Short report

Transcription factor gene MNX1 is a novel cause of permanent neonatal diabetes in a consanguineous family

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Abstract

Aim. – Permanent neonatal diabetes mellitus (PNDM) is a rare monogenic form of non-autoimmune diabetes. Genetic defects have been identified in ~ 60% of cases, with mutations in ABCC8, KCNJ11 and INS being the most frequent causes of PNDM. Recognition of genetic subtypes strongly impacts on both patients’ care and family counseling. This study aimed to identify the genetic aetiology of PNDM in a diabetic girl born of consanguineous parents.

Methods. – DNA samples from both the proband and her non-diabetic parents were analyzed for homozygosity mapping, using Illumina Infinium 660 K SNP microarrays, focusing on the runs of homozygosity (ROHs) detected only in the patient. Standard Sanger sequencing of candidate genes (MNX1 and GATA6) present in the ROHs was subsequently performed, as well as expression analyses on human embryonic and adult pancreatic islet samples.

Results. – A putative causal homozygous mutation in the transcription factor gene MNX1 (c.816C>A/p.Phe272Leu) was identified in the PNDM patient, who was clinically diagnosed as a typical case of PNDM with no developmental pancreatic defects or other clinical features. The probable deleterious mutation was located within the MNX1 homeodomain helix 2 that is highly conserved between species. In human embryonic pancreatic islet samples, it has been shown that MNX1 expression is significantly enriched in pancreatic epithelium compared with mesenchyme, suggesting a role for MNX1 in human pancreatic beta-cell development.

Conclusion. – This study found a new putative cause of PNDM in a consanguineous family. Replication in other cohorts would help to clarify the clinical spectrum of MNX1 mutations in PNDM patients.

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Keywords: Genetics; Homozygosity mapping; MNX1; Neonatal diabetes mellitus; Transcription factor

Résumé

Le facteur de transcription MNX1 est une nouvelle cause de diabète néonatal permanent dans une famille consanguine.

Objectif. – Le diabète néonatal permanent (DNP) est une forme rare, monogénique de diabète non-autoimmune. Un défaut génétique a été identifié dans ~ 60 % des cas (les mutations des gènes ABCC8, KCNJ11 et INS étant les principales causes de DNP). Connaître le sous-type génétique chez les patients a un impact important sur le plan thérapeutique et pour le conseil génétique aux familles. Notre étude a eu pour but d’identifier l’étiologie génétique du DNP chez un patient né de parents consanguins, ne présentant pas d’autres anomalies.

Abbreviations: MNX1, motor neuron and pancreas homeobox protein 1; NDM, neonatal diabetes mellitus; ROH, run of homozygosity; SNP, single nucleotide polymorphism.

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Méthodes. – Les échantillons ADN du patient et des deux parents non diabétiques ont été analysés au moyen d’une puce ADN de haute densité (Illumina Infinium 660K-SNP BeadChip) afin de rechercher les régions d’homozygotie (ROH) spécifiques à l’enfant. Un séquençage ciblé des gènes MNX1 et GATA6, localisés dans ces ROHs uniques au patient, a été réalisé. Nous avons intégré à notre analyse les données d’expression génique obtenues à partir d’échantillons humains d’îlots pancréatiques embryonnaires et adultes.

Résultats. – Une mutation homozygote du gène MNX1 (c.816C>A/p.Phe272Leu) a été identifiée chez le patient avec DNP sans anomalie de développement du pancréas ni autre malformation. Cette mutation, prédite délétère et potentiellement causale, est localisée dans un domaine protéique (hormodomaine) très conservé entre espèces. En outre, nous montrons que le facteur de transcription MNX1 est fortement exprimé dans l’épithélium pancréatique embryonnaire humain (en comparaison au mésenchyme), impliquant MNX1 dans le développement de la cellule beta-pancréatique chez l’homme.

Conclusion. – Nous rapportons une nouvelle cause de DNP à partir de l’étude d’une famille consanguine. La réplication de ce résultat dans d’autres cohortes de patients permettra de mieux évaluer le spectre clinique des mutations MNX1 associées au DNP.

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Mots clés : Génétique ; Homozygotie ; MNX1 ; Diabète néonatal ; Facteur de transcription

1. Introduction

Neonatal diabetes mellitus (NDM), defined by mild-to-severe hyperglycaemia within the first few months of life, is a rare monogenic disease (affecting ~1:100,000 live births) with various clinical presentations and numerous genetic aetiologies [1]. NDM may be permanent (PNDM) or transient (TNDM), although TNDM patients can relapse as chronic diabetes several years following the initial remission [1]. The most frequent causes of PNDM are mutations in the ABCC8 and KCNJ11 genes, encoding two subunits of the pancreatic ATP-dependent potassium channel, and in the INS gene encoding insulin [1,2]. Rarer genetic aetiologies have been reported in syndromic forms of PNDM associated with pancreatic developmental defects and/or malformations of other organs [1]. These include recessively inherited mutations in the transcription factor genes PDX1 and PTF1A (causing pancreatic agenesis/hypoplasia) and RFX6 (causing the Mitchell–Riley syndrome), and in EIF2AK3/PERK (causing the Wolcott–Rallison syndrome) [1,3]. Recently, homozygous mutations in GATA6 have also been reported in NDM patients presenting with pancreatic agenesis/hypoplasia, heart defects and sometimes other clinical features [4,5]. In consanguineous families, NDM is mostly due to homozygous mutations mapping to large runs of homozygosity (ROHs) encompassing several megabases (Mb) [3,6].

