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COMMENTARY

Not for the eyes only: *PAX6* and glucose metabolism

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Abbreviations

GLP	Glucagon-like peptide
IGT	Impaired glucose tolerance
PAX6	Paired box 6
PC1/3	Prohormone convertase 1/3

Paired box 6 (PAX6) is a member of the *PAX* multigene family of transcription factors: this family regulates embryonic differentiation. Nine unlinked *PAX* genes dispersed throughout the genome encode proteins that each include a 128-amino-acid sequence-specific highly conserved DNA-binding domain, the *Pa*ired bo*x*, which can regulate the expression of other genes [1]. *PAX6* also contains another common DNA-binding element: the homeobox that encodes a 60-amino-acid homeodomain responsible for target sequence recognition [2]. The paired domain and homeodomain interact cooperatively to recognise multiple DNA binding sites [3].

Pax6 is expressed in the developing nervous system and in developing eyes [4], and also has important functions in the development of the endocrine pancreas. Genetic studies indicate that all eyes share a similar developmental cascade: mutations in *Pax6* disrupt eye development in both mammals and insects [4]. *Pax6* appears to be necessary for the correct execution of beta cell differentiation [5] and is essential for the normal expression of insulin [6].

PAX6 is required for the differentiation of pancreatic islet alpha cells, and competes with *PAX4* in binding to a common element in the glucagon, insulin and somatostatin promoters [7]. Mice lacking both *Pax4* and *Pax6* fail to develop any mature endocrine cells, suggesting that both genes are required for endocrine development of the pancreas [8]. As well as binding to a common element in the glucagon, insulin and somatostatin promoters, *Pax6* also transactivates the glucagon and insulin promoters [6]. *Pax6*-deficient mice have reductions in both insulin- and glucagon-expressing cells. Human *PAX6* is transcribed as a 2.7 kb mRNA and encodes a 422-amino-acid protein. *PAX6* extends to over 22 kb: it contains 14 exons and intron sequences and is highly conserved among vertebrates and lower animals.

Mutations in *PAX6* in humans cause aniridia type II, a bilateral panocular disorder characterised by a complete or partial absence of the iris and fovea, and malformations of the lens and anterior chamber [2]. About one-third of the cases are sporadic and two-thirds are familial, with autosomal dominant inheritance and high penetrance. There is a PAX6 dosage effect in aniridia, ranging from mild loss of visual acuity and cataracts, to severe nervous system defects and anophthalmia [7]. Using magnetic resonance imaging techniques and smell testing, Sisodiya et al. [9] showed with that reduced olfaction was present in a large proportion of aniridia patients, indicating that *PAX6* haploinsufficiency causes more widespread human neurodevelopmental anomalies.

Yasuda et al. [10] were the first to demonstrate that carriers of the *PAX6* mutation might have abnormal glucose

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tolerance. They described patients with aniridia who had mutations in *PAX6*. Oral glucose tolerance tests revealed that all the patients with a *PAX6* gene mutation had impaired glucose tolerance (IGT), characterised by impaired insulin secretion. Diabetes was co-segregated with aniridia in the family, and a single-nucleotide polymorphism in intron 9 of *PAX6* was correlated with the disorders, suggesting that a mutation, possibly located in an uncharacterised portion of *PAX6*, could explain both diabetes and aniridia in this family. However, the mechanisms for IGT remain unclear. Nishi et al. [11] reported the first case of *PAX6* mutation (2 bp deletion, c.402del2) with early-onset diabetes mellitus.

