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# Origin of *de Novo KCNJ11* Mutations and Risk of Neonatal Diabetes for Subsequent Siblings

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## Abstract

**Context—**Activating mutations in the *KCNJ11* gene, which encodes the Kir6.2 subunit of the pancreatic β-cell $K_{ATP}$ channel, result in permanent and transient neonatal diabetes. The majority of *KCNJ11* mutations are spontaneous, but the parental origin of these mutations is not known.

**Objective—**Our objective was to determine the parental origin of *de novo KCNJ11* mutations and investigate the possibility of mosaicism in transmitting parents.

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**Design—**We identified 68 index cases with a *KCNJ11* mutation where neither parent was known to be affected. DNA was available from both parents of 41 probands. The parental origin of the mutation was determined in 18 families by examination of pedigrees, microsatellite analysis, or allele-specific PCR.

**Results—**A nonsignificant excess of paternally derived mutations was found with 13 of 18 (72%) shown to have arisen on the paternal allele. There was no evidence to suggest an association with increased age at conception. In two families, there were half-siblings with permanent neonatal diabetes born to an unaffected father, suggesting germline mosaicism that was confirmed by the presence of the R201C mutation in one father's semen. Somatic mosaicism was detected in one unaffected mother, and this mutation will also be present in her germ cells.

**Conclusion—** *De novo KCNJ11* mutations can arise either during gametogenesis or embryogenesis. The possibility of germline mosaicism means that future siblings are at increased risk of neonatal diabetes, and we recommend that molecular genetic testing is routinely offered at birth for subsequent siblings of children with *de novo KCNJ11* mutations.

NEONATAL DIABETES MELLITUS (NDM) is a rare disorder that can be permanent (PNDM) or transient (TNDM) (1). Heterozygous activating mutations in the *KCNJ11* and *ABCC8* genes, which encode the $K_{ATP}$ channel subunits Kir6.2 and SUR1, have recently been identified (2–4). *KCNJ11* mutations are most common, accounting for 47% of PNDM (4, 5) and a few cases of TNDM (6, 7). Most of these patients achieve improved glycemic control after transfer from insulin injections to sulfonylurea tablets (8).

Approximately 84% of patients with *KCNJ11* mutations have no family history of neonatal diabetes, and their diabetes results from a *de novo* (spontaneous) mutation (9). *De novo* mutations arise in the parent either during gametogenesis (germline *de novo* mutations) or as an early postzygotic event. We previously reported a family with paternal germline mosaicism where two paternal half-siblings were affected with PNDM (10). If a postzygotic mutational event happens before the separation between somatic and germinal lineages, the individual will show both somatic and germline mosaicism, although the level of mosaicism will vary between tissues and may not be detectable in leukocyte DNA. The percentage of mosaicism in the germ cells will depend upon the timing of the mutation, but if all primordial germ cells are affected, then the recurrence risk for future siblings can be up to 50% (11).

The aim of this study was to determine the origin of *de novo KCNJ11* mutations causing neonatal diabetes. The possibility of somatic mosaicism (and hence germline mosaicism) was investigated in the transmitting parents using a quantitative real-time PCR assay.

## Subjects and Methods

### Subjects

We identified 68 probands with a *KCNJ11* mutation and no family history of neonatal diabetes (12). DNA from both parents was available for 41 cases. The majority were recruited after a request for referrals to the International Society of Pediatric and Adolescent Diabetes (ISPAD). Informed consent was obtained from all subjects.

### Genetic analysis of KCNJ11

Leukocyte DNA was extracted using standard methods. DNA was extracted from semen as described previously (13).

Haplotype analysis and family relationship confirmations were performed by analyzing microsatellites D11S902, D11S419, D11S1397, D11S1901, D11S921, and D11S1888. Gender identity of parental samples was confirmed by analysis of the amelogenin locus.

The *KCNJ11* gene was sequenced as described previously (13) in probands and parental samples to confirm that the mutations occurred spontaneously in the proband and to genotype the coding single-nucleotide polymorphisms (SNPs) E23K (rs5219), A190A (rs5218), and I337V (rs5215) for the molecular haplotyping assay.

