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Further evidence that mutations in *INS* can be a rare cause of Maturity-Onset Diabetes of the Young (MODY)

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Abstract

Background: Insulin gene (*INS*) mutations have recently been described as a common cause of permanent neonatal diabetes (PNDM) and a rare cause of diabetes diagnosed in childhood or adulthood.

Methods: *INS* was sequenced in 116 maturity-onset diabetes of the young (MODYX) patients ($n = 48$ Danish and $n = 68$ Czech), 83 patients with gestational diabetes mellitus (GDM), 34 type 1 diabetic patients screened negative for glutamic acid decarboxylase (GAD), and 96 glucose tolerant individuals. The control group was randomly selected from the population-based sampled Inter99 study.

Results: One novel heterozygous mutation c.17G>A, R6H, was identified in the pre-proinsulin gene (*INS*) in a Danish MODYX family. The proband was diagnosed at 20 years of age with mild diabetes and treated with diet and oral hypoglycaemic agent. Two other family members who carried the *INS* R6H were diagnosed with diabetes when 51 years old and with GDM when 27 years old, respectively. A fourth mutation carrier had normal glucose tolerance when 20 years old. Two carriers of *INS* R6H were also examined twice with an oral glucose tolerance test (OGTT) with 5 years interval. They both had a ~30% reduction in beta-cell function measured as insulinogenic index. In a Czech MODYX family a previously described R46Q mutation was found. The proband was diagnosed at 13 years of age and had been treated with insulin since onset of diabetes. Her mother and grandmother were diagnosed at 14 and 35 years of age, respectively, and were treated with oral hypoglycaemic agents and/or insulin.

Conclusion: Mutations in *INS* can be a rare cause of MODY and we conclude that screening for mutations in *INS* should be recommended in MODYX patients.

Background

Insulin gene (*INS*) mutations have recently been described as a common cause of permanent neonatal diabetes (PNDM) and a rare cause of diabetes diagnosed in childhood or adulthood [1-4]. Heterozygous mutations in *INS* account for 15 - 20% of cases of PNDM [4,5]. Gene discovery can lead to recognition of novel phenotypes [6] and recognition of novel clinical subgroups. For example, MODY was initially clinically defined as autosomal dominantly inherited, non insulin dependent, early-onset diabetes, but now there are at least eight distinct genetic subgroups of MODY, most of

which have a discrete phenotype and specialized treatment needs [6]. An R46Q *INS* mutation was recently described in a Norwegian study of 62 probands fulfilling conventional MODY criteria. In addition, they examined 223 patients from the population-based Norwegian Childhood Diabetes Registry and found an R55C *INS* mutation. One hundred blood donors were screened negative for these mutations [7]. The Italian study group on early onset diabetes has detected two *INS* mutations (A23S and G23S) in children negative for 5 type 1 diabetes (T1D) autoantibodies [1]. In addition, an R6C mutation was identified in an English MODY family and a L68M mutation was described in a family of Turkish origin with young-onset type 2 diabetes [3].

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Furthermore, a recent screening for *INS* mutations in 252 patients diagnosed clinically with T1D between 6 months and 17 years of age identified 2 de novo heterozygous mutations G32S and R89C among the 25 (8%) antibody-negative patients [8]. To our knowledge, no studies have screened women with gestational diabetes mellitus (GDM) for *INS* mutation. GDM was defined as an abnormal glucose tolerance diagnosed for the first time in pregnancy.

We aimed to evaluate the prevalence and the disease-associated phenotype of *INS* mutations among diabetic patients diagnosed with MODY, anti-body negative T1D or GDM.

Methods

INS was sequenced in 116 unrelated MODYX probands: 48 Danish, age at diagnosis (mean \pm SD) 24 ± 19 years, BMI 24.8 ± 5.5 kg/m², and 68 Czech, age at diagnosis 18 ± 8 years, BMI 22.6 ± 5.0 kg/m², 83 Danish diabetic patients previously diagnosed with GDM, age at examination 40 ± 7 years, BMI at examination 28.1 ± 6.5 kg/m², 34 GAD autoantibody-negative T1D patients, age at diagnosis 20 ± 16 years and 96 glucose tolerant control individuals, age at examination 46 ± 7 years, BMI 26.4 ± 4.4 kg/m². All MODYX probands were from families fulfilling the conventional criteria of MODY [5] defined by: diabetes diagnosed before 25 years of age in at least one of the family members. No treatment with insulin and/or measurable C-peptide at least one year after diagnosis. Autosomal dominant inherited diabetes with known diabetes in at least two consecutive generations.

