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RESEARCH LETTER

Neurogenin 3 is important but not essential for pancreatic islet development in humans

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Abbreviations

bHLH Basic helix-loop-helix
NEUROG3 Neurogenin 3

To the Editor: Based on the findings that *Neurog3*-null mice fail to develop pancreatic islets [1] and lack enteroendocrine cells [2], this basic helix-loop-helix (bHLH) transcription factor is considered to be essential for differentiation of endocrine cells both in the pancreas and in the gut [3]. Limited data from human fetuses suggest that transient expression of *NEUROG3* in pancreatic progenitor cells from approximately 7 to 8 weeks post conception is followed by beta cell differentiation and immature islet formation by week 12 of fetal development [4, 5].

Homozygous loss-of-function mutations in *NEUROG3* were first identified in patients with a rare form of congenital

malabsorptive diarrhoea that was due to enteric anendocrinosis [6]. Unexpectedly, the patients did not present with neonatal diabetes, suggesting that pancreatic islet development was at least partially spared, although they developed diabetes during late childhood. The authors hypothesised that an unidentified factor might compensate for the absence of functional neurogenin 3 (*NEUROG3*) and result in the production of some functional beta cells. Further studies showed that the reported mutations were hypomorphic rather than null mutations. Therefore, differences in *NEUROG3* gene dosage requirements could explain the observed differential effect on the intestinal and pancreatic phenotypes [7].

We recently identified biallelic null *NEUROG3* mutations in a patient with enteric anendocrinosis and permanent neonatal diabetes [8]. Although pancreatic tissue was not available for direct analysis, the presence of detectable blood C-peptide indicated that there was some endogenous insulin production at 4.5 years of age. Similar findings were later reported in a different patient [9]. Consequently, we hypothesised that, despite the pivotal role of *Neurog3* in pancreatic endocrine development in mice, complete *NEUROG3* deficiency does not necessarily imply an absolute insulin deficiency in humans and, therefore, affected patients may present with diabetes at any age.

To test this hypothesis, the single coding exon of *NEUROG3* was amplified and sequenced from genomic DNA as previously described [8] in a further three probands with congenital malabsorptive diarrhoea and diabetes, regardless of the age at diagnosis of the latter. This study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee. Written informed consent was obtained from the parents/guardians of the patients. Previously described homozygous missense mutations were identified in the three cases and a non-diabetic sibling of proband 3. Clinically unaffected siblings of probands 1 and 2 (one each) were unavailable for evaluation. Genetic findings

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Table 1 Clinical and molecular findings in patients with NEUROG3 deficiency

| | Proband 1 | Proband 2 | Proband 3 | Sibling of proband 3 | Ref. [6] | Ref. [6] | Ref. [6] | Ref. [8] | Ref. [9] | Ref. [10] | Ref. [11] |
|---|--|-----------------------|-----------------------|-----------------------|--|-----------------------|-----------------------|-----------------------------------|---|-------------------------|---|
| Sex | Female | Male | Female | Male | Male | Male | Male | Female | Female | Female | Female |
| Age at report | 24 years | 17 years | 18 years | 23 years | Deceased at 2 years 11 months (sepsis following orthotopic liver-intestinal transplant at 2 years) | 8 years | 9 years | 7 years | Deceased at 10 months (chronic cholestatic liver disease secondary to parenteral nutrition) | 20 months | 3 months |
| Consanguinity | No | No | Yes | Yes | Yes | Not reported | Not known | No | Denied but from same region in Ecuador | Yes | Yes |
| Mutations in NEUROG3 gene | c.404T>C/ c.404T>C | c.404T>C/ c.404T>C | c.319C>A/ c.319C>A | c.319C>A/ c.319C>A | c.319C>A/ c.319C>A | c.278G>T/ c.278G>T | c.278G>T/ c.278G>T | c.82G>T/ c.404T>C | c.367G>T/c.367G>T | c.510dupG/ c.510dupG | Homozygous mutation, not reported |
| Predicted change on NEUROG3 protein | L135P/L135P | L135P/L135P | R107S/R107S | R107S/R107S | R107S/R107S | R93L/R93L | R93L/R93L | E28X/L135P | E123X/E123X | S171fsX68/ S171fsX68 | Not reported |
| Birthweight (g) | 2,250 | 2,250 | 2,575 | 3,100 | 2,530 | 2,720 | 2,334 | 1,910 | 1,960 | 3,040 | Not reported |
| Congenital malabsorptive diarrhoea | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Enteric anendocrinosis | Unknown | Unknown | Unknown | Unknown | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Pancreatic exocrine insufficiency | Yes | Yes | No | No | No | No | No | No | Yes | Yes | Not reported |
| Pancreatic imaging | Hypoplastic pancreas | Normal | No | No | Not reported | Not reported | Not reported | Normal | Normal | Not reported | Not reported |
| Diabetes mellitus | Yes | Yes | Yes | No | No | Yes | Yes | Yes | Yes | No | Not reported |
| Age at presentation | 3 weeks | 13 years | 12 years | – | – | 8 years | 8 years | 3 weeks | 5 months | – | – |
| Subtype | Transient neonatal diabetes, relapsed at 6 years | Permanent diabetes | Permanent diabetes | – | – | Permanent diabetes | Permanent diabetes | Permanent neonatal diabetes | Permanent neonatal diabetes | – | – |
| Serum C-peptide (nmol/l) | 0.04 (postprandial) | 0.4 (postprandial) | 0.4 (fasting) | 0.5 (fasting) | Not reported | Not reported | Not reported | 0.5 (postprandial) | 0.6 (feeding status not reported) | Not reported | Not reported |