In this issue of *Diabetologia*, Wen et al. [12] identified a mutation R240Stop of *PAX6* in a single pedigree including 19 patients with aniridia. This mutation resulted in a truncated polypeptide that prematurely terminated at residue 240, and lacked the entire transactivation domain and a small portion of homeodomain. In an oral glucose tolerance test, younger patients with aniridia (aged 11–33 years) had normal glucose tolerance, whereas older patients had IGT or diabetes. The authors found that the total insulin level was reduced, whereas the proinsulin:insulin ratio was greater in patients with aniridia than in a control group. No insulin resistance was found, suggesting that the patients were likely to have defects in both insulin production and processing. Since cleavage of proinsulin mainly depends on prohormone convertases, the authors hypothesised that PAX6 may regulate production of beta cell prohormone convertases 1/3 (PC1/3).

As is very often the case in human genetic studies, the authors had to perform mechanistic studies using a mouse model to test their hypothesis. They screened *N*-ethyl-*N*-nitrosourea (ENU) mutagen-induced mice for *Pax6* gene mutations based on the typical 'small eye phenotype' of *Pax6* mutants, and identified a strain of R266Stop-mutant mice. Older mice exhibited abnormal glucose tolerance, and the proinsulin:total insulin ratio was significantly greater than that in control mice. The authors demonstrated that protein levels of PC1/3 and the expression of the gene that encodes PC1/3 were significantly decreased in pancreatic islets of the R266Stop-mutant mice. Thus, the *Pax6* mutation caused PC1/3 deficiency and defective proinsulin processing. To gain further evidence for their hypothesis, the authors showed that hypothalamus adrenocorticotropic hormone (ACTH) and alpha-melanoctye stimulating hormone (α-MSH) levels were reduced (this was to be expected, since PC1/3 coverts pro-opiomelanocortin into ACTH, which is further converted into α-MSH). In a previous study, a complete loss of PAX6 in the islets was associated with disturbed islet architecture and diabetes. Surprisingly, the levels of PC1/3 were maintained in that study, suggesting that the reduction in insulin levels in *Pax6*-deficient mice was probably due to direct changes in insulin expression and/or the inability of these cells to respond to elevated glucose levels [13].

Most of the new genes identified recently in connection with the aetiology of diabetes regulate insulin secretion [14]. Therefore, the findings by Wen et al. [12] confirming the role of *Pax6* in insulin secretion will stimulate further studies on the role of this gene in disturbances of glucose metabolism and in diabetes. *PAX6* has a central role among the genes regulating beta cell development (Fig. 1). *NGN3* expression is critical for establishing the endocrine cell programme not only in the rodent pancreas, but also in the human pancreas [15]. Inactivation of *NGN3* results in a complete loss of endocrine cells, whereas the lack of *PAX4*

