A novel *INS* mutation in a family with MODY: variable insulin secretion and putative mechanisms

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Running title *INS* mutation in MODY

A novel *INS* mutation in a family with MODY: variable insulin secretion and putative mechanisms

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Abstract

Background: Insulin gene (*INS*) mutations are a rare form of maturity-onset diabetes of the young (MODY), a heterogeneous group of autosomal dominant diabetes with at least fourteen confirmed causative genes. Here we describe a family with MODY from a novel *INS* mutation detected using massively parallel sequencing (MPS).

Subjects: The proband presented aged 11 years with mild diabetic ketoacidosis. She was negative for IA2 and GAD antibodies. She had a strong family history of diabetes, including both siblings and her mother, none of whom had ketosis but who were considered to have type 1 diabetes and managed on insulin; and her maternal grandfather, who was managed for decades on sulfonylureas. Of note, her younger sister had insulin deficiency but an elevated fasting proinsulin:insulin ratio of 76% (ref 5-30%). Sanger sequencing of *HNF4A*, *HNF1A* and *HNF1B* in the proband was negative.

Methods: The proband underwent targeted MPS using custom-designed amplicon panel and sequenced on an Illumina MiSeq.

Results: A heterozygous *INS* mutation c.277G>A (p.Glu93Lys) was detected. Sanger sequencing confirmed the variant segregated with diabetes within the family. Structural analysis of this variant suggested a disruption of a critical hydrogen bond between insulin and the insulin receptor; however the clinical picture in some individuals also suggested abnormal insulin processing and insulin deficiency.

Conclusion: This family has a novel *INS* mutation and demonstrated variable insulin deficiency. MPS represents an efficient method of MODY diagnosis in families with rarer gene mutations.

Key words

Maturity-onset diabetes on the young

Monogenic diabetes

Genetic sequencing

Massively parallel sequencing

Insulin gene

Maturity-onset diabetes of the young (MODY) is a heterogeneous group of disorders, characterised by autosomal dominant inheritance of beta cell dysfunction. Approximately 2% of children with diabetes mellitus have MODY¹; however, many cases of MODY are not recognised². To date, fourteen genes are associated with MODY, with mutations identified most commonly in *HNF4A*, *GCK*, *HNF1A* and *HNF1B*^{2,3}. Several genes associated with MODY are also associated with neonatal diabetes (NDM, usually defined as diabetes presenting <6 months of age), including, *INS*, *GCK*, *PDX1*, *HNF1B*, *NEUROD1*, *ABCC8* and *KCNJ11*.

Traditionally, genetic confirmation of MODY has involved iterative Sanger sequencing, starting with the most common and/or clinically likely genes. Consequently, even individuals with compelling clinical histories often have incomplete genetic screening. Undoubtedly, this results in mis-classification of children with MODY as type 1 or type 2 diabetes². However, the rapid expansion of massively parallel sequencing (MPS, also known as next generation sequencing) from the research to the clinical setting means comprehensive MODY screening can now occur much more efficiently and cost-effectively than traditional methods^{4,5}.

Here, we report a family with MODY caused by a novel *INS* mutation, identified using targeted MPS.

Methods

Case

The proband (individual IV:i from pedigree in Figure 1) presented aged 11 years with mild diabetic ketoacidosis (glucose 15 mmol/L, pH 7.25, bicarbonate 11 mmol/L.) and HbA1c 15.2%. Her non-fasting C-peptide was low at 0.14 nmol/L (fasting reference range 0.3 – 0.7), consistent with insulin deficiency. Testing for anti-pancreatic antibodies (GAD and IA-2) and

insulin antibodies was negative. She was commenced on multiple daily injections of insulin aspart and glargine. Initially she required insulin doses of 0.6units/kg to maintain HbA1c 5.7-6.3%. Her insulin dose -adjusted HbA1c (IDAA1c) was 8.1- 8.7, suggesting partial remission ($IDAA1c = HbA1c (\%) + [4 \times \text{insulin dose (U/Kg/24h)}^6]$); over the next four years she required a gradual increase in insulin to 0.8 units/kg/day to maintain HbA1C <6.5%. She is now aged 18 years.