### Molecular haplotyping

PCR primers were designed to amplify a single copy of the *KCNJ11* gene using a primer complementary to one allele of a heterozygous SNP or the heterozygous mutation (Table 1). Sequence analysis of the PCR amplicon revealed the coinherited allele and hence the parental origin of the mutation.

### Real-time PCR quantification

Genomic DNA from leukocytes was investigated for low-level mosaicism using an allelic discrimination assay as described previously (10) with the addition of a preamplification stage of 10 cycles using fragment 1 or 2 primers (13) for analysis of the V59M, E227K, and G53R mutations.

### Restriction enzyme digest

The restriction endonuclease *Afl*III (New England Biolabs Ltd., Hitchin, Hertfordshire, UK) was used to digest the normal allele from 1 *μg* semen or leukocyte DNA for 24 h at 37 C using 20 U enzyme. The digested DNA was then subject to 35 cycles of amplification using fragment 2 primers (12). This amplicon was then sequenced.

## Results

*De novo KCNJ11* mutations were proven in 41 families where neither parent was shown to carry the mutation, and microsatellite analysis of markers flanking the *KCNJ11* locus confirmed family relationships. There were four families where after an initial spontaneous mutation, there were then two generations affected with neonatal diabetes (Fig. 1). Genotyping and microsatellite analysis established that these R201H and E227K mutations originated as *de novo* events in the grandparental generation and arose in two families on the paternal allele (one R201H and one E227K) and in two families on the maternal haplotype (Fig. 1 and Table 2). We previously reported a family (ISPAD 58) with two affected half-siblings born to an unaffected father (10). In this study, a second family was identified (ISPAD 115) where the proband and her affected paternal half-sibling were heterozygous for the R201C mutation, but the father was not affected.

An allele-specific PCR assay was used to determine the chromosome of origin in 12 of the remaining 35 families in whom informative SNPs were located within 500 base pairs of the mutation. The molecular haplotyping method was validated by confirming the paternal origin of the R201H mutation in family ISPAD 19 (Fig. 2). We found that nine of these mutations had arisen on the paternal allele, and three were of maternal origin (Table 2). The parental origin of *KCNJ11* mutations was therefore determined in a total of 18 families and demonstrated a moderate but not significant bias toward a paternal origin (13 of 18 mutations; $\chi^2$ $P = 0.17$).

A quantitative real-time PCR assay was used to investigate the possibility of low-level somatic mosaicism in the transmitting parent. The limit of sensitivity for the TaqMan probes was calculated as more than 0.4% for the R201C allele-specific probe (10), whereas all others had a probe sensitivity of more than 0.8% (data not shown). There was no evidence of somatic mosaicism in leukocyte DNA from 13 parents (Table 2), but the I182V mutation was present at 2.9% in the mother of a child with TNDM (ISPAD 50).

Real-time PCR analysis of leukocyte and semen DNA samples from the unaffected father in family ISPAD 115 did not show somatic or germline mosaicism for the R201C mutation present in his two affected children. We investigated this further by using an *Afl*III restriction digest of genomic DNA to specifically cleave the normal sequence and thus enrich for the mutant R201C allele. These digested DNA samples were then subject to PCR amplification, and sequencing of the resulting PCR products showed the presence of the mutant allele in the paternal semen DNA sample but not in his leukocyte DNA (Fig. 3). It was not possible to perform this analysis in the father of family ISPAD 58 because he had undergone a vasectomy.

The median parental age at conception was 31 yr (range, 19–49 yr) for *KCNJ11* mutations of paternal origin and 25 yr (range, 19–39 yr) for maternal origin ($P = 0.5$, Mann-Whitney *U* test). Comparison with the population median age at conception was not possible due to the small sample size as a result of the diversity of populations represented within this study (Table 2).

In addition to the 41 cases where a *de novo* mutation was proven by molecular genetic testing, we identified *KCNJ11* mutations in 27 probands in whom there was no parental history of neonatal diabetes but DNA samples from one or both parents were not available for testing. In only the two families described above was there a second sibling affected with neonatal diabetes (two of 68, 3% of families).