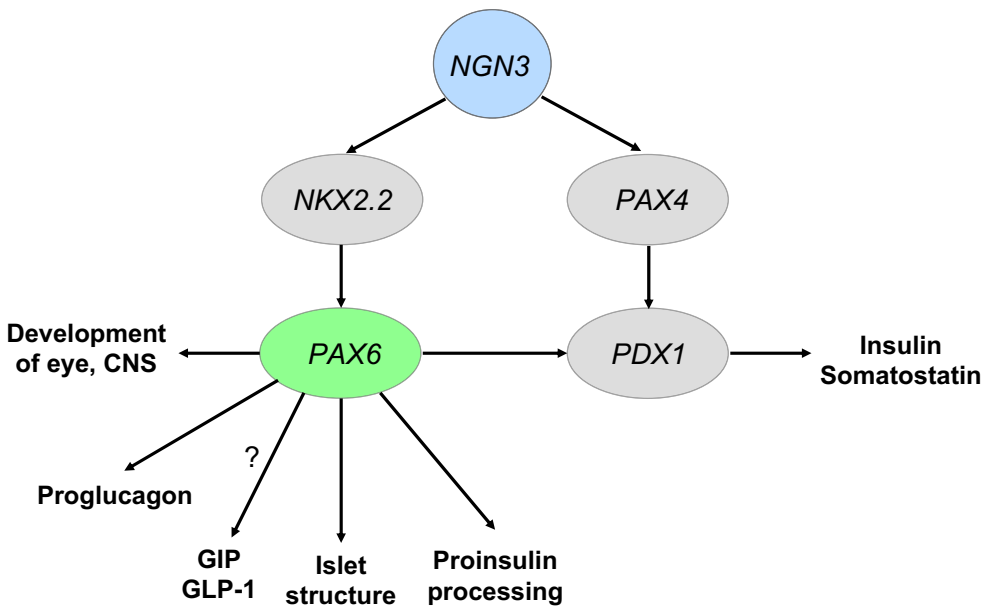

Fig. 1 *PAX* genes regulating beta cell development. CNS, central nervous system; GIP, gastric inhibitory polypeptide

expression prevents the formation of mature pancreatic insulin-producing beta cells [16]. Endocrine cell-specific transcription factors, including NK2 homeobox 2 (NKX2.2), are direct regulators of *PAX6* during pancreatic development. In turn, *PAX6* is a direct regulator of *PDX1* [17], which regulates insulin secretion and somatostatin gene expression in the endocrine pancreas [15].

Pax6-knockout mice lack glucagon-producing alpha cells and a proper islet structure [8]. Hamasaki et al. [18] examined the pancreatic phenotype of a small eye rat strain with a point mutation in the transactivation domain of the *Pax6* locus, resulting in truncated PAX6 proteins. Islet architecture was maintained, but these rats had impaired insulin response to glucose, although glucagon secretion was unaffected. Therefore, insulin-secreting beta cells require greater expression of *Pax6* than do glucagon-secreting alpha cells for normal function.

PAX6 is required for normal proglucagon gene expression in both the pancreas and intestine [19]. Thiazolidinediones inhibit glucagon gene transcription through binding to peroxisome proliferator activated receptor gamma (PPARγ) and inhibition of the transcriptional activity of *PAX6*, which is required for cell-specific activation of the glucagon gene [20]. In addition, retinoid X receptor (RXR) agonists inhibit glucagon gene transcription in a PPARγ-dependent manner. A ligand-bound PPARγ–RXR heterodimer interacts with promoter-bound PAX6 to inhibit glucagon gene transcription.

PAX6 could also be important for incretin hormone action. In rodents, the deletion of *Pax6* eliminates glucagon-like peptide (GLP)-1- and GLP-2-producing cells in the distal intestine [21] and gastric inhibitory polypeptide (GIP)-producing cells in the duodenum [22]. This might not be the case in humans, since the expression of *PAX6* is small in the intestine [23]. Wen et al. [12] did not find any difference in GLP-1 levels between carriers and non-carriers of the *PAX6* mutation, which suggests that incretin hormone action does not explain their findings. More studies are needed, however, to clarify this issue.

In the era of genome-wide scans it is important to note that the study by Wen et al. [12] demonstrates the strength of a single pedigree in genetic studies of diabetes. The authors identified the *PAX6* R240Stop mutation in one family and characterised the underlying mechanisms in *Pax6* R266Stop-mutant small-eye mice, resembling the human mutation. Both mutations led to PC1/3 deficiency, and consequently to defective proinsulin processing. This raises the question of whether defective proinsulin processing is also linked to other genes regulating insulin secretion or not. In order to exclude the latter possibility, a defect in proinsulin processing should be kept in mind in studies of all new genes associated with impaired insulin secretion and abnormal glucose metabolism.

Duality of interest The author declares that there is no duality of interest associated with this manuscript.

