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A novel mutation in *INS* gene linked to permanent neonatal diabetes mellitus

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Abstract

Purpose Neonatal diabetes mellitus (NDM) is caused by mutations in the genes responsible for pancreatic β cell mass or function. This study aimed to screen the mutations in the *KCNJ11*, *ABCC8*, and *INS* genes in a Chinese patient with clinical features of NDM.

Methods The entire coding sequence and exon/intron boundaries of *KCNJ11*, *ABCC8*, and *INS* genes were detected by Sanger sequencing. The pathogenicity of the mutation was determined by using online prediction programs SIFT and Mutation Taser. The conformational alterations which contribute to the change of protein function were analyzed at the structural level.

Results A novel mutation L35Q (B11) of the *INS* gene was discovered in the patient. As L35 residue contributes to its hydrophobic core of the protein, the L35Q substitution is predicated to affect B19-A20 disulfide bond and therefore disrupt the folding of the proinsulin, which ultimately results in beta cell apoptosis by inducing ER stress.

Conclusions This case could help us understand the role of the *INS* mutation in the development of diabetes.

Keywords Neonatal diabetes mellitus · *INS* mutation · Diabetes related complications · Molecular model

Introduction

Neonatal diabetes mellitus (NDM), currently defined as diabetes with onset within 6 months of birth, is a rare monogenic disorder due to genetic defects of pancreatic β cell mass or function. It consists of two types of diabetes according to clinical features, transient neonatal diabetes mellitus (TNDM), and permanent neonatal diabetes mellitus (PNDM). A few diabetes will remit within a few weeks or months, but half will relapse during childhood or adolescence and still require lifelong treatment in patients with TNDM. Appropriate molecular genetic diagnosis is crucial to define the subtype and clinical care for NDM. For example, patients with PNDM, which were caused by heterozygous activating mutations in *KCNJ11* encoding the ATP-sensitive K channel, were suggested to be treated with oral sulfonylureas instead of insulin [1]. In addition, clarifying the underlying mechanism of the mutations in NDM can provide the important insight into the monogenic diabetes. Even though a growing number of monogenic

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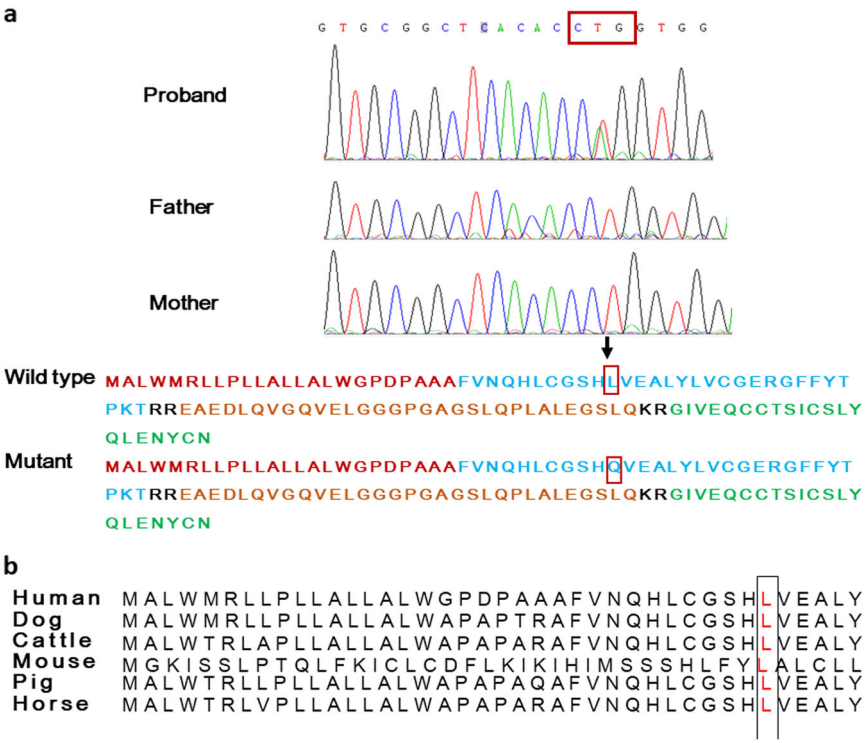