The present study aimed to identify the genetic aetiology of PNDM in an unelucidated patient, who was born of consanguineous parents, through high-resolution homozygosity mapping and targeted gene sequencing.

2. Patient and methods

2.1. Clinical features of the proband

The proband, a girl of Egyptian origin, was diagnosed at 17 days of life with diabetes requiring insulin therapy up till now (insulin dosage: 0.8 units/kg/day at her last examination at 18 months of age). At birth she presented with severe intrauterine growth retardation (Table S1; see supplementary material associated with this article online). Pancreatic morphology was normal on ultrasound scan, and no other malformations were reported. Her parents are first cousins and are not known to be diabetic, although their glucose tolerance was not tested. The proband has no siblings (Fig. S1; see supplementary material associated with this article online). This family was referred to the French NDM study group. Informed consent for genetic analysis was obtained from the parents.

2.2. Previous genetic screening

An initial search for mutations in ABCC8, KCNJ11 and INS, and for abnormalities in chromosome 6q24, identified no putative causal mutations or abnormalities in the proband.

2.3. Homozygosity mapping

Hybridization of genomic DNA from the patient and both parents was performed using Infinium 660 K SNP microarrays according to the manufacturer’s instructions (Illumina Inc., San Diego, CA, USA). After applying standard quality-control procedures, the number of SNPs available for analysis was 547,731, with a genotype call rate of >99%. Detection of ROHs was performed using PLINK v1.07 (‘homozyg’ option). In particular, the focus was on ROHs >2.5 Mb and 100 SNPs in length, as these are more likely to result from consanguineous parents [7]. Subsequently, the focus was on ROH segments that were identified only in the child, as these were more likely to harbour the causal recessive mutation. Finally, the gene content in these regions was investigated.

2.4. Sanger sequencing of MNX1 and GATA6

MNX1 is located on human chromosome 7q36 and encodes a 401 or 189 amino-acid protein (NM_005515.3 [3 coding exons] → NP_005506.3 or NM_001165255.1 [3 coding exons] → NP_001158727.1). GATA6 is located on human chromosome 18q11.1-q11.2 and encodes a 595 amino-acid protein (NM_005257.4 [5 coding exons] → NP_005248.2). All exons, as well as the flanking exon-intron boundaries and proximal promoters, boundaries and proximal promoters were amplified by polymerase chain reaction (PCR) and bidirectionally sequenced using the automated Applied Biosystems 3730xl DNA Analyzer (Life Technologies Corp., Carlsbad, CA, USA). Electrophoregram readouts were assembled and analyzed using the Applied Biosystems Variant Reporter software (Life
Human embryonic pancreases and expression analysis

Human fetal pancreases were extracted from tissue fragments obtained immediately after elective termination of pregnancy. Terminations were performed by aspiration at between 7 and 11 weeks of fetal development in compliance with French legislation and the guidelines of our institution, as described elsewhere [8]. Epithelium and mesenchyme were mechanically separated using needles. Total RNA was extracted using the RNeasy Micro Kit (Qiagen, Hilden, Germany) and used for Affymetrix analysis using an HG-U133_Plus_2.1 microarray, as per the manufacturer’s protocols. Expressions were computed using the Affymetrix GeneChip Robust Multi-Array Analysis method, and normalization was performed using the quantile method. Both methods were implemented using the Bioconductor software package in R. One probe was analyzed for MNX1 (214614_at).

3. Results

Using SNP microarray analysis, it was found that the percentage of genomic ROH segments > 1 Mb in the proband, which estimates the inbreeding coefficient F(ROH), was 5.7%, confirming that both parents were indeed first cousins (range: 5–20%) [7]. A total of 17 genomic ROHs > 2.5 Mb (totaling 117.8 Mb) was found in the proband’s genome after filtering ROHs that overlapped with those of either her father or mother (Table S2; see supplementary material associated with this article online).

The 885 genes present in these 17 regions were listed and examined for possible candidates based on the following criteria:

- genes previously reported in NDM;
- genes known to play a key role in insulin production and secretion from pancreatic beta-cells;
- genes known to be involved in the development of pancreatic beta-cells.

