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RESEARCH LETTER

GATA6 inactivating mutations are associated with heart defects and, inconsistently, with pancreatic agenesis and diabetes

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Abbreviations
GATA6 GATA-binding protein 6
NDM Neonatal diabetes mellitus

To the Editor: Pancreatic agenesis is an extremely rare cause of permanent neonatal diabetes mellitus (NDM) in humans. It can be associated with severe intrauterine growth retardation as well as a plethora of abnormalities or malformations in the heart, biliary tract, gut, thyroid or brain [1–3]. To date, mutations in three genes have been shown to cause pancreatic agenesis, namely, *PDX1* [1], *PTF1A* [2], and most recently *GATA6* [3], all three of which encode transcription factors. The *GATA6* study reported heterozygous coding mutations in a dozen individuals with permanent NDM presumed to be due to pancreatic agenesis [3]. The genetic evidence supporting the pathogenicity of these mutations seemed quite strong as the reported mutations had arisen de novo and none of these mutations was present in the 1,000 Genome Project database [3]. The authors concluded that GATA6 may have a key role in human pancreatic development, with strong implications for beta cell regenerative medicine in diabetes [3]. In the

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present study, we aimed to qualify this conclusion by reporting a case study of a non-consanguineous family.

We analysed the whole exome of two sisters from a French non-consanguineous family (electronic supplementary material [ESM] Methods). The youngest sister presented with severe intra-uterine growth retardation, pancreatic agenesis (with permanent NDM and exocrine pancreatic insufficiency requiring insulin treatment and enzyme replacement therapy), ventricular septal defects requiring surgery during the neonatal period, gall bladder agenesis, bicornuate uterus and neonatal pancytopenia (Table 1). Her older sister presented with patent ductus arteriosus requiring surgery when she was 1 year old, gall bladder agenesis and proctorrhagia (Table 1). Contrary to her sister, she did not present with NDM. At 13 years, she showed normal fasting plasma glucose level (5.4 mmol/l) but she had high glucose values 2 h after an oral glucose challenge (14.2 mmol/l). MRI examination revealed a small pancreas, suggesting pancreatic hypoplasia, but no enzyme replacement therapy was needed. Of note, another pregnancy of the mother was terminated at 22 weeks of amenorrhea, as the male fetus presented with severe hypoplasia of the left heart. The mother has mild valvular abnormalities whereas the father is healthy (Table 1). We had previously searched for mutations in several genes known to be involved in NDM in the two sisters (ESM Methods). By whole-exome sequencing, we identified in both sisters a novel heterozygous deletion of two nucleotides in the fifth exon of *GATA6* (c.1504_1505del; p.Lys502Aspfs*5), which leads to a premature termination codon (ESM Fig. 1). The presence of the mutation was investigated in parents, the mother's parents and 378 French adults with normal fasting glucose (ESM Methods). The mutation was not present in the controls. Interestingly, the mother carries the frameshift mutation (ESM Fig. 1), however neither the father nor the mother's parents are carriers, implying that the mutation had arisen de novo in the mother, who is not diabetic. Indeed, since we started to study the family in 1998 (she is now 49), her metabolic profile has always been normal. Therefore, in contrast to the study by Allen et al [3], we demonstrate that *GATA6* mutations do not always occur de novo in affected children. Furthermore, the penetrance of the p.Lys502Aspfs*5 frameshift mutation for diabetes is incomplete, whereas it seems complete for heart anomalies (Table 1). In fact, there is a spectrum of phenotypes in the three carriers of the family. This statement is supported by several studies that have reported loss-of-function mutations in *GATA6* causing human congenital heart diseases (e.g. persistent or patent truncus arteriosus, atrial septal defects, pulmonary valve stenosis, tetralogy of Fallot) [4, 5]. Importantly, diabetes was never reported in the analysed carriers [4, 5]. It shows that when patients with *GATA6* mutations have been primarily selected for congenital cardiac diseases but not for NDM, they are not usually diabetic. In the study by Allen et al it is noteworthy that 14 out of 15 carriers of a *GATA6* mutation presented with severe cardiac malformations [3]. The only patient without a heart anomaly carried a non-coding splice-site mutation, of which deleterious functionality was not proven [3].

