Brief Genetics Report

Activating Mutations in the *KCNJ11* Gene Encoding the ATP-Sensitive K$^+$ Channel Subunit Kir6.2 Are Rare in Clinically Defined Type 1 Diabetes Diagnosed Before 2 Years

Emma L. Edghill,[1] Anna L. Gloyn,[1] Kathleen M. Gillespie,[2] A. Paul Lambert,[2] Neil T. Raymond,[3] Peter G. Swift,[4] Sian Ellard,[1] Edwin A.M. Gale,[2] and Andrew T. Hattersley[1]

We have recently shown that permanent neonatal diabetes can be caused by activating mutations in *KCNJ11* that encode the Kir6.2 subunit of the β-cell ATP-sensitive K$^+$ channel. Some of these patients were diagnosed after 3 months of age and presented with ketoacidosis and marked hyperglycemia, which could have been diagnosed as type 1 diabetes. We hypothesized that *KCNJ11* mutations could present clinically as type 1 diabetes. We screened the *KCNJ11* gene for mutations in 77 U.K. type 1 diabetic subjects diagnosed under the age of 2 years. One patient was found to be heterozygous for the missense mutation R201C. She had low birth weight, was diagnosed at 5 weeks, and did not have a high risk predisposing HLA genotype. A novel variant, R176C, was identified in one diabetic subject but did not cosegregate with diabetes within the family. In conclusion, we have shown that heterozygous activating mutations in the *KCNJ11* gene are a rare cause of clinically defined type 1 diabetes diagnosed before 2 years. Although activating *KCNJ11* mutations are rare in patients diagnosed with type 1 diabetes, the identification of a *KCNJ11* mutation may have important treatment implications. *Diabetes* 53:2998–3001, 2004

W e have recently shown that heterozygous mutations in the Kir6.2 (*KCNJ11*) gene, on chromosome 11p15.1, accounts for approximately one-third of cases of permanent neonatal diabetes mellitus (PNDM) (1). The gene encodes a subunit of the β-cell ATP-sensitive K$^+$ channel (K$_{ATP}$

© 2004 by the American Diabetes Association.

channel) known as the inwardly rectifying K$^+$ channel (Kir6.2). The K$_{ATP}$ channel plays a critical role in coupling metabolism to membrane electrical events, which bring about closure of the K$_{ATP}$ channel and initiate insulin secretion. Activating mutations in *KCNJ11* cause familial and sporadic PNDM by decreasing β-cell insulin secretion through reduced ATP sensitivity of the K$_{ATP}$ channel (1). Subjects presented with marked hyperglycemia and ketoacidosis at a mean age of 7 weeks (range 0–26), with 15% presenting outside 3 months. We hypothesized that *KCNJ11* activating mutations could present in young children with a clinical diagnosis of type 1 diabetes.

The *KCNJ11* gene, encoding Kir6.2, is located close to the large *IDDM2* linkage peak (logarithm of odds [LOD] = 4.28) at 11p15.5 seen in the combined analysis of the U.S. and the U.K. type 1 diabetes genome-wide scans (2). *IDDM2* is primarily explained by the insulin gene variable number tandem repeat (*INS VNTR*) region, located upstream of the insulin gene. However, after allowing for the *INS VNTR*, the resulting LOD score for chromosome 11 in this region was still suggestive of linkage (LOD = 2.53), and it was suggested by Cox et al. (2) that there could be a second type 1 diabetes locus in this region. A subset of families linked to *KCNJ11* might explain this. A recent large study did not find any association with type 1 diabetes of the single nucleotide polymorphism E23K in *KCNJ11* (3).