She had a striking family history of autosomal dominant inheritance of diabetes spanning four generations (Figure 1). Her younger brother (individual IV:ii) was diagnosed with diabetes aged 8 years, concomitant with her own diagnosis. At presentation, he was non-ketotic, with glucose 19.2 mmol/L and random C-peptide 0.32nmol/L. He also was negative for GAD, IA2 and insulin antibodies. He remained in partial remission for two years, as evidenced by an insulin requirement of 0.3 units/kg/day, to maintain an HbA1c between 5.2 – 5.4% (IDAA1c 6.4-6.6); and he also required gradual increase in dose (after four years, insulin requirement 0.7 units/kg/day). Their sister (individual IV:iii) subsequently presented also aged 8 years, with mild polyuria and polydipsia and had nonketotic hyperglycemia. Her oral glucose tolerance test revealed a fasting glucose of 5.6 mmol/L and two-hour glucose 17.8 mmol/L. Her serum insulin was reduced in the context of hyperglycemia (fasting 3.5 mIU/L rising to only 10 mIU/L at two hours). She had an elevated intact fasting proinsulin of 18.4 pmol/L (reference range <13.3 pmol/L) and an elevated proinsulin:insulin ratio of 76% (reference range 5-30%⁷). She commenced insulin at diagnosis remained in partial remission, requiring 0.2 units/kg/day insulin for the first 2 years, maintaining HbA1c <6%. Three years after diagnosis, her insulin requirement was 0.7 units/kg/day. Neither sibling has developed ketones since diagnosis (brother now aged 14 years; sister now aged 12 years).

Their mother, aged 43 years, (individual III:iii) was diagnosed aged 11 years with presumed antibody-negative type 1 diabetes, and had been on insulin from diagnosis. Their maternal grandfather, aged 66 years, (individual II:i) was diagnosed with presumed type 2 diabetes in his 30s. He was initially managed with a sulphonylurea for over two decades but subsequently transitioned to insulin. He has never had ketosis. He had recently been diagnosed with metastatic pancreatic neuroendocrine tumour (NET).

The autosomal dominant nature of diabetes in this family alerted the caring clinician to the possibility of MODY.

Genetic screening

No mutations in *HNF4A*, *HNF1A* or *HNF1B* were detected in the proband by Sanger sequencing. Subsequently, targeted MPS was performed. Sequence libraries were constructed using an Illumina TruSeq DNA custom Amplicon panel (Illumina, San Diego, CA USA) which included *HNF4A*, *GCK*, *HNF1A*, *PDX1*, *HNF1B*, *INS*, *KCNJ11* and *ABCC8*, and sequenced using an Illumina MiSeq. CLC Bio was used to demultiplex data, trim and align the data and generate a variant file. Single nucleotide polymorphisms and small insertion/deletions were filtered using Cartagenia to identify high-quality, non-synonymous variants in coding regions. Variants were further filtered to exclude those predicted to be benign.

Results

A heterozygous variant (NM_000207.2: c.277G>A; p.Glu93Lys) was detected in *INS*. This variant has not been previously reported in Exome Aggregation Consortium (ExAC), dbSNP, 1000 Genomes, LOVD, HGMD or PubMed and is predicted to be damaging by protein prediction algorithms PolyPhen, MutationTaster and SIFT. The variant was confirmed by

Sanger sequencing and segregated with disease in all family members tested (genotypes shown in Figure 1).

The variant lies in a highly conserved region and affects amino acid residue Glu⁹³ located in one of the alpha helices of the insulin peptide. Glu⁹³ in insulin makes hydrogen bond interaction with Asn⁷¹¹ of the insulin receptor. Inspection of the recently determined insulin:receptor complex structure ⁸ suggests that a Glu to Lys exchange at this position would be expected to disrupt this interaction, likely leading to impaired binding of insulin to its receptor (Supplementary Figure).

Discussion

Here we have identified a novel heterozygous variant mutation in *INS* in a family with MODY.

INS mutations cause a heterogeneous group of monogenic diabetes. To date over 50 mutations have been described in *INS*, with both recessive and dominant inheritance ⁹⁻¹¹. Most *INS* mutations are associated with NDM (both autosomal recessive and dominant disease); indeed, *INS* mutations are the second most common cause of NDM in non-consanguineous families ⁹. Dominant *INS* mutations can cause NDM and MODY phenotypes, with the same mutation associated with both phenotypes even within the same family - hence the collective term Mutant *INS*-gene Induced Diabetes of Youth (MIDY) ¹¹. However, overall, *INS* mutations are a rare cause of MODY ⁹, as mutations in other MODY genes are much more prevalent in the MODY population.

