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GATA6 haploinsufficiency causes pancreatic agenesis in humans

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Abstract

Understanding the regulation of pancreatic development is key for efforts to develop new regenerative therapeutic approaches for diabetes. Rare mutations in *PDX1* and *PTF1A* can cause pancreatic agenesis, however, most instances of this disorder are of unknown origin. We report *de novo* heterozygous inactivating mutations in *GATA6* in 15/27 (56%) individuals with pancreatic agenesis. These findings define the most common cause of human pancreatic agenesis and establish a key role for the transcription factor GATA6 in human pancreatic development.

The genetic basis for most instances of pancreatic agenesis is unknown; mutations in *PDX1* (MIM#260 370) and *PTF1A* (MIM#609 069) have been reported in only five families[1-3]. We studied a cohort of 27 individuals with pancreatic agenesis, defined as neonatal diabetes requiring insulin treatment and exocrine pancreatic insufficiency requiring enzyme replacement therapy, born to non diabetic parents. In all subjects for whom pancreatic imaging was performed (*n*=21), there was a complete absence (*n*=16) or marked hypoplasia of the pancreas. We found one affected subject to have a homozygous *PTF1A* splice site mutation, but we identified no mutations in *PDX1* in this cohort. A common recessive

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AUTHOR CONTRIBUTIONS: S.E., S.E.F., J.F. and A.T.H. designed the study. R.C. performed the exome sequencing and the structural modeling. H.L.A. did the bioinformatic analyses. E.D.F. and S.E.F. did the Sanger sequencing analysis and the interpretation of the resulting data. C.S.-S. and A.T.H. analyzed the clinical data. I.A. and J.F. performed the functional studies. H.L.A., C.S.-S., J.F., A.T.H. and S.E. prepared the draft manuscript. All authors contributed to the discussion of the results and the manuscript preparation.

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etiology was unlikely in the remaining 26 affected subjects, as only 5 were known to have consanguineous parents, and none had affected siblings. We therefore hypothesized that pancreatic agenesis resulted, at least in some individuals, from *de novo* heterozygous mutations.

We initially sought to find *de novo* mutations by sequencing the exomes of two affected individuals and their unaffected parents. We performed exome capture by in-solution hybridization followed by massively parallel sequencing (Supplementary Methods) and generated between 4.2 and 7.0 billion bases covering 87-90% of the targeted Consensus Coding Sequence bases with at least ten reads (Supplementary Table 1). We called the variants using the Genome Analysis Toolkit and filtered them by removing synonymous variants, variants present in the dbSNP or 1000 Genomes Project databases and variants identified in either parent. This filtering reduced the number of potentially pathogenic *de novo* mutations to a single heterozygous *GATA6* mutation in each subject (Supplementary Table 2). The two variants seen in *GATA6* were a missense substitution (p.Thr452Ala) and an 8-bp frameshift deletion (c.1448_1455del). Sanger sequencing confirmed that the mutations were present in the two affected subjects but not in their unaffected parents.

We identified a further 12 heterozygous mutations in 13 affected individuals by sequencing the coding exons and intron-exon boundaries (exons 2-7; the primer sequences used are listed in Supplementary Table 3) of *GATA6* in the remaining 24 individuals with pancreatic agenesis of unknown genetic cause. We found these to be missense mutations, frameshift insertions and/or deletions, or splicing mutations (Fig. 1a and Supplementary Table 4). Therefore, we found *GATA6* mutations to be present in the majority (15/27 (56%)) of subjects with pancreatic agenesis.

The genetic evidence to support the pathogenicity of the *GATA6* mutations is very strong. First, in 12/15 affected subjects, both parental DNA samples were available, and a combination of Sanger sequencing and a microsatellite analysis (Supplementary Methods) established that in all of these subjects, the mutations had arisen *de novo*. Second, five mutations are insertions or deletions resulting in a premature termination codon and three are predicted to cause aberrant splicing (Supplementary Methods). Third, none of the mutations has been reported in 1,094 population controls (from the 1000 Genomes Project database).