Type 1 diabetes is defined by an absolute deficiency of insulin secretion (4). The most common form is immune-mediated diabetes resulting from autoimmune destruction of the pancreatic β-cells. Pancreatic auto-antibodies are present in 85–90% of type 1 diabetic patients at diagnosis (4). Another indicator of the autoimmune nature of type 1 diabetes is the presence of the high risk HLA haplotypes (DRB1*03-DQA1*0501-DQB1*0201 and DRB1*04-DQA1*0301-DQB1*0302) in the majority of Caucasian type 1 diabetic subjects, while the protective haplotype DRB1*15-DQA1*102/DQB1*0602 is rarely found (5). In contrast to type 1 diabetes, patients with *KCNJ11* mutations do not have pancreatic auto-antibodies (1). HLA studies have not been performed in patients with *KCNJ11* mutations, but children diagnosed with permanent diabetes before 6
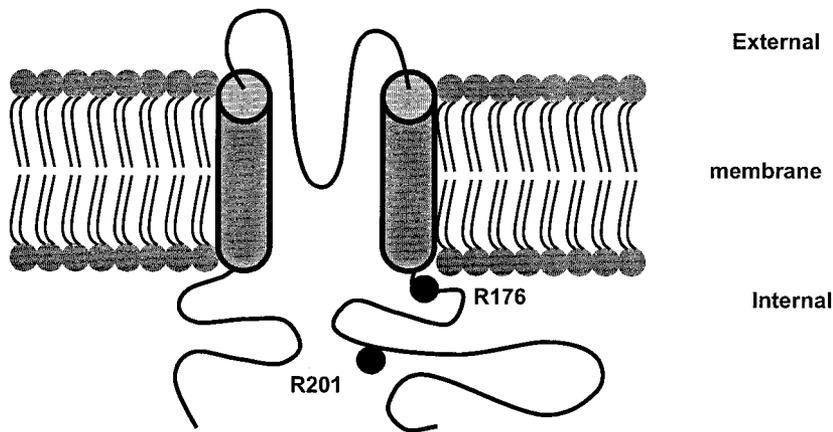
FIG. 1. Topology of Kir6.2 subunit showing the mutated residues R201 and R176 within the cytosolic COOH-terminal domain. External and internal are marked relative to the β-cell membrane.

months have the same frequency of high risk HLA haplotypes as that found in the normal population (6).

Recent studies have shown that some patients with a clinical diagnosis of type 1 diabetes have maturity-onset diabetes of the young due to *HNF-1α* mutations (7–10). We previously reported a patient with an *HNF-1α* mutation who was GAD antibody negative and homozygous for a protective HLA haplotype, not supporting the diagnosis of type 1 diabetes. The identification of a specific genetic etiology can have implications for treatment; in this case, an affected family member was taken off insulin and is now treated with a sulfonylurea (7).

To test whether *KCNJ11* activating mutations could present outside the neonatal period and be mistaken for clinical type 1 diabetes, we analyzed samples from 77 individuals clinically diagnosed with type 1 diabetes before the age of 2 years from two U.K.-based cohorts. The *KCNJ11* gene was sequenced in 19 subjects from the Barts & Oxford (BOX) study (11) and 58 subjects from the British Diabetic Association (BDA) 1972–1981 cohort (12).

We identified possible heterozygous mutations in two cases from the BDA 1972–1981 cohort and no mutations in subjects from the BOX study. In addition, we identified several previously recognized polymorphisms (E23K, A190A, L267L, L270V, I337V, K381K, and S385C). The potential mutations were the substitution of arginine by cystine at codon 201 (CGT>TGT, R201C) and the substitution of arginine by cystine at codon 176 (CGC>TGC, R176C). Neither of these variants was identified in 100 normal chromosomes, and both residues are conserved in rat, mouse, and bullfrog. R201 and R176 both lie within the COOH-terminal of the Kir6.2 (Fig. 1). The arginine residue encoded by codon 201 is also conserved across 10 members of the Kir channel family, supporting a critical role for this residue in channel function.

R201C is likely to be an etiological mutation, as it has previously been reported in a subject with PNDM (1). The amino acid position R201 has been reported as the most common mutated residue (R201H) in *KCNJ11* PNDM subjects. The residue R201 includes a CpG dinucleotide, which is a mammalian "hot spot" for gene mutations. Functional studies have shown that mutating this residue to histidine reduces the ATP sensitivity by ~40-fold in the homozygous state (1). The R201 residue is thought to influence ATP binding indirectly by acting as an allosteric modifier. The R201C mutation has not been studied, but other substitutions at this site have also shown a reduction

of ATP sensitivity (13). DNA was not available from other members of this family, but the absence of diabetes in the parents is consistent with a spontaneous mutation in the proband, as previously reported with this mutation (1).