All MODY probands were screened negative for mutations in the MODY genes *HNF4A*, *GCK* and *HNF1A*. Women with GDM and all GAD autoantibody-negative T1D patients had a positive family history of diabetes including a diabetic parents and or child with diabetes. The control group was randomly selected from the

population-based study Inter99 [9]. Additional family members of the probands with an *INS* mutation were examined and screened for the family-specific *INS* mutation. The participants and examined family members gave their informed written consent, and the study protocols were approved by the ethical committees. The genomic sequence of *INS* was analysed in two segments covering the two exons, exon-intron boundaries and UTRs. Sequencing analyses were performed as described [4]. Analyses of the *INS* sequence revealed four low-frequency (minor allele frequency = 0.05) and three frequent (minor allele frequency > 0.05) variants (Table 1).

Results and Discussion

One novel mutation c.17G>A, R6H, was identified in *INS* in a Danish MODYX family (Figure 1A). The mutation is located in the signal peptide of proinsulin and was not present in 96 control individuals. The proband, M132-1, had been diagnosed at 20 years of age with mild diabetes treated with diet and oral hypoglycaemic agent (OHA). His 20-years-old son, M132-2, carried the mutation but had normal glucose tolerance. The brother of the proband, M132-4, carried the mutation and had been diagnosed with diabetes at 51 years of age. A daughter, M132-5, of the brother carried the mutation and had been diagnosed with IGT at the age of 26 and with GDM at the age of 27 years. Two carriers of *INS* R6H, M132-2 and M132-4, were also examined with an OGTT at age 15 years and 46 years, respectively. Interestingly, they both had a ~30% reduction in beta-cell function over a period of 5 years measured as insulinogenic index calculated from OGTT data (s-insulin 30 min (pmol/l) - s-insulin 0 min (pmol/l)/p-glucose 30 min (mmol/l). Insulinogenic index of M132-2 declined from 50 to 33 and for M132-4 from 19 to 13. BMI for M132-2 increased from 19.7 to 24.8 kg/m²; for M132-4 BMI was unchanged, 24.2 kg/m², over the 5-year period.

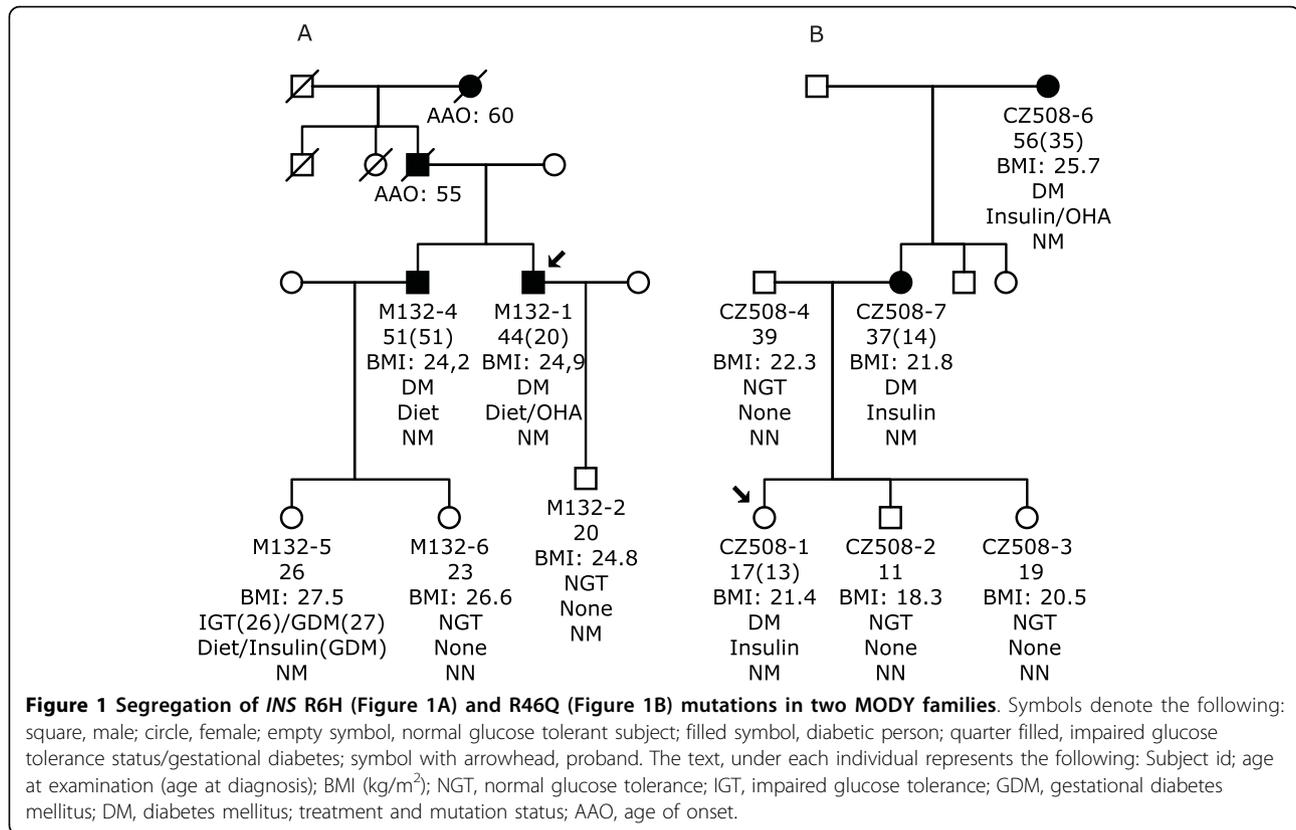
Table 1 *INS* gene variants identified in MODY (n = 116), T1DM (n = 34), GDM (n = 83) and controls (n = 96)