## Discussion

The majority (29 of 41, 71%) of *de novo* mutations identified in this study were substitutions at CpG nucleotides that are likely to be the result of the spontaneous deamination of methylcytosine to thymine. The most common *KCNJ11* mutations (V59M, R201H, R201C, E227K, and E229K) all occur within the context of a CpG dinucleotide and encode C→T or G→A transitions.

It was possible to determine the parent of origin in 18 families, and a moderate excess of paternal mutations (13 of 18) was observed. In two families, the inheritance pattern suggested paternal germline mosaicism because two half-siblings had PNDM, but neither parent was affected. Paternal germline mosaicism was confirmed in one family by analysis of semen DNA. The R201C mutation was present at a very low level (less than 0.4%), which was unexpected because two of his three children are affected with neonatal diabetes. The germline mosaicism is likely to be the result of a postzygotic mutation during embryonic development, and therefore the mosaicism may not be restricted to the germline. Although there was no evidence of somatic mosaicism in either father's leukocyte DNA, samples from other tissues (*e.g.* buccal cells, hair roots, or fibroblasts) were not available for testing, and hence somatic mosaicism cannot be excluded.

Somatic mosaicism was detected in a single transmitting parent of 14 tested. This mother (ISAPD 50) was mosaic for the I182V *KCNJ11* mutation. The presence of the mutation in leukocyte DNA is consistent with a postzygotic mutation occurring early in embryonic development. The I182V mutation will also be present in the mother's germ cells, although it is not possible to measure the mutation load and hence the risk to future siblings. However, if the mutation arose early enough to affect all the primordial germ cells, then the recurrence risk could be up to 50% (11). This is the first report to suggest that neonatal diabetes may be caused by maternal germline mosaicism for a *KCNJ11* mutation.

The occurrence of both paternal and maternal mutations is consistent with *KCNJ11* mutations resulting from postzygotic mutations during embryogenesis in an unaffected parent and from mitotic errors during gametogenesis. Other genes showing a similar pattern of *de novo* mutations include APC, *VHL, NF1, NF2,* and *EFNB1* mutations (causing familial adenomatous polyposis, Von Hippel-Lindau, neurofibromatosis, or craniofrontonasal syndrome) (14–16). Postzygotic mutations occurring during embryogenesis will show no sex bias because they are random events, and no correlation with increased parental age at conception is predicted.

The situation for *KCNJ11* mutations is distinct from the exclusive paternal origin of *de novo FGFR2* and *FGFR3* mutations in Apert syndrome and achondroplasia where there is a significant association with increased paternal age at conception (17, 18). Recent evidence suggests that these gain-of-function mutations confer a selective advantage to spermatogonial cells leading to their accumulation over time (13, 19).

Germline mosaicism was indicated in two families by pedigree analysis and in one of 12 additional parents tested for somatic mosaicism. Within our cohort of 68 families with presumed or proven *de novo KCNJ11* mutations, a second affected child was born to two families. From this series, the empiric recurrence risk for affected siblings due to parental germline mosaicism is 3%. Although this may be an overestimate due to the increased likelihood of referral for genetic testing for families with two affected children, it is important that the possibility of germline mosaicism is discussed during counseling. In view of the increased risk of neonatal diabetes compared with the population, we recommend that the parents of all children with *KCNJ11* mutations are offered molecular genetic testing for future siblings at birth. A cord blood sample can be tested quickly (within 3 d), and a

normal result will provide reassurance. In the unlikely event of a mutation being identified, monitoring of blood glucose levels can be initiated promptly to ensure early diagnosis and appropriate treatment to achieve good glycemic control from the outset.