R176C has not previously been found to be mutated in PNDM. It occurs at a residue that is involved in PIP$_2$ (phosphatidylinositol 4,5 bisphosphate) binding to the K$_{ATP}$ channel, which causes the channel to open (14). This arginine residue has been extensively analyzed by functional studies (14–16). The R176C variant was shown to reduce PIP2 binding, which would tend to result in channel closure and increased insulin release (16). Family studies showed that the R176C variant did not cosegregate with diabetes; her diabetic father did not have the variant, but the proband's two unaffected sisters (one of which is her nonidentical twin) and mother were heterozygous carriers of the variant. Hyperinsulinism is the clinical phenotype associated with mutations that increase channel closure, but there were no clinical features to suggest this in the R176C carriers. The discrepancy between the functional studies and the clinical phenotype may reflect that in the functional studies all of the channels were mutated, but in the family members, as the mutation was heterozygous, only 50% of Kir6.2 channel subunits are mutated. We therefore conclude that R176C, despite being rare, conserved across species and in a functionally important position is a noncausal variant.

The clinical characteristics of the subjects with R201C and R176C are shown in Table 1. The subject with the R201C *KCNJ11* mutation has similar characteristics to previously described patients with activating *KCNJ11* mutations (1): she was born with a low birth weight (2.67 kg) and diagnosed with diabetes at 5 weeks of age, and GAD and IA-2 antibody testing performed at age 26 years were negative. This was the only patient in the cohort diagnosed before 6 months of age. According to current criteria, this patient would be diagnosed with PNDM (17), but at the time of diagnosis in 1977, neonatal diabetes was defined as presentation during the 1st month of life (the neonatal period). In contrast, the subject with the R176C variant did not have low birth weight, was diagnosed at 17 months, and is likely to have type 1 diabetes despite having a rare *KCNJ11* variant.

HLA typing of the patient with the R201C *KCNJ11* mutation identified her genotype as DRB1*02-DQB1*0502/ DRB1*03-DQB1*0201. This genotype has recently been shown in a U.K. cohort (BOX) to have a neutral risk of type

TABLE 1
Clinical details and investigations for patients BDA1 and BDA2 with R201C and R176C *KCNJ11* mutations

	BDA1	BDA2
Mutation/rare variant	R201C	R176C
Clinical details		
Sex	Female	Female
Birth weight (kg) (gestation week)	2.67 (42)	3.18 (37)
Age of diagnosis (months)	1.25	17
Presentation	Polyuria and failure to thrive	Polyuria, polydipsia, and poor growth
Treatment	Insulin from diagnosis	Insulin from diagnosis
Family history of diabetes	None	Father diagnosed with diabetes at 16 years
Age at follow-up examination (years)	26	26
BMI (kg/m^2)	23	22
Insulin dose (units/kg)	0.94	0.37
Investigations		
HbA$_{1c}$ (%) (normal range 4–6%)	8.9	7.4
GADA (cut off 1.6)	0.49	0.52
IA-2A (cut off 0.9)	0.29	0.25

GADA, GAD antibody; IA-2A, IA-2 antigen.

1 diabetes despite carrying one disease-associated haplotype (15). The patient with the R176 variant had the neutral genotype DRB1*04-DQB1*0301/DRB1*07-DQB1*02. Ninety percent (69 of 77) of the diabetic patients had high risk HLA genotypes for type 1 diabetes, with 48% having the highest risk HLA genotype DRB1*03-DQB1*0201/DRB1*04-DQB1*0302, as expected in a young type 1 diabetic cohort. Only 10% (8 of 77) of individuals were positive for protective or neutral haplotypes, and it was from this group that the *KCNJ11* mutation was identified. HLA analysis may help to identify a subset of individuals who are more likely to have *KCNJ11* mutations. However, in this and our previous study (1), age of diagnosis (before 6 months) is more specific. Further studies are needed to assess whether age of diagnosis and HLA testing are helpful in identifying patients likely to have *KCNJ11* mutations.

In summary, we have identified a heterozygous activating *KCNJ11* mutation in a subject classified as having type 1 diabetes diagnosed under the age of 2 years. We also report a novel rare variant R176C, which family studies show to be unlikely to be a pathogenic mutation illustrating the importance of confirming cosegregation of a variant with diabetes in the family. There is difficulty differentiating this genetic subtype from type 1 diabetes on clinical criteria at presentation because patients can present with marked hyperglycemia and ketoacidosis. This and other studies (1,6) suggest that age of diagnosis (<6 months) and possibly HLA genotype (nonpredisposing) may help select subjects for testing for Kir6.2 mutations. Additional studies are required to test this further. The identification of a single subject (1 of 77, 1.3%) diagnosed at 5 weeks does not support an important role of *KCNJ11* mutations in subjects diagnosed with type 1 diabetes under the age of 2 years. However, if, as suggested by physiological studies (1), sulfonylurea therapy proves effective in patients with activating *KCNJ11* mutations, then this low pick-up rate may still warrant genetic testing of subjects with a clinical diagnosis of type 1 diabetes, particularly when diagnosed before 6 months of age.