Fig. 1 Shows the location of the mutation residues. The black arrow in **a** indicates the heterozygous mutation L35Q in *INS* gene (signal peptide–red, B-chain–blue, C-peptide–orange, and A-chain–dark green). **b** shows sequence alignment of preproinsulin in various species

factors, including *HNF1B*, *GLS3*, *GCK*, *ABCC8*, *KCNJ11*, *SUR1*, *INS*, *EIF2AK3*, and et al., have been uncovered to be likely to cause NDM [2], the genetic molecular bases of the disease remain unknown. Here, we conducted a genetic sequencing of a patient with PNDM and identified a novel mutation in *INS* gene.

Clinical features

We identified a 25-year-old male patient with PNDM, whose birth weight was 2600 g. No history of gestational diabetes during pregnancy for his mother. The diabetes manifests itself 10 months after his birth, including polydipsia, polyuria, polyphagia, and mild malnutrition. He was referred to hospital because of diabetic ketoacidosis and treated with basal and preprandial insulin therapy. He has been treated with an insulin pump until now. The recent evaluation in our hospital indicated that BMI was 17.8 kg/m^2, hemoglobin A1c (HbA1c) was 6.2% (normal range 4.0–6.0%), or 44 mmol/mol (normal range 20–42 mmol/mol). The fasting C-peptide level was 0.056 ng/ml and islet autoantibody tests were negative. He has been diagnosed with bilateral cataracts in 2005. The eye exam noted visual acuity of 0.8 in the right eye and 1.0 in the left eye. No neurological abnormalities, such as developmental delay or epilepsy were observed in his clinical history. He never have suffered from any other chronic diabetic complications (e.g. diabetic nephropathy, diabetic retinopathy).

Molecular genetic analysis

Written informed consent was given by the patient and his parents and the genetic mutation testing was under the Declaration of Helsinki and approved by the ethic committee of the Second Affiliated Hospital of Soochow University. Genomic DNA was extracted from blood sample and the *KCNJ11*, *ABCC8*, and *INS* were sequenced. The results revealed a heterozygous missense mutation CTG>CAG (c.T104A) in the proband, leading to the amino acid replacement p.Leu35Gln (L35Q) (as shown in Fig. 1). We found no information when we searched the variant in the ExAC browser (http://exac.broadinstitute.org). And L35Q mutation was not identified in 100 non-diabetic controls of Han Chinese subjects. It also appeared de novo since the mutation was not found in his parents without diabetes. The fasting plasma glucose levels of his father and mother were both 5.1 mmol/L, and HbA1c levels were 5.1 and 6.0%, respectively. In addition, L35Q mutation was predicted to be deleterious by using online prediction programs SIFT and Mutation Taser.

Construction of human proinsulin molecular model

The L35Q mutation located in an invariant residue of the B chain, which is highly conserved across all species (as shown in Fig. 1). To investigate whether L35Q mutation

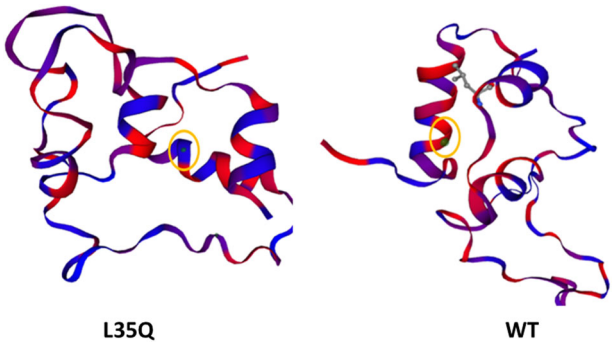

Fig. 2 Secondary structure computer modeling of wildtype (WT) and mutant proinsulin. Different color scheme indicates the different hydrophobic feature. A color transition from blue to red indicates the change from the least hydrophobic part to most hydrophobic part. The yellow circle shows the amino acid residue of WT and mutant proinsulin

could lead to conformational alterations which contribute to the change of protein function, we analyzed the mutation at the structural level. The location of L35 was modeled in the central α-helix (also called hydrophobic core), contributing to clustering of non-polar residues. We predicted that a substitution of this leucine by glutamine would affect the hydrophobic core of the protein and in further destabilize the entire central α-helix (Fig. 2).