GATA6 is a transcription factor that includes two tandem GATA zinc fingers that together function as a DNA-binding domain. All six missense mutations that we found affect residues on the DNA binding surface (Supplementary Fig. 1) that are conserved across vertebrate orthologs of GATA6 and in all human GATA proteins (Supplementary Fig. 2). Consistent with this observation, GATA6 proteins carrying four different missense mutations did not bind to GATA6 recognition sites and were unable to activate a GATA6-responsive promoter (Fig. 1b-d). Thus, genetic and molecular studies indicate that pancreatic agenesis is caused by inactivating *GATA6* mutations.

In addition to pancreatic agenesis (Supplementary Fig. 3), we frequently observed other phenotypes in individuals with *GATA6* mutations (Fig. 2). The most common phenotypes

(seen in 14/15 affected subjects) were congenital cardiac defects, particularly outflow tract malformations such as atrial septal defects, ventral septal defects or tetralogy of Fallot. A clinical syndrome of pancreatic agenesis and congenital heart malformations has been previously described in six individuals[4], but the only prior reports of *GATA6* mutations were in nine individuals with congenital heart malformations who accounted for 1.5% of all individuals tested in those studies[5-7]. There is no previous report of endocrine or exocrine pancreatic failure in any individual with a *GATA6* mutation. Other features present in our cohort included congenital biliary tract anomalies, gut developmental disorders including hernia, neurocognitive abnormalities and additional endocrine abnormalities (Supplementary Table 4). These findings, therefore, implicate GATA6 in the development of multiple organ systems, including the biliary tract, gut, pituitary and thyroid, as well as the pancreas.

It was notable that the 11 affected subjects without *GATA6* mutations rarely (2/11) had extra-pancreatic features and were more likely to be born to consanguineous parents than individuals with these mutations (5/11 subjects without *GATA6* mutations compared to 0/15 subjects with *GATA6* mutations), suggesting there is at least one as yet unidentified recessive subtype of isolated pancreatic agenesis.

This work uncovers an essential function of GATA6 in human pancreas development. In contrast to the observations reported here, *Gata6* heterozygous-null mice show no obvious phenotype[8], whereas *Gata6* homozygous-null mice die during gastrulation, thus precluding the investigation of the role of this transcription factor in mouse pancreatic organogenesis[8]. Earlier studies have nevertheless provided indirect evidence that *Gata6* has a role in mouse pancreas development. *Gata6* is expressed in embryonic pancreatic multipotent progenitors[9], and one study using tetraploid complementation revealed fewer cells expressing *Pdx1* in the ventral foregut of *Gata6*$^{-/-}$ embryos compared to heterozygous embryos[9]. Another study showed that transgenic overexpression of a chimeric protein formed by Gata6 and the engrailed repressor inhibits pancreas development[10]. A conclusive demonstration that Gata6 is essential for the development of mouse pancreas requires further work.

The functional studies of missense DNA-binding-domain mutations and the identification of frameshift and splicing mutations suggest that heterozygous *GATA6* mutations result in loss of function and cause pancreatic agenesis through haploinsufficiency. This is in contrast to *PDX1* and *PTF1A* mutations, where pancreatic agenesis results from a complete absence of functional protein as a result of homozygous inactivating mutations. Homozygous-null mouse models closely resemble the human phenotypes caused by biallelic mutations in *PDX1, PTF1A, NEUROD1* and *NEUROG3* (refs. 1,2,11,12). In contrast, as is seen with GATA6, discrepant phenotypes between mouse and man are seen in haploinsufficient forms of monogenic diabetes resulting from mutations in the transcription factor genes *HNF1A*, *HNF4A* or *HNF1B* (reviewed in ref. 13).

Current efforts to develop replacement therapies for diabetes focus on inducing functional endocrine cells from adult somatic cells through the expression of key transcription factors[14] or through recapitulation of human pancreatic development from pluripotent cells[15]. Both strategies have largely exploited the knowledge of transcriptional regulators of pancreatic