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## Abbreviations

| | |
|---|---|
| **ISPAD** | International Society of Pediatric and Adolescent Diabetes |
| **NDM** | neonatal diabetes mellitus |
| **PNDM** | permanent NDM |
| **SNP** | single-nucleotide polymorphism |
| **TNDM** | transient NDM |

## References

1. Polak M, Shield J. Neonatal and very-early-onset diabetes mellitus. Semin Neonatol. 2004; 9 :59–65. [PubMed: 15013476]
2. Proks P, Arnold AL, Bruining J, Girard C, Flanagan SE, Larkin B, Colclough K, Hattersley AT, Ashcroft FM, Ellard S. A heterozygous activating mutation in the sulphonylurea receptor SUR1 (ABCC8) causes neonatal diabetes. Hum Mol Genet. 2006; 15 :1793–1800. [PubMed: 16613899]
3. Babenko AP, Polak M, Cave H, Busiah K, Czernichow P, Scharfmann R, Bryan J, Aguilar-Bryan L, Vaxillaire M, Froguel P. Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. N Engl J Med. 2006; 355 :456–466. [PubMed: 16885549]
4. Gloyn AL, Pearson ER, Antcliff JF, Proks P, Bruining GJ, Slingerland AS, Howard N, Srinivasan S, Silva JM, Molnes J, Edghill EL, et al. Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med. 2004; 350 :1838–1849. [PubMed: 15115830]
5. Hattersley AT, Ashcroft FM. Activating mutations in Kir6.2 and neonatal diabetes: new clinical syndromes, new scientific insights, and new therapy. Diabetes. 2005; 54 :2503–2513. [PubMed: 16123337]
6. Gloyn AL, Reimann F, Girard C, Edghill EL, Proks P, Pearson ER, Temple IK, Mackay DJ, Shield JP, Freedenberg D, Noyes K, et al. Relapsing diabetes can result from moderately activating mutations in KCNJ11. Hum Mol Genet. 2005; 14 :925–934. [PubMed: 15718250]
7. Colombo C, Delvecchio M, Zecchino C, Faienza MF, Cavallo L, Barbetti F. Transient neonatal diabetes mellitus is associated with a recurrent (R201H) KCNJ11 (KIR6.2) mutation. Diabetologia. 2005; 48 :2439–2441. [PubMed: 16205880]
8. Pearson ER, Flechtner I, Njolstad PR, Malecki MT, Flanagan SE, Larkin B, Ashcroft FM, Klimes I, Codner E, Iotova V, Slingerland AS, et al. Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. N Engl J Med. 2006; 355 :467–477. [PubMed: 16885550]
9. Slingerland AS, Hattersley AT. Mutations in the Kir6.2 subunit of the KATP channel and permanent neonatal diabetes: new insights and new treatment. Ann Med. 2005; 37 :186–195. [PubMed: 16019717]

10. Gloyn AL, Cummings EA, Edghill EL, Harries LW, Scott R, Costa T, Temple IK, Hattersley AT, Ellard S. Permanent neonatal diabetes due to paternal germline mosaicism for an activating mutation of the KCNJ11 gene encoding the Kir6.2 subunit of the β-cell potassium adenosine triphosphate channel. J Clin Endocrinol Metab. 2004; 89 :3932–3935. [PubMed: 15292329]
11. Zlotogora J. Germ line mosaicism. Hum Genet. 1998; 102 :381–386. [PubMed: 9600231]
12. Flanagan SE, Edghill EL, Gloyn AL, Ellard S, Hattersley AT. Mutations in KCNJ11, which encodes Kir6.2, are a common cause of diabetes diagnosed in the first 6 months of life, with the phenotype determined by genotype. Diabetologia. 2006; 49 :1190–1197. [PubMed: 16609879]
13. Goriely A, McVean GA, Rojmyr M, Ingemarsson B, Wilkie AO. Evidence for selective advantage of pathogenic FGFR2 mutations in the male germ line. Science. 2003; 301 :643–646. [PubMed: 12893942]
14. Aretz S, Uhlhaas S, Caspari R, Mangold E, Pagenstecher C, Propping P, Friedl W. Frequency and parental origin of de novo APC mutations in familial adenomatous polyposis. Eur J Hum Genet. 2004; 12 :52–58. [PubMed: 14523376]
15. Richards FM, Payne SJ, Zbar B, Affara NA, Ferguson-Smith MA, Maher ER. Molecular analysis of *de novo* germline mutations in the von Hippel-Lindau disease gene. Hum Mol Genet. 1995; 4 :2139–2143. [PubMed: 8589692]
16. Twigg SR, Matsumoto K, Kidd AM, Goriely A, Taylor IB, Fisher RB, Hoogeboom AJ, Mathijssen IM, Lourenco MT, Morton JE, Sweeney E, et al. The origin of EFNB1 mutations in craniofrontonasal syndrome: frequent somatic mosaicism and explanation of the paucity of carrier males. Am J Hum Genet. 2006; 78 :999–1010. [PubMed: 16685650]
17. Moloney DM, Slaney SF, Oldridge M, Wall SA, Sahlin P, Stenman G, Wilkie AO. Exclusive paternal origin of new mutations in Apert syndrome. Nat Genet. 1996; 13 :48–53. [PubMed: 8673103]
18. Bellus GA, Hefferon TW, Ortiz de Luna RI, Hecht JT, Horton WA, Machado M, Kaitila I, McIntosh I, Francomano CA. Achondroplasia is defined by recurrent G380R mutations of FGFR3. Am J Hum Genet. 1995; 56 :368–373. [PubMed: 7847369]
19. Tiemann-Boege I, Navidi W, Grewal R, Cohn D, Eskenazi B, Wyrobek AJ, Arnheim N. The observed human sperm mutation frequency cannot explain the achondroplasia paternal age effect. Proc Natl Acad Sci USA. 2002; 99 :14952–14957. [PubMed: 12397172]
20. Gloyn AL, Diatloff-Zito C, Edghill EL, Bellanne-Chantelot C, Nivot S, Coutant R, Ellard S, Hattersley AT, Robert JJ. KCNJ11 activating mutations are associated with developmental delay, epilepsy and neonatal diabetes syndrome and other neurological features. Eur J Hum Genet. 2006; 14 :824–830. [PubMed: 16670688]