Discussion

In our study, a non-obese male patient, whose hyperglycemia was noted during the 10 months of his life, has been treated with insulin injection since then. There was no gestational or familial diabetes. He fulfilled neither the classical maturity onset diabetes in young (MODY) criteria (non-obesity, absence of autoantibodies, at least one patient with onset aged <25 years, and a family history of diabetes for at least three consecutive generations) nor the minimal MODY criteria (two consecutive generations of type 2 diabetes with at least one member diagnosed aged <25 years). Based on the onset age of diabetes and negative autoantibodies testing, he was diagnosed with PNDM. Moreover, we discovered that a novel heterozygous mutation in the *INS*-gene was likely to cause PNDM. The patient had no extra-pancreatic comorbidities and hyperglycemia complications with a follow-up of 28 years, except for the first diabetic ketoacidosis at diagnosis.

Prior studies have discovered that an increasing number of *INS*-mutations participated in a variety of different steps of insulin biosynthesis, which manifests itself with different clinical severity, ranging from mild adult type 2 diabetes to severe NDM. Since Tager et al. first reported the mutant insulin (insulin Los Angeles) in patients with hyperinsulinemia in 1983 [3], the discovery of mutant insulin or proinsulin was quickly favored by researchers. And these years preceded the progress of research in identifying *INS*-gene mutations with monogenic diabetes by genome technology innovation (e.g. microarray and sequencing). To date, over 56 *INS*-gene mutations [3–12] have been identified in the untranslated regions as well as the sequence of preproinsulin signal peptide, B-chain, C-peptide, A-chain, the cleavage sites for prohormoneconvertases PC1/3 and PC2, and the proteolytic cleavage sites of signal peptidase. These *INS* mutations present differently in clinical features, ranging from mild adult onset to severe neonatal diabetes. Most of the *INS*-mutations were inherited in an autosomal dominant model, which were attributed to a gain-of-toxic function from the mutant gene product [13]. Prior evidence indicated that cysteine-related mutations, which introduce or remove a cysteine, lead to pancreatic β-cell dysfunction and diabetes, while non-cysteine-related mutations might impair the efficiency of chain combination [14]. In contrast, Garin et al. reported a set of recessive INS-mutations with NDM and elucidated that these mutations caused NDM because of decreased insulin biosynthesis through varied mechanisms, including gene deletion, lack of the translation initiation signal, and altered mRNA stability in functional studies [15]. Recessive *INS* gene mutations could cause a decrease of 80% in insulin production, therefore they were considered as "loss-of-function" mutations. The patients with such recessive *INS*-mutations are diagnosed earlier and have a lower birth weight than those with heterozygous *INS*-mutations [4, 15]. These indicated the different roles of mutant proinsulin in the pathogenesis of diabetes.

The *INS* gene encodes a 110 amino acid protein, which is named preproinsulin, and then undergoes subsequent forming proinsulin by cleavage of the signal sequence in the endoplasmic reticulum (ER) of pancreatic beta cells. The folding action of newly synthesized proinsulin is processed and three native disulfide bonds (B7-A7, B19-A20, and A6-A11) are formed once the C-peptide is cleaved [14]. It is noted that B-chain starts acquisition of the central B-chain α-helix running from B9-B19, which facilitates the formation of B19-A20 disulfide bond [16]. As the B-chain α-helix formed by the amino acid residues B9–B19 is involved in the stabilization of its hydrophobic core, the L35Q (B11) substitution presumably destabilizes hydrophobic core of the molecular and in further affects B19-A20 disulfide bond, which ultimately leads to beta cell apoptosis by impairing proinsulin folding and severe ER stress. Previous research has identified the missense mutation L35P (B11) in PNDM diagnosed in infancy (the first 12 months of life), which was in line with our findings [16]. The molecular biology is needed to be elucidated in functional studies.

Genetic factors, duration of diabetes, poor glycaemic control, and several metabolic factors are considered to contribute to the hyperglycemia complications [17]. Our

patient did not suffer from the classic complications (e.g. diabetic nephropathy, diabetic retinopathy) in the long duration of NDM. Routine ophthalmological examination, however, noted that he was presented with bilateral cataracts. Only three cases have been reported with bilateral cataracts in monogenic diabetes so far [12, 18, 19], which were likely secondary to hyperglycemia. It is not clear why our patient did not suffer from the classic chronic complications. We presumed that it might depend both on the biological behavior of the *INS* mutants themselves and other environmental factors.

