Mutations in *HHEX* are not a common cause of monogenic forms of beta cell dysfunction

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Abbreviations

HEX haematopoietically expressed homeobox protein
HI hypoglycaemia of infancy
HNF1 α hepatocyte nuclear factor 1 α
PDX1 pancreatic and duodenal homeobox protein 1
PNDM permanent neonatal diabetes

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To the Editor: Haematopoietically expressed homeobox protein (HEX), a divergent homeobox transcription factor, is encoded by the *HHEX* gene and is required for organogenesis of the ventral pancreas [1]. Mouse embryos lacking *HHEX* show a complete failure of ventral pancreatic specification [1] and have deletions of the forebrain, midbrain and rostral hindbrain [2]. HEX functions from very early in embryogenesis (E4.5) through adulthood and is involved in the regulation of genes at multiple developmental states, including mature tissue and cell type [3]. *HHEX* is expressed before the gene encoding the key pancreatic transcription factor, pancreatic and duodenal homeobox protein 1 (*PDX1*), and mouse embryos lacking *HHEX* do not express *PDX1* in the ventral pancreas, whereas expression in the dorsal pancreas is unaffected [1]. HEX has also been shown to activate hepatocyte nuclear factor 1 α (HNF1 α), an important transcription factor not only in beta cell development but also in the regulation of insulin secretion [4].

Mutations in key transcription factors for pancreatic development are responsible for monogenic forms of beta cell dysfunction, resulting in both hyper- and hypoglycaemia [5, 6]. Homozygous or compound heterozygous mutations in *PDX1* cause permanent neonatal diabetes (PNDM) with pancreatic agenesis [5], whilst heterozygous mutations result in MODY [5]. Mutations in the genes

encoding HNF1 α (*HNF1A*, also known as *TCF1*), HNF1 β (*HNF1B*, also known as *TCF2*) and HNF4 α (*HNF4A*) also cause MODY [5]. It has recently been shown that mutations in *HNF4A* can also cause transient hypoglycaemia [6]. A number of cases of PNDM have extra-pancreatic features, including cerebellar agenesis [7] and other neurological abnormalities [5], supporting a role in disease pathogenesis for additional genes essential to both pancreatic and brain development.

There is considerable overlap between the genetic aetiology of monogenic forms of beta cell dysfunction and the more common polygenic type 2 diabetes. It is well established that rare mutations in key genes involved in pancreatic beta cell development and function result in monogenic or early-onset diabetes, whilst common genetic variation in the same genes predisposes individuals to adult-onset type 2 diabetes [8]. A genome-wide scan for association, performed in subjects with type 2 diabetes, revealed that *HHEX* is an excellent candidate susceptibility gene for type 2 diabetes [9]. A linkage disequilibrium block on chromosome 10q23 containing the insulin-degrading enzyme (*IDE*), kinesin family member 11 (*KIF11*) and *HHEX* genes has been shown to be associated with type 2 diabetes [9]. Given the essential role of *HHEX* in the development of the pancreas, its possible role in susceptibility to type 2 diabetes, and our knowledge of the overlap in the genetic aetiologies of type 2 diabetes and monogenic forms of pancreatic beta cell dysfunction, we hypothesised that mutations in *HHEX* may play a role in the pathogenesis of PNDM, MODY and hypoglycaemia of infancy (HI).

To evaluate the putative contribution of *HHEX* in monogenic forms of beta cell dysfunction we screened all four exons (including the intron–exon junctions) the 5' and 3' untranslated regions and the promoter of *HHEX* in unrelated subjects with unexplained PNDM ($n=30$), MODY ($n=32$) or HI ($n=45$). The clinical characteristics of the patients screened are shown in Table 1. *HHEX* is

located on chromosome 10q23.33 and encodes a 270 amino acid protein. The exon structure of *HHEX* was established using the GenBank sequence NM_002729.3 (for GenBank database see <http://www.ncbi.nlm.nih.gov/Genbank/>, last accessed in June 2007). M13-tailed primers were designed to amplify *HHEX* in five fragments (primer sequences are presented in Supplementary Table 1). Amplicons were sequenced on an ABI3700 DNA analyser using standard protocols. Sequence variants were confirmed by re-amplifying a fresh dilution of DNA. No mutations were identified that explained PNDM, MODY or HI. A total of eight rare SNPs were identified (Table 2). In one subject with HI a novel variant (c. 431C>G, p. F129L) was detected. The mutated residue is conserved across species and is located close to the homeobox region (K137–L196). To establish the frequency of this variant in normal controls, exons 2 and 3 were sequenced in samples from 96 blood donors of European descent from a panel of commercially available DNA. The F129L variant was not present in the 192 normal chromosomes tested. DNA was available from the affected mother and unaffected father. The F129L variant was present in the father but not the affected mother. Gender assignment of parental DNA was confirmed by an in-house sex-determining region Y (SRY) PCR assay (details available on request) to exclude the possibility of sample mix up. Therefore, since co-segregation with HI could not be demonstrated, it is not the cause of beta cell dysfunction in these individuals and therefore unlikely to be pathogenic. Seven other rare, silent or intronic variants were identified: c.–137G>A; c.–46C>A; c.–11G>A; c.–5C>G; c.318G>A, p.T106T; c.541–29T>G, c.592–22C>T. According to the dbSNP build 126 database (see <http://www.ncbi.nlm.nih.gov/projects/SNP/>, last accessed in June 2007) an rs number has been assigned to one of the eight variants identified (rs2275729, c.541–29T>G). The presence and frequency of these variants was determined in 96 blood donors of European descent. Both the c.–5C>G and c.–137G>A variants were not present in the control population. Parental DNA was available