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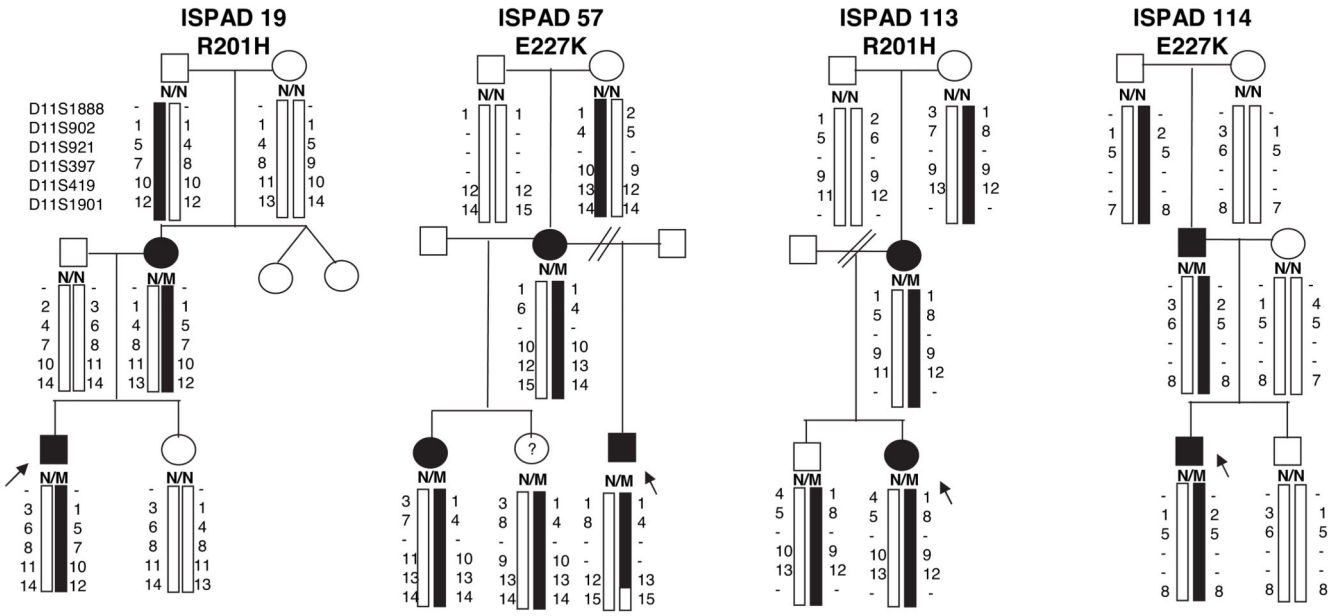


**Fig. 1.**

Microsatellite analysis of markers flanking the *KCNJ11* gene in four families with two generations affected with neonatal diabetes. *Black bars* indicate the haplotype cosegregating with neonatal diabetes on which the *de novo* mutation has arisen. *Squares* denote males, *circles* females, and *black symbols* show individuals affected with neonatal diabetes. N/M, Heterozygous mutation; N/N, mutation not present. The *KCNJ11* gene is located between markers D11S902 and D11S921. *Arrows* point to the proband in each family.