Table 1 Clinical data of the three carriers of the *GATA6* p.Lys502Aspfs*5 mutation

Clinical characteristics	Proband	Older sister	Mother
Pancreas agenesis	x	–	–
Diabetes	x (permanent NDM)	x (onset at 13 years)	–
Cardiac anomaly	x (requiring surgery)	x (requiring surgery)	x
Gall bladder agenesis	x	x	?
Bicornuate uterus	x	–	–
Intra-uterine growth retardation	x	–	–
Neonatal pancytopenia	x	–	–
Proctorrhagia	–	x	–
p.Lys502Aspfs*5 heterozygous mutation	x	x	x

The father is healthy and does not carry the mutation

Based on these findings, we needed to question the proposed key role of GATA6 in pancreatic beta cell development using public and in-house expression databases. First, according to public databases, *GATA6* expression is unambiguously weak in human whole pancreas (Gene Portal System, http://biogps.org/, last accessed March 2012). In our experiments on isolated human pancreatic islets and beta cells (ESM Methods), this was also the case (ESM Fig. 2a). In a rat model of pancreatic endocrine cell differentiation and proliferation (ESM Methods), *Gata6* expression is constantly weak from precursor cells to mature beta cells (ESM Fig. 2b). Moreover, between embryonic days E13.5 and E17, there is no significant change in *Gata6* expression in pancreatic epithelium (ESM Fig. 2c). *GATA6* expression is also not significantly different between pancreatic epithelium and mesenchyme in rat and human samples, at embryonic day E12 and at ~7–11 weeks of development, respectively (ESM Methods, ESM Fig. 2d, e). Therefore, our results do not suggest a key role of GATA6 in the specific beta cell lineage, but these results would need further investigation as they are limited to gene expression analyses. In mice, in which GATA6 has been further studied, expression-based analyses suggest that GATA6 could play a role in very early pancreas specification [6–8]. Nevertheless, it has been reported that transgenic mouse embryos expressing a GATA6–engrailed dominant repressor fusion protein in the pancreatic epithelium and in islets revealed two distinct phenotypes, either pancreatic agenesis or a simple reduction in pancreatic tissue [6], implying a putative spectrum of phenotypes, as we noted in our

family. Therefore, the role of GATA6 in pancreatic morphogenesis is very variable, which could be due to various epigenetic effects or partial replacement of GATA6 by other transcription factors from the GATA family that are expressed in the pancreas (e.g. GATA4).

Taken together, our results and other genetic studies indicate *GATA6* inactivating mutations are associated with heart defects and, inconsistently, with pancreatic agenesis and diabetes. We suggest that in carriers of a *GATA6*-inactivating mutation, diabetes (when present) is due to a severe multi-tissue developmental defect rather than a specific impairment of beta cell lineage. The present study is limited by a lack of functional analyses of our identified frameshift mutation. Nevertheless, a de novo deletion which leads to a premature termination codon at the 514th amino acid has previously been reported in an NDM patient with pancreatic agenesis [3]. Our deletion, which leads to a termination codon at the 507th amino acid, is likely to have a functional effect similar to that reported by Allen et al [3].

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Contribution statement AB and PF designed the study, interpreted the data and wrote the manuscript; MV, BG, MP and PC analysed the clinical data and revised the manuscript; LR, JK-C, FP and RS contributed to acquisition of gene expression data (in rat and/or in human) and revised the manuscript; OS and FD performed the bioinformatic analyses and revised the manuscript; ED and MH performed the sequencing and revised the manuscript; PF is the guarantor of the manuscript. All authors of the paper have read and approved the final version submitted.

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