rs number	SNP	Position on chr. 11	Part of gene	MAF (%)			
				MODY (n = 116)	T1D ab ⁻ (n = 34)	GDM (n = 83)	Controls (n = 92)
Novel	c.-218A>C	2.138.995	5'UTR	0.4	0	0	0
rs689	c.-23A>T	2.138.800	Intron	27	10	33	28
rs5505	c.-9C>T	2.138.786	5'UTR	0.9	0	0.6	0 ^A
Novel	c.17G>A, p.R6H	2.138.761	Exon	0.4	0	0	0
Novel	c.137 G>A, p.R46Q	2.138.641	Exon	0.4	0	0	0
rs3842752	c.*9C>T	2.137.649	3'UTR	20	7	27	21
rs3842753	c.*22C>A	2.137.636	3'UTR	26	10	34	28

SNP locations (-strand) are displayed counting from the first translated nucleotide in pre-proinsulin (*INS*). Base-pair positions are displayed counting from the p-arm telomere of chromosome 11 (according to the Base position feature in the Human (*Homo sapiens*) Genome Browser Gateway Human Mar. 2006 [hg18] assembly (<http://genome.ucsc.edu/cgi-bin/hgGateway>, assessed 19 June 2009). Minor allele frequencies (MAF) are given as a percentage.

^A Subsequent genotyping of 198 population-based individuals revealed a MAF of 0.7%

Control individuals were collected as a subset of the population based study Inter99 of middle-aged Danish individuals.



The R6 residue is conserved across mammalian species (rhesus monkey, elephant, mouse, dog and horse) and xenopus but is not conserved in lizard (S), platus (G) or stikleback (Q). A mutation in the same codon, R6C, was recently described in an English MODY family affecting the proband, the mother and the maternal grandmother [3], underlining a putative importance of this residue for proper function of the signal peptide.

In a Czech MODY proband a previously described R46Q mutation was found (Figure 1B). The mutation cosegregates with diabetes in two affected relatives and is not present in any of the non-diabetic family members or 96 controls. The proband, a 17-year old girl, was diagnosed with diabetes at 13 years of age and had been treated with insulin since onset of disease. Her mother was diagnosed at 14 years of age with polyuria and polydipsia, and had been treated with insulin since onset of diabetes. The grandmother was diagnosed with diabetes at 35 years of age and treated with OHA and insulin. No coding mutations were found in GDM women or GAD-negative type 1 diabetic patients.

It is very likely that the R46Q and R6H mutations are disease causing and not just rare neutral polymorphisms. In already published studies [1,3,7,8] a total of 1560

individuals primarily of Northern European ancestry have been screened for *INS* mutations. The R46Q mutation was identified in one Norwegian MODY family [7]. The mutation segregated with diabetes in the family. The R6H mutation has not been identified in the 1560 examined individuals indicating that this mutation is not a rare polymorphism. Furthermore, we have screened additional 74 Danish individuals (data not shown) without identifying the R46Q and R6H mutations.

Conclusion

Mutations in *INS* can be a rare cause of MODY and we conclude that screening for mutations in *INS* should be recommended in MODYX patients.

Abbreviations

GAD: Glutamic acid decarboxylase; GDM: Gestational diabetes mellitus; *INS*: Insulin gene; MODY: Maturity-onset diabetes of the young; PNDM: Permanent neonatal diabetes mellitus.

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Authors' contributions

The concept and idea regarding the study belong to EAA, TWEB, OP and TH. The collection of study subjects was planned and performed by SP, OC, BO, JL, JL, PD, RB, FP, CP, TWEB, OP and TH.

The original hypothesis regarding the genetic study was conceived by EAA and TWEB, and approved by OP and TH. Detail planning of analyses and study design was performed by EAA and TWEB, and approved by FB, OP and TH. EAA, TWEB, OP and TH contributed to the establishment of study population databases specific for this study. Statistical analyses were performed by EA and TWEB. The first manuscript was written by TWEB and EAA (equal contributions) and the final draft was produced by TWEB, OP and TH. All authors revised the manuscript and contributed to the discussion.

Competing interests

T.W Boesgaard, E.A Andersson, R Bergholdt, F Pociot, O Pedersen and T Hansen hold stock in Novo Nordisk, and O Pedersen and T Hansen have received lecture fees from pharmaceutical companies. All other authors declare that there is no competing interest associated with this manuscript.

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