Table 1 Clinical characteristics of subjects studied

Variable	PNDM	MODY	HI
<i>n</i>	30	32	45
Sex (% male)	70	31	49
Age at diagnosis	7.4 $\pm$ 8.2 ^a	17.1 $\pm$ 6.4 ^b	9.9 $\pm$ 0.06 ^a
Age at examination (years)	NA	27.8 $\pm$ 13.6	5.11 $\pm$ 6.08
Fasting glucose (mmol/l)	NA	NA	1.91 $\pm$ 0.56
BMI (kg/m ²)	NA	24.7 $\pm$ 13.6	NA
Current treatment (%)			
Insulin/OHA/diet/none/not known	100/0/0/0/0	50/15/28/0/6	
Diazoxide/pancreatectomy/octreotide/diet/none/not known			49/31/5/2/2/11
Birth weight (g)	2,256 $\pm$ 853	NA	3,436 $\pm$ 879
Under tenth percentile (%)	42	–	–
Over 70th percentile (%)	–	–	58

Data presented as means $\pm$ SD

NA data not available, OHA

oral hypoglycaemic agent

^a Age at diagnosis in weeks

^b Age at diagnosis in years

Table 2 *HHEX* gene variants detected in patients with PNDM ($n=30$), MODY ($n=32$) or HI ($n=45$)

SNP	Exon	Minor allele frequency (MAF)			rs number
c.-137G>A	Promoter	PNDM	0/60	0.00	NA
		MODY	1/64	0.01	
		HI	0/90	0.00	
		Controls	0/192	0.00	
c.-46C>A	Promoter	PNDM	2/60	0.03	NA
		MODY	1/64	0.01	
		HI	1/90	0.01	
		Controls	6/192	0.03	
c.-11G>A	Exon 1	PNDM	3/60	0.05	NA
		MODY	1/64	0.01	
		HI	8/90	0.09	
		Controls	6/192	0.03	
c.-5C>G	Exon 1	PNDM	1/60	0.01	NA
		MODY	0/64	0.00	
		HI	0/90	0.00	
		Controls	0/192	0.00	
c.318G>A, p.T106T	Exon 1	PNDM	1/60	0.01	NA
		MODY	1/64	0.01	
		HI	0/90	0.00	
		Controls	6/192	0.03	
c.431C>G, p.F129L	Exon 2	PNDM	0/60	0.00	NA
		MODY	0/64	0.00	
		HI	1/90	0.01	
		Controls	0/192	0.00	
c.541-29T>G	Intron 2	PNDM	7/60	0.12	rs2275729
		MODY	10/64	0.16	
		HI	8/90	0.09	
		Controls	17/192	0.09	
c.592-22C>T	Intron 3	PNDM	0/60	0.00	NA
		MODY	1/64	0.01	
		HI	0/90	0.00	
		Controls	1/192	<0.01	

NA not available

for the proband with the c.-5C>T variant and sequencing demonstrated that the variant was present in the unaffected father. Unfortunately, parental DNA was not available for the proband with the c.137G>A variant, and we are therefore unable to exclude a role for this variant in the aetiology of MODY.

Linkage to chromosome 10q23 has not been reported in the UK MODYX families previously included in a genome-wide scan for linkage [10]. Family samples and data are not available for the PNDM and HI samples, and so linkage to chromosome 10q23 cannot be excluded. It is therefore possible that some of our cases are linked to this region, which would suggest a role of known and unknown regulatory regions of *HHEX* in the pathogenesis of beta cell dysfunction.

In conclusion, despite the convincing emerging evidence for a role of common genetic variation at the *HHEX* locus in susceptibility to type 2 diabetes, our data suggest that it is unlikely that mutations in *HHEX* are a common cause of unexplained MODY, PNDM or HI.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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