutation (*R121W*) in *PAX4* and type 2 diabetes in a Japanese population [6]. The mutant allele occurred at a low frequency (2.0%) within this population [6], and Kanatsuka and coworkers described the same *PAX4* mutation in patients with late-onset type 2 diabetes [7]. Interestingly, this mutation was associated with impaired first-phase insulin secretion, providing further evidence for the involvement of *PAX4* in beta cell function [7]. However, it is not clear from these studies whether the *R121W* *PAX4* missense mutation, whose functional effects have not been demonstrated in insulin-producing cells, may affect beta cell mass in these patients.

What is the situation in type 1 diabetes? Through microsatellite analysis of Scandinavian multiplex families, Holm and co-workers demonstrated a linkage between type 1 diabetes and a region on chromosome 7 (7q32) that carries *PAX4*, but they were unable to identify variation within the coding region of the gene [8]. There is thus evidence that variations in *PAX4* could be associated with susceptibility to type 1 diabetes, but the nature of this *PAX4* variation and its functional consequences are yet to be established.

The paper by Bion-Laubert and coworkers in this issue reports on the identification of *PAX4* variants within the coding region of the gene and their functional effects in insulin-producing cells [3]. The authors screened 1,468 individuals for mutations in *PAX4* through allelic discrimination and sequence analysis. Subjects included 379 schoolchildren with type 1 diabetes, 1,070 non-diabetic controls, and 19 schoolchildren who tested positive for anti-islet cell antibodies, but did not develop type 1 diabetes. A single nucleotide polymorphism, *A1168C*, was identified in exon 9 of *PAX4*. The *A* variant corresponded to a proline residue at position 321 of the protein, and the *C* variant coded for histidine at this position. It should be noted that this is the *PAX4* variant that could not be linked to MODY diabetes in French families [5]. In the study of Bion-Laubert

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et al., 77% (Swiss cohort) and 72% (German cohort) of the schoolchildren with type 1 diabetes carried the *C/C* genotype, whereas only 30% of the control subjects and 10% of the antibody-positive non-diabetic children were *C/C* carriers. The risk of developing type 1 diabetes was calculated to be 3.75-fold higher for individuals carrying the *C/C* genotype than for individuals carrying the *A/C* or *A/A* genotype. It is of particular interest that antibody-positive children with the *A/C* genotype had a significantly lower risk of developing manifest type 1 diabetes than those with other genotypes.

The authors next investigated the involvement of PAX4 variants in the development and function of endocrine cells. Human PAX4C and PAX4A variants were overexpressed in cell lines with a beta cell phenotype (β TC3), an alpha cell phenotype (α TC1.9) and a non-endocrine phenotype (NIH3T3) [3]. Compared with PAX4A and the combination of the two variants, PAX4C repressed the transcription of a proinsulin promoter reporter construct to a lesser degree than PAX4A and the combination of the two variants, and showed impaired binding to DNA target sequences in electrophoretic mobility shift assays. Glucose-dependent cell proliferation was lower in β TC3 cells, but not in α TC1.9 or NIH3T3 cells, after overexpression of the PAX4C variant. From these data, PAX4C could be interpreted as a 'diabetic' variant that limits the proliferation of beta cells in response to glucose stimulation. It should, however, be noted that these results were obtained using permanent cell lines. One of the crucial characteristics of these cell lines is their immortalisation, which results in constitutive cell growth. Consequently the results do not necessarily reflect the situation in primary beta cells, either during development or regeneration in the adult state. It would be useful to prove the importance of PAX4C for cell function and regeneration in primary beta cells, e.g. by using viral vector systems and by generating transgenic mice. These studies would help to verify PAX4C as a 'diabetic' variant of this homeobox transcription factor.

What are the implications of these data for experimental and clinical type 1 diabetes? First, they should focus our attention on defects in beta cell regeneration pathways in diabetic individuals and animal models of type 1 diabetes. The regenerative potential of the endocrine pancreas is generally accepted, but it remains unclear whether defects in regenerative capacity really exist in diabetic patients or in animal models. It therefore remains to be determined whether these defects are crucial for susceptibility to the development of type 1 diabetes. Second, genes encoding proteins involved in beta cell differentiation and regeneration could serve as prognostic markers, either for suscep-

tibility to type 1 diabetes or for progression of the disease. Biason-Laubier and coworkers showed that antibody-positive children carrying the *A/C* genotype of the *A1168C* single nucleotide polymorphism in *PAX4* did not develop a diabetic syndrome [3]. The authors speculate that an improved regenerative potential might counterbalance the autoimmune destruction of beta cells, and this plausible hypothesis should be verified by prospective genetic studies on larger cohorts of antibody-positive children. Third, as an ultimate goal, an understanding of defects in pathways affecting the regenerative capacity of beta cells may open the door for new therapeutic interventions. This does not necessarily mean the correction of 'diabetic' variants of a gene by the use of viral vectors or physical gene transfer. In the era of modern drug design, compounds that increase the activity of the PAX4C variant could be developed in order to restore the regenerative capacity of beta cells. Biason-Laubier and coworkers have elegantly shown that defective beta cell regeneration may be an essential and—so far—underestimated component of the pathogenesis of autoimmune diabetes [3]. It would be worthwhile to employ this experimental strategy to study other genes involved in beta cell differentiation to gain an insight into the role of regeneration in susceptibility to type 1 diabetes.

References

1. Eizirik DL, Mandrup-Poulsen T (2001) A choice of death—the signal-transduction of immune-mediated beta-cell apoptosis. *Diabetologia* 44:2115–2133
2. Homo-Delarche F, Drexhage HA (2004) Immune cells, pancreas development, regeneration and type 1 diabetes. *Trends Immunol* 25:222–229
3. Biason-Laubier 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:DOI 10.1007/s00125-005-1723-5
4. Sosa-Pineda B (2004) The gene *Pax4* is an essential regulator of pancreatic beta-cell development. *Mol Cells* 18:289–294
5. Dupont S, Vionnet N, Chevre JC et al (1999) No evidence of linkage or diabetes-associated mutations in the transcription factors *BETA2/NEUROD1* and *PAX4* in Type II diabetes in France. *Diabetologia* 42:480–484
6. Shimajiri Y, Sanke T, Furuta H et al (2001) A missense mutation of *Pax4* gene (R121W) is associated with type 2 diabetes in Japanese. *Diabetes* 50:2864–2869
7. Kanatsuka A, Tokuyama Y, Nozaki O, Matsui K, Egashira T (2002) Beta-cell dysfunction in late-onset diabetic subjects carrying homozygous mutation in transcription factors *NeuroD1* and *Pax4*. *Metabolism* 51:1161–1165
8. Holm P, Rydlander B, Luthman H, Kockum I (2004) Interaction and association analysis of a type 1 diabetes susceptibility locus on chromosome 5q11-q13 and the 7q32 chromosomal region in Scandinavian families. *Diabetes* 53:1584–1591