Diabetes-associated *INS* mutations lead to the production of structurally abnormal preproinsulin or proinsulin. Many identified mutations impair cleavage of the signal peptide and/or proinsulin folding. Thus misfolded proinsulin is retained in the ER, causing ER stress which activates the unfolded protein response and ultimately resulting in beta cell apoptosis¹². This mechanism of beta cell apoptosis from ER stress is also seen in another monogenic form of diabetes, Wolfram syndrome, caused by mutations in *WFS1*^{13,14}. Of note, the insulin deficiency seen in the diabetic Akita mouse (resulting from a heterozygous Cys96Try *INS* mutation) predates beta cell apoptosis and is not completely accounted for by the degree of apoptosis¹⁵. This mutation has a dominant negative effect, such that wild type insulin folding is directly affected by both the mutant gene product and the change in the ER environment. This affects trafficking of the wildtype protein in addition to the mutant protein. Thus the combination of beta cell loss and blockage of wild type insulin secretion results in insulin deficiency. The reduced serum insulin and C-peptide, and elevated proinsulin-to-insulin ratio, in individual IV:iii is consistent with these mechanisms, with both impaired insulin processing and evolving beta-cell failure.

Additionally, although less commonly, *INS* mutations can affect binding of insulin to its receptor. Defective binding of mutant insulins to the insulin receptor can impair insulin clearance, increasing half-life and increasing circulating insulin levels¹⁶. In silico analysis of this particular mutation suggests any circulating insulin will have impaired binding to the insulin receptor. This mechanism will be of little clinical relevance in individuals with little/no residual insulin secretion (e.g. individuals III:iii, IV:i, IV:ii and IV:iii); but it may be of relevance in the grandfather (individual II:i) who was managed without insulin for years.

Given these differing mechanisms, it is not surprising that the clinical presentation of *INS*-MODY can be variable, both between and within families (as seen here), ranging from milder

diabetes managed with diet or oral hypoglycemic agents through to diabetes presenting with diabetic ketoacidosis requiring full insulin replacement ¹⁷. This is dissimilar to other forms of MODY. According to the International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines, a diagnosis of MODY should be considered when there is “low or no insulin requirements five years after diagnosis (stimulated C-peptide >200 pmol/L)” ¹⁸. This is not the case for most cases of *INS*-MODY and was not evident in several members of this family. The longer honeymoon period experienced by the two siblings of the proband may be related to the earlier diagnosis in the disease process. Replacing insulin and decreasing demand for endogenous insulin production may lessen the production of mis-folded proinsulin and minimise ongoing ER stress and beta cell apoptosis. “Beta cell rest” is also responsible for the honeymoon period in type 1 diabetes ¹⁹. However, the natural rate of progression of beta cell failure for cases of *INS*-MODY is not known, and therefore it is impossible to conclusively say whether earlier intervention affected the natural history.

This family manifest a similar phenotype to that seen in a previously reported family with a mutation at the adjacent codon causing a Val92Leu mutation (*Insulin Wakayama*).

Individuals had evidence of both hyperproinsulinemia and impaired binding of insulin to the insulin receptor ²⁰. A variant resulting in a different amino acid change at the same location has been described. It is classified as “likely pathogenic” in ClinVar, however, the supporting evidence is not available.

Limitations of this study include the lack of biochemical data on family members other than individual IV:iii, as all other affected individuals were already established on insulin at the time that MODY was considered. While individuals III:iii, IV:I and IV:ii appear to have a similar clinical picture to IV:iii, this is speculative. Insulin and C-peptide levels would have confirmed if the grandfather (II:i) had insulin deficiency or resistance. Assessment of beta-

cell insulin processing and/or ER stress might have been possible had (non-tumourous) pancreatic islets cells been evident in the grandfather's pancreatic biopsy – unfortunately this tissue was not available. Mutagenesis studies to elucidate the mechanism in this case are beyond the scope of this short report; however, the reporting of variants in *INS* may prompt future studies by others. Lastly, any possible association between the *INS* mutation and neuroendocrine pancreatic tumorigenesis in the grandfather is interesting but speculative. There have been no reported cases of NET or other pancreatic tumours from *INS* mutations; however, ER stress is known to play a role in NET development ²¹.

In conclusion, this case highlights the utility of MPS in diagnosing cases of MODY, particularly in rarer forms. Unlike some forms of MODY that can be managed with sulphonylureas (e.g. *HNF4A*, *HNF1A*, *ABCC8* and *KCNJ11*), MODY arising from *INS* mutations usually require insulin. Future directions might include reducing ER stress, with consequent reduced beta cell apoptosis and improved insulin secretion.

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Abbreviations

MODY mature onset diabetes of the young

ER endoplasmic reticulum

MPS massively parallel sequencing

MIDY mutant *INS*-gene Induced Diabetes of Youth

NET neuroendocrine tumour

INS insulin gene

Figure 1. Pedigree of family with *INS* variant.

Individuals without diabetes are indicated by white symbols; individuals with diabetes by black symbols, males are represented by squares, females by circles. The *INS* genotype of each individual, if known, is indicated below the symbol: WT wild type; c.277G>A variant in *INS*. OHG denotes treatment with oral hypoglycaemia agents, INS denotes individuals treated with insulin.

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