In conclusion, we discovered a novel mutation p. Leu35Gln (L35Q) in the *INS* gene, which was likely to cause PNDM in the patient. It is predicted that the substitution L35Q mutation might lead to conformational alterations, thus impair β cell function by inducing ER stress.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. E.R. Pearson, I. Flechtner, P.R. Njolstad, M.T. Malecki, S.E. Flanagan, B. Larkin, F.M. Ashcroft, I. Klimes, E. Codner, V. Iotova, A.S. Slingerland, J. Shield, J.J. Robert, J.J. Holst, P.M. Clark, S. Ellard, O. Sovik, M. Polak, A.T. Hattersley, Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. N. Engl. J. Med. **355**(5), 467–477 (2006). https://doi.org/10.1056/NEJMoa061759
2. S.A. Greeley, R.N. Naylor, L.H. Philipson, G.I. Bell, Neonatal diabetes: an expanding list of genes allows for improved diagnosis and treatment. Curr. Diab. Rep. **11**(6), 519–532 (2011). https://doi.org/10.1007/s11892-011-0234-7
3. S. Shoelson, M. Fickova, M. Haneda, A. Nahum, G. Musso, E.T. Kaiser, A.H. Rubenstein, H. Tager, Identification of a mutant human insulin predicted to contain a serine-for-phenylalanine substitution. Proc. Natl. Acad. Sci. USA. **80**(24), 7390–7394 (1983)
4. J. Stoy, D.F. Steiner, S.Y. Park, H. Ye, L.H. Philipson, G.I. Bell, Clinical and molecular genetics of neonatal diabetes due to mutations in the insulin gene. Rev. Endocr. Metab. Disord. **11**(3), 205–215 (2010). https://doi.org/10.1007/s11154-010-9151-3
5. H. Sakura, Y. Iwamoto, Y. Sakamoto, T. Kuzuya, H. Hirata, Structurally abnormal insulin in a diabetic patient. Characterization of the mutant insulin A3 (Val—Leu) isolated from the pancreas. J. Clin. Invest. **78**(6), 1666–1672 (1986). https://doi.org/10.1172/jci112760
6. S.J. Chan, S. Seino, P.A. Gruppuso, R. Schwartz, D.F. Steiner, A mutation in the B chain coding region is associated with impaired proinsulin conversion in a family with hyperproinsulinemia. Proc. Natl. Acad. Sci. USA. **84**(8), 2194–2197 (1987).
7. F. Barbetti, N. Raben, T. Kadowaki, A. Cama, D. Accili, K.H. Gabbay, J.A. Merenich, S.I. Taylor, J. Roth, Two unrelated patients with familial hyperproinsulinemia due to a mutation substituting histidine for arginine at position 65 in the proinsulin molecule: identification of the mutation by direct sequencing of genomic deoxyribonucleic acid amplified by polymerase chain reaction. J. Clin. Endocrinol. Metab. **71**(1), 164–169 (1990). https://doi.org/10.1210/jcem-71-1-164
8. H. Yano, N. Kitano, M. Morimoto, K.S. Polonsky, H. Imura, Y. Seino, A novel point mutation in the human insulin gene giving rise to hyperproinsulinemia (proinsulin Kyoto). J. Clin. Invest. **89**(6), 1902–1907 (1992). https://doi.org/10.1172/jci115795
9. M.G. Warren-Perry, S.E. Manley, D. Ostrega, K. Polonsky, S. Mussett, P. Brown, R.C. Turner, A novel point mutation in the insulin gene giving rise to hyperproinsulinemia. J. Clin. Endocrinol. Metab. **82**(5), 1629–1631 (1997). https://doi.org/10.1210/jcem.82.5.3914
10. R. Courtney, C. Gamble, M.L. Arango, A. Shah, N.I. Rubio, J. Nguyen, D. Rodriguez-Buritica, Novel homozygous likely-pathogenic intronic variant in INS causing permanent neonatal diabetes in siblings. J. Pediatr. Endocrinol. Metab. **29**(9), 1089–1093 (2016). https://doi.org/10.1515/jpem-2016-0040
11. J. Yan, F. Jiang, R. Zhang, T. Xu, Z. Zhou, W. Ren, D. Peng, Y. Liu, C. Hu, W. Jia, Whole-exome sequencing identifies a novel INS mutation causative of maturity-onset diabetes of the young 10. J. Mol. Cell Biol. **9**(5), 376–383 (2017). https://doi.org/10.1093/jmcb/mjx039
12. X. Xiao, L. Liu, Y. Xiao, Z. Xie, L. Li, H. Zhou, W. Tang, S. Liu, Z. Zhou, Novel frameshift mutation in the insulin (INS) gene in a family with maturity onset diabetes of the young (MODY). J. Diabetes **11**(1), 83–86 (2019). https://doi.org/10.1111/1753-0407.12849
13. M. Liu, J. Sun, J. Cui, W. Chen, H. Guo, F. Barbetti, P. Arvan, INS-gene mutations: from genetics and beta cell biology to clinical disease. Mol. Asp. Med. **42**, 3–18 (2015). https://doi.org/10.1016/j.mam.2014.12.001
14. M. Liu, M.A. Weiss, Biosynthesis, structure, and folding of the insulin precursor protein. Ann. N. Y Acad. Sci. **20**(Suppl 2), 28–50 (2018). https://doi.org/10.1111/dom.13378
15. I. Garin, E.L. Edghill, I. Akerman, O. Rubio-Cabezas, I. Rica, J.M. Locke, M.A. Maestro, A. Alshaikh, R. Bundak, G. del Castillo, A. Deeb, D. Deiss, J.M. Fernandez, K. Godbole, K. Hussain, M. O'Connell, T. Klupa, S. Kolouskova, F. Mohsin, K. Perlman, Z. Sumnik, J.M. Rial, E. Ugarte, T. Vasanthi, K. Johnstone, S.E. Flanagan, R. Martinez, C. Castano, A.M. Patch, E. Fernandez