RESEARCH DESIGN AND METHODS

All 77 subjects (42 male) had been diagnosed with diabetes before the age of 2 years and were considered by their clinicians to have type 1 diabetes on the basis of clinical features. A total of 58 subjects were from the BDA 1972–1981 cohort, a collection of individuals diagnosed under the age of 2 years established from the BDA register (12). The clinical data were collected from questionnaires sent out to the affected individual, the general practitioner, and the consultant. These data included HbA$_{1c}$, creatinine, cholesterol, HDL, and LDL. Blood sampling was carried out for GAD and IA-2 antibody testing and DNA collection. An additional cohort of 19 subjects from the BOX study were selected because they were diagnosed before the age of 2 years (11,18). The mean age at diagnosis was 16.2 months; 1 (1.3%) child was diagnosed before 6 months, 12 (15.6%) between 6 and 12 months, and 64 (83%) over 12 and under 24 months. Twenty-one children (27.2%) had a first-degree relative with diabetes.

***KCNJ11* sequencing.** The entire coding region and intron-exon boundaries of the *KCNJ11* gene were amplified by PCR using genomic DNA and sequence specific primers as previously described (1). Both strands were sequenced in forward and reverse using the Big Dye Terminator Cycler Sequencing Kit (Applied Biosystems, Warrington, U.K.) according to the manufacturer's instructions. Reactions were analyzed on an ABI 3100 Capillary DNA sequencer (Applied Biosystems). Sequences were compared with the published sequence (NM_000525) using Sequence Navigator Software (Applied Biosystems). Changes in the sequence were checked against published polymorphisms and mutations.

Microsatellite analysis. Family relationships were confirmed using a panel of six microsatellites (19).

HLA class II genotyping. HLA-DRB1, -DQA1, and -DQB1 typing (including subtyping of DRB*04) was performed by PCR using sequence specific primers as previously described (20,21).

Pancreatic auto-antibody analysis. Antibodies to GAD$_{65}$ and IA-2$_{ic}$ were measured by radioimmunoassay as previously described (11).

REFERENCES

1. Gloyn AL, Pearson ER, Antcliff JF, Proks P, Bruining GJ, Slingerland AS, Howard N, Srinivasan S, Silva JM, Molnes J, Edghill EL, Frayling TM, Temple IK, Mackay D, Shield JP, Sumnik Z, van Rhijn A, Wales JK, Clark P, Gorman S, Aisenberg J, Ellard S, Njolstad PR, Ashcroft FM, Hattersley AT: Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. *N Engl J Med* 350:1838–1849, 2004

2. Cox NJ, Wapelhorst B, Morrison VA, Johnson L, Pinchuk L, Spielman RS, Todd JA, Concannon P: Seven regions of the genome show evidence of linkage to type 1 diabetes in a consensus analysis of 767 multiplex families. *Am J Hum Genet* 69:820–830, 2001

3. Eftychi C, Howson JM, Barratt BJ, Vella A, Payne F, Smyth DJ, Twells RC, Walker NM, Rance HE, Tuomilehto-Wolf E, Tuomilehto J, Undlien DE, Ronningen KS, Guja C, Ionescu-Tiirgoviste C, Savage DA, Todd JA: Analysis of the type 2 diabetes–associated single nucleotide polymorphisms in the genes IRS1, KCNJ11, and PPARG2 in type 1 diabetes. *Diabetes* 53:870–873, 2004

4. American Diabetes Association: Diagnosis and classification of diabetes mellitus (Position Statement). *Diabetes Care* 27 (Suppl. 1):S5–S10, 2004

5. Pugliese A, Kawasaki E, Zeller M, Yu L, Babu S, Solimena M, Moraes CT, Pietropaolo M, Friday RP, Trucco M, Ricordi C, Allen M, Noble JA, Erlich HA, Eisenbarth GS: Sequence analysis of the diabetes-protective human leukocyte antigen-DQB1*0602 allele in unaffected, islet cell antibody-positive first degree relatives and in rare patients with type 1 diabetes. *J Clin Endocrinol Metab* 84:1722–1728, 1999