References

1. Mark M, Rijli FM, Chambon P (1997) Homeobox genes in embryogenesis and pathogenesis. Ped Res 42:421–429
2. Glaser T, Walton DS, Maas RL (1992) Genomic structure, evolutionary conservation and aniridia mutations in the human PAX6 gene. Nat Genet 2:232–239
3. Jun S, Desplan C (1996) Cooperative interactions between paired domain and homeodomain. Development 122:2639–2650
4. Kozmik Z (2008) The role of Pax genes in eye evolution. Brain Res Bull 75:335–339
5. Marsich E, Vetere A, Di Piazza M, Tell G, Paoletti S (2003) The PAX6 gene is activated by the basic helix–loop–helix transcription factor NeuroD/BETA2. Biohem J 376:707–715
6. Sander M, Neubuser A, Kalamaras J, Ee HC, Martin GR, German MS (1997) Genetic analysis reveals that PAX6 is required for normal transcription of pancreatic hormone genes and islet development. Genes Dev 11:1662–1673
7. Glaser T, Jepeal L, Edwards JG, Young SR, Favor J, Maas RL (1994) PAX6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central nervous system defects. Nat Genet 7:463–471
8. St-Onge L, Sosa-Pineda B, Chowdhury K, Mansouri A, Gruss P (1997) Pax6 is required for differentiation of glucagon-producing alpha-cells in mouse pancreas. Nature 387:406–409
9. Sisodiya SM, Free SL, Wlliamson KA et al (2001) PAX6 haploinsufficiency causes cerebral malformation and olfactory dysfunction in humans. Nat Genet 28:214–216
10. Yasuda T, Kajimoto Y, Fujitani Y et al (2002) PAX6 mutation as a genetic factor common to aniridia and glucose intolerance. Diabetes 51:224–230
11. Nishi M, Sasahara M, Shono T et al (2005) A case of novel de novo paired box gene 6 (PAX6) mutation with early-onset diabetes mellitus and aniridia. Diabet Med 22:641–644
12. Wen JH, Chen YY, Song SJ et al (2008) Paired box 6 (PAX6) regulates glucose metabolism via proinsulin processing mediated by prohormone convertase 1/3 (PC1/3). Diabetologia. doi: 10.1007/s00125-008-1210-x
13. Ashery-Padan R, Zhou X, Marquardt T et al (2004) Conditional inactivation of Pax6 in the pancreas causes early onset of diabetes. Dev Biol 269:479–488
14. Frayling TM (2007) Genome-wide association studies provide new insights into type 2 diabetes aetiology. Nat Rev Genet 8:657–662
15. Lyttle BM, Li J, Krishnamurthy M, Fellows F et al (2008) Transcription factor expression in the developing human fetal endocrine pancreas. Diabetologia 51:1169–1180
16. Wang J, Elghazi L, Parker SE et al (2004) The concerted activities of Pax4 and Nkx2.2 are essential to initiate pancreatic beta-cell differentiation. Dev Biol 266:178–189
17. Sosa-Pineda B (2004) The gene Pax4 is an essential regulator of pancreatic beta-cell development. Mol Cells 18:289–294
18. Hamasaki A, Yamada Y, Kurose T et al (2007) Adult pancreatic islets require different pax6 gene dosage. Biochem Biophys Res Commun 353:40–46
19. Schonhoff SE, Giel-Moloney M, Leiter AB (2004) Minireview: development and differentiation of gut endocrine cells. Endocrinology 145:2639–2644
20. Krätzner R, Frölich F, Lepler K et al (2008) A peroxisome proliferator-activated receptor gamma-retinoid X receptor hetero-

dimer physically interacts with the transcriptional activator PAX6 to inhibit glucagons gene transcription. Mol Pharmacol 73:509–517
21. Hill ME, Asa SL, Drucker DJ (1999) Essential requirement for Pax6 in control of enteroendocrine proglucagon gene transcription. Mol Endocrinol 13:1474–1486
22. Larsson LI, St-Onge L, Hougaard DM, Sosa-Pineda B, Gruss P (2008) Pax4 and 6 regulate gastrointestinal endocrine cell development. Mech Dev 79:153–159
23. Cao X, Flock G, Choi C, Irwin DM, Drucker DJ (2003) Aberrant regulation of human intestinal proglucagon gene expression in the NCI-H716 cell line. Endocrinology 144:2025–2033