and the most significant clinical features of these patients are depicted in Table 1, along with similar data from all previous case reports on children with biallelic *NEUROG3* mutations for comparison [6, 8–11]. Early-onset severe malabsorptive diarrhoea was a common finding in the 11 patients, with enteric anendocrinosis demonstrated in all cases where this condition was investigated on intestinal biopsies. Absence of enteroendocrine cells was not specifically investigated in any of the four patients included in this study, but it has previously been reported in patients with the same *NEUROG3* mutations [6, 8]. Typically, the intestinal failure requires long-term parenteral nutrition initially, but oral feedings are increasingly tolerated and parenteral nutrition can eventually be discontinued. Low fecal elastase or trypsin levels have been reported in some but not all cases, suggesting that pancreatic exocrine insufficiency represents a functional defect secondary to a lack of cholecystokinin and secretin, the enteric hormones that stimulate pancreatic exocrine secretion [10].

No evident genotype–phenotype correlation exists between *NEUROG3* mutations and pancreatic endocrine function. *NEUROG3* deficiency can lead to a number of different phenotypes of diabetes, including permanent neonatal diabetes, relapsing transient neonatal diabetes and childhood-onset permanent diabetes. In either case, insulin production is partially spared, as indicated by detectable, although relatively low, C-peptide levels. Furthermore, none of the diabetic patients has ever presented with severe hyperglycaemia or ketosis, not even during acute intercurrent illness, which also supports the persistence of some residual endogenous insulin production. This relative insulin deficiency might be due in part to a lack of incretin hormones (glucagon-like peptide 1, glucose-dependent insulinotropic peptide) from the gut, a hypothesis that has not been tested in *NEUROG3*-deficient patients so far. However, diabetes does not universally present during childhood or young adulthood, as evidenced by the non-diabetic 23-year-old male patient, although it might develop later on. In addition, the same genotype can cause different diabetic phenotypes both between and within kindreds. Overall, the data available suggest that either genetic or non-genetic modifiers are likely to play a role in the penetrance and expressivity of diabetes in *NEUROG3* biallelic mutation carriers.

The presence of some residual endogenous insulin secretion in patients with *NEUROG3* deficiency suggests that, in contrast to mice [3], a redundant *NEUROG3*-independent pancreatic endocrine developmental pathway might exist in humans so that *NEUROG3* is not absolutely required for differentiation of pancreatic progenitors into islet cell precursors. In this sense, it has recently been reported that *ASCL1B*, another bHLH transcription factor, initiates the endocrine cell differentiation programme instead of *NEUROG3* in zebrafish [12]. Further research in this area will help clarify the exact role of *NEUROG3* in human pancreatic development.

In summary, *NEUROG3* deficiency produces a rare clinical syndrome characterised by severe malabsorptive diarrhoea from early life and mild diabetes with a variable age of onset. This finding suggests that *NEUROG3* is important but not essential for beta cell development and function in humans.

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Contribution statement ORC, SEF, SE, and ATH designed the study. ORC, EC, SEF, and JLG acquired and/or analysed the data. ORC wrote the manuscript. All authors reviewed and revised the manuscript critically. All authors approved the final version of the manuscript. ATH is the guarantor of this work and, as such, had full access to all of the study data and takes full responsibility for the integrity of the data.

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