6. Iafusco D, Stazi MA, Cotichini R, Cotellessa M, Martinucci ME, Mazzella M, Cherubini V, Barbetti F, Martinetti M, Cerutti F, Prisco F: Permanent diabetes mellitus in the first year of life. *Diabetologia* 45:798–804, 2002

7. Lambert AP, Ellard S, Allen LI, Gallen IW, Gillespie KM, Bingley PJ, Hattersley AT: Identifying hepatic nuclear factor-1α mutations in children and young adults with a clinical diagnosis of type 1 diabetes. *Diabetes Care* 26:333–337, 2003

8. Moller AM, Dalgaard LT, Pociot F, Nerup J, Hansen T, Pedersen O: Mutations in the hepatocyte nuclear factor-1alpha gene in Caucasian families originally classified as having type 1 diabetes. *Diabetologia* 41:1528–1531, 1998

9. Hathout EH, Cockburn BN, Mace JW, Sharkey J, Chen-Daniel J, Bell GI: A case of hepatocyte nuclear factor-1α diabetes/MODY3 masquerading as type 1 diabetes in a Mexican-American adolescent and responsive to a low dose of sulphonylurea (Letter). *Diabetes Care* 22:867–868, 1999

10. Kawasaki E, Sera Y, Yamakawa K, Abe T, Ozaki M, Uotani S, Ohtsu N, Takino H, Yamasaki H, Yamaguchi Y, Matsuura N, Eguchi K: Identification and functional analysis of mutations in the hepatocyte nuclear factor-1alpha gene in anti-islet autoantibody-negative Japanese patients with type 1 diabetes. *J Clin Endocrinol Metab* 85:331–335, 2000

11. Bingley PJ, Bonifacio E, Williams AJ, Genovese S, Bottazzo GF, Gale EA: Prediction of IDDM in the general population: strategies based on combinations of autoantibody markers. *Diabetes* 46:1701–1710, 1997

12. Botha JL, Parker H, Raymond NT, Swift PG: Diabetes diagnosed before the age of 2 years: mortality in a British cohort 8–17 years after onset. *Int J Epidemiol* 21:1132–1137, 1992

13. Ribalet B, John SA, Weiss JN: Molecular basis for Kir6.2 channel inhibition by adenine nucleotides. *Biophys J* 84:266–276, 2003

14. Fan Z, Makielski JC: Anionic phospholipids activate ATP-sensitive potassium channels. *J Biol Chem* 272:5388–5395, 1997

15. Shyng SL, Cukras CA, Harwood J, Nichols CG: Structural determinants of PIP(2) regulation of inward rectifier K(ATP) channels. *J Gen Physiol* 116:599–608, 2000

16. John SA, Weiss JN, Ribalet B: Regulation of cloned ATP-sensitive K channels by adenine nucleotides and sulfonylureas: interactions between SUR1 and positively charged domains on Kir6.2. *J Gen Physiol* 118:391–405, 2001

17. Shield JPH: Neonatal diabetes. In *Childhood Diabetes*. Shield JPH, Baum JD, Eds. London, Bailliere Tindall, 1996, p. 681–740

18. Gardner SG, Bingley PJ, Sawtell PA, Weeks S, Gale EA: Rising incidence of insulin dependent diabetes in children aged 5 years in the Oxford region: time trend analysis: the Bart's-Oxford Study Group. *BMJ* 315:713–717, 1997.

19. Nestorowicz A, Inagaki N, Gonoi T, Schoor KP, Wilson BA, Glaser B, Landau H, Stanley CA, Thornton PS, Seino S, Permutt MA: A nonsense mutation in the inward rectifier potassium channel gene, Kir6.2, is associated with familial hyperinsulinism. *Diabetes* 46:1743–1748, 1997

20. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D: The structure of haplotype blocks in the human genome. *Science* 296:2225–2229, 2002

21. Gillespie KM, Valovin SJ, Saunby J, Hunter KM, Savage DA, Middleton D, Todd JA, Bingley PJ, Gale EA: HLA class II typing of whole genome amplified mouth swab DNA. *Tissue Antigens* 56:530–538, 2000