From this gene list, two possible candidate genes were identified:

- GATA6, encoding the GATA-binding protein transcription factor 6, known to be involved in severe forms of PNDM [4,5];
- MNX1, encoding the motor neuron and pancreas homeobox protein 1 [also known as ‘homeobox HB9 (HLXB9)], shown

Fig. 1. The MNX1 p.Phe272Leu mutation identified in the patient’s family and its position in the homeobox domain of MNX1. Upper: electropherograms of the MNX1 mutation (red arrows) found in a homozygous state in the PNDM patient and heterozygous state in both her parents. Lower: schematic representations of the MNX1 gene (NM_005515.3) and protein (NP_005506.3) showing interspecies conservation of the MNX1 homeobox domain helix 2 (mutation indicated by red stars).
to be involved in pancreas development and beta-cell specification in mice and zebrafish [9].

The exons, and the exon-intron junctions and proximal promoter of both GATA6 and MNX1 genes were sequenced in the proband. No rare mutations were identified in GATA6, whereas a novel homozygous missense mutation was detected in the second exon of MNX1: c.816C>A/p.Phe272Leu (transcript number: NM_005515.3; Fig. 1). Both parents were found to carry the same mutation in a heterozygous state. The genomic location of MNX1 was on chromosome 7q36 (chr7:156,797,547-156,803,347 from UCSC/GRCh37/hg19), which mapped to an ROH segment of ∼4 Mb detected in the patient’s DNA (Table S2; see supplementary material associated with this article online).

Such an MNX1 p.Phe272Leu mutation has previously been reported by either the 1000 Genomes Project or the NHLBI Exome Sequencing Project, or in 88 other patients in the French NDM cohort (data not shown here). The homozygous mutation results in a substitution of a proline residue within helix 2 of the MNX1 homeodomain, which is highly conserved among mammalian species and some invertebrates (Fig. 1). According to Align-GVGD, PolyPhen-2 and SIFT software [10–12], the mutation is thought to be possibly damaging or deleterious.

No additional rare coding MNX1 mutations were found in the 88 unelucidated NDM patients in the French NDM study cohort.

In publicly available gene-expression databases (Gene Portal System, www.biogps.org), MNX1 is expressed in human pancreatic islets. In addition, according to the present authors’ previous whole-genome expression experiments [13], MNX1 is expressed in human pancreatic islets and flow-sorted beta-cells (data not shown). Moreover, human embryonic pancreatic islet samples have shown that MNX1 expression is significantly enriched in pancreatic epithelium compared with mesenchyme at ∼7 to 11 weeks of development ($P=0.00019$; Fig. S2; see supplementary material associated with this article online), thereby suggesting a key role of MNX1 in human pancreatic beta-cell development.

4. Discussion

In the present study using a combination of high-resolution homozygosity mapping and candidate gene sequencing, a putative causal homozygous mutation in MNX1 (p.Phe272Leu) was identified in a PNDM patient from a consanguineous family. It is noteworthy that this patient was clinically diagnosed as a typical case of PNDM with no developmental defects of the pancreas or other clinical features (Similar independent findings on the putative contribution of MNX1 to PNDM were presented by Professor A.T. Hattersley at the Third Meeting of the European Association for the Study of Diabetes Study Group on the Genetics of Diabetes (EASD–SGGD) in Slovakia in 2011).

Previous reports demonstrated that MNX1 was prominently expressed in adult human pancreatic islets as well as in developing and mature mouse pancreatic beta-cells [9,14]. Mnx1-deficient mice showed pancreatic dorsal-lobe agenesis and small pancreatic islets [9,15], while Mnx1 gain-of-function in the pancreas led to aberrant pancreatic development, suggesting tight temporal regulation of Mnx1 expression during the early stages of pancreatic development [16]. In Mnx1-deficient zebrafish, beta-cell precursors gave rise to alpha-cells, suggesting that Mnx1 might promote beta-cell fate and inhibit alpha-cell fate in endocrine progenitors [17]. Furthermore, a recent study showed that Mnx1 is a major direct target of PTF1A in pancreatic progenitors [18]. Altogether these data strongly support a critical role of Mnx1 in the functional network of transcription factors acting on pancreas development and beta-cell specification. This suggests that MNX1 may be considered a strong biological candidate gene for pancreatic beta-cell dysfunction and diabetes in humans.

Apart from the pancreas, MNX1 is known to be involved in caudal development and motor neuron differentiation, and is the major susceptibility locus for dominantly inherited sacral agenesis (known as Currrario syndrome (CS); OMIM No. 176450) [19]. More than 70 heterozygous mutations (including cytogenetic anomalies) have been identified in CS, but no studies have reported a possible association with either hyperglycaemia or diabetes [19]. In the family in our study, as no specific investigation was performed, a minor form of CS in the proband or her parents cannot be excluded.

In conclusion, the present study has identified a new putative cause of PNDM in a consanguineous family. Replication in other NDM cohorts would help to clarify the clinical spectrum of MNX1 mutations in NDM patients.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Appendix A. Supplementary material

Supplementary materials (Figs. S1 and S2, and Tables S1 and S2) associated with this article can be found at http://www.sciencedirect.com at http://dx.doi.org/10.1016/j.diabet.2013.02.007.
References