differentiation in mice. The key role of GATA6 in the development of the human pancreas provides new knowledge to develop tools for regenerative medicine in diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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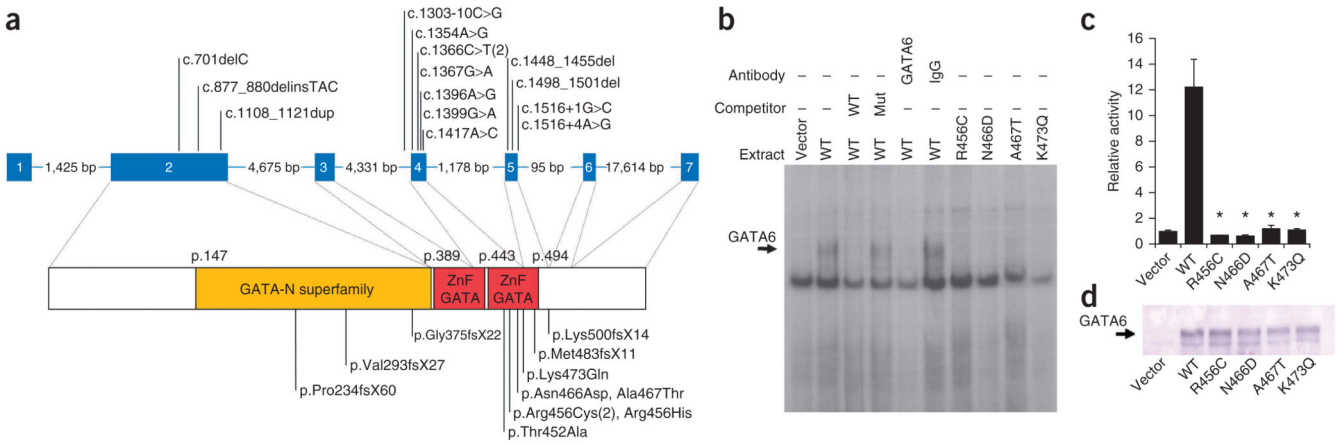

Figure 1. *GATA6* mutations causing pancreatic agenesis

(a) Genomic and protein positions of the 14 *GATA6* mutations. (b) Electrophoretic mobility shift assay showing that mutations abolish the binding to a predicted GATA6 binding sequence in the pancreatic *HNF4A* proximal promoter. We used nuclear extracts from cells transfected with a control vector or vectors expressing GATA6 wild-type (WT) or mutant (Mut) proteins (described at the level of the protein changes shown in fig. 1a). Only wild-type GATA6 formed a retardation complex (arrow) that disappeared after preincubation with unlabeled wild-type but not mutated double-stranded oligonucleotide probes (competitor) and with GATA6 antiserum. Identical results were observed with *in vitro* translated wild-type and mutant GATA6 proteins using the TFF2 GATA6 binding site (data not shown). (c) Mutated GATA6 does not activate the GATA6-responsive *WNT2* promoter-luciferase gene in HeLa cells. *Statistically significant difference in activity as compared to wild type ($P < 0.0001$). (d) Protein blot showing comparable expression of wild-type and mutant GATA6 proteins.

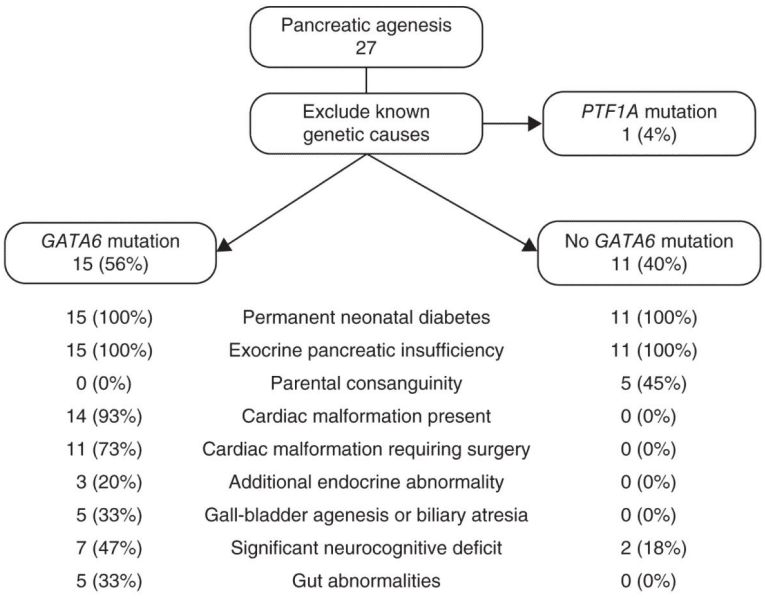

Figure 2. Clinical characteristics of the pancreatic agenesis cohort

In addition to pancreatic agenesis, *GATA6* mutations cause several other phenotypes. The precise malformations seen in each subject are listed in Supplementary Table 4.

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