-Rebollo, K. Raile, N. Morgan, L.W. Harries, L. Castano, S. Ellard, J. Ferrer, G. Perez de Nanclares, A.T. Hattersley, Recessive mutations in the INS gene result in neonatal diabetes through reduced insulin biosynthesis. Proc. Natl. Acad. Sci. USA. **107**(7), 3105–3110 (2010). https://doi.org/10.1073/pnas.0910533107

16. E.L. Edghill, S.E. Flanagan, A.M. Patch, C. Boustred, A. Parrish, B. Shields, M.H. Shepherd, K. Hussain, R.R. Kapoor, M. Malecki, M.J. MacDonald, J. Stoy, D.F. Steiner, L.H. Philipson, G.I. Bell; G. Neonatal Diabetes International Collaborative, A.T. Hattersley, S. Ellard, Insulin mutation screening in 1044 patients with diabetes: mutations in the INS gene are a common cause of neonatal diabetes but a rare cause of diabetes diagnosed in childhood or adulthood. Diabetes **57**(4), 1034–1042 (2008). https://doi.org/10.2337/db07-1405
17. A. Girach, D. Manner, M. Porta, Diabetic microvascular complications: can patients at risk be identified? a review Int. J. Clin. Pract. **60**(11), 1471–1483 (2006). https://doi.org/10.1111/j.1742-1241.2006.01175.x
18. H. Wasserman, R.B. Hufnagel, V. Miraldi Utz, K. Zhang, C.A. Valencia, N.D. Leslie, N.A. Crimmins, Bilateral cataracts in a 6-yr-old with new onset diabetes: a novel presentation of a known INS gene mutation. Pediatr. Diabetes **17**(7), 535–539 (2016). https://doi.org/10.1111/pedi.12335
19. K. Raile, M. O'Connell, A. Galler, G. Werther, P. Kuhnen, H. Krude, O. Blankenstein, Diabetes caused by insulin gene (INS) deletion: clinical characteristics of homozygous and heterozygous individuals. Eur. J. Endocrinol. **165**(2), 255–260 (2011). https://doi.org/10.1530/eje-11-0208