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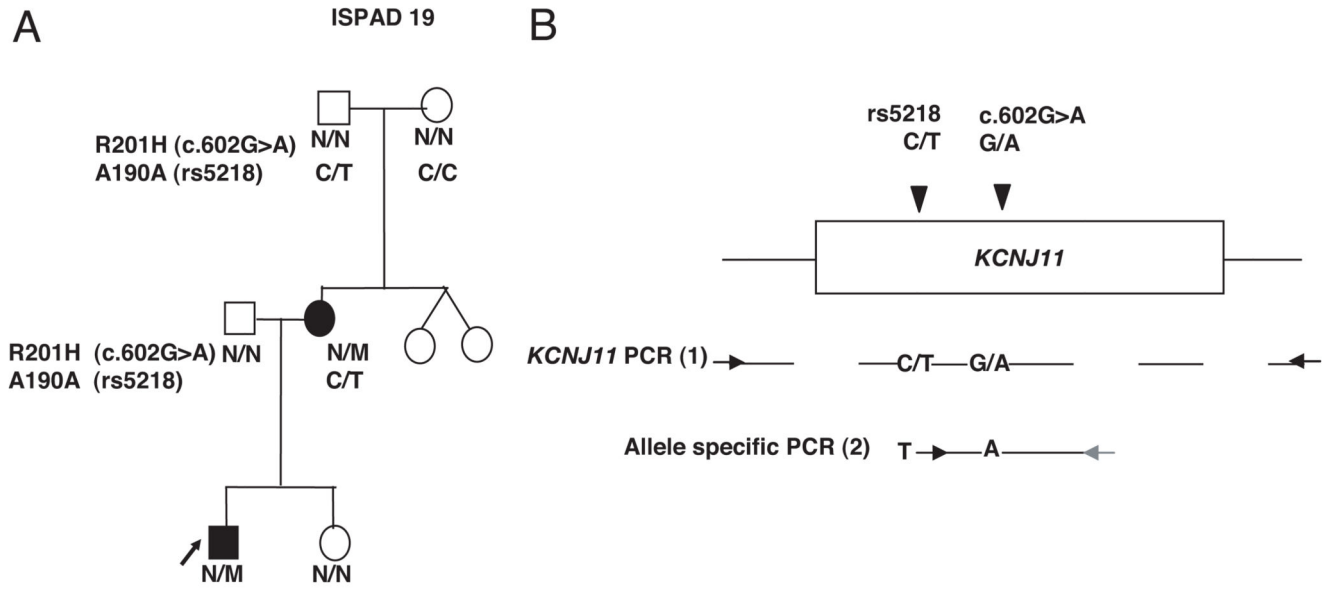


**Fig. 2. Allele-specific PCR to determine chromosome of origin in family ISPAD 19.**

A, The heterozygous R201H (c.602G→A) mutation arose spontaneously in the proband's mother, who is also heterozygous for SNP rs5218 (C/T; A190A). Her mother is homozygous and her father heterozygous for this SNP. B, Standard *KCNJ11* PCR detected the heterozygous mutation and polymorphism in the mother (product 1). Allele-specific PCR using a specific primer for the T allele of the SNP amplifies a single product (product 2). Sequencing of this allele-specific PCR product revealed an A at nucleotide 602. This demonstrates that the mutant allele is *in cis* with the T allele of the rs5218 SNP, and therefore the R201H mutation arose on the paternal chromosome. *Squares* denote males, *circles* females, and *black symbols* show individuals affected with neonatal diabetes. An *arrow* is used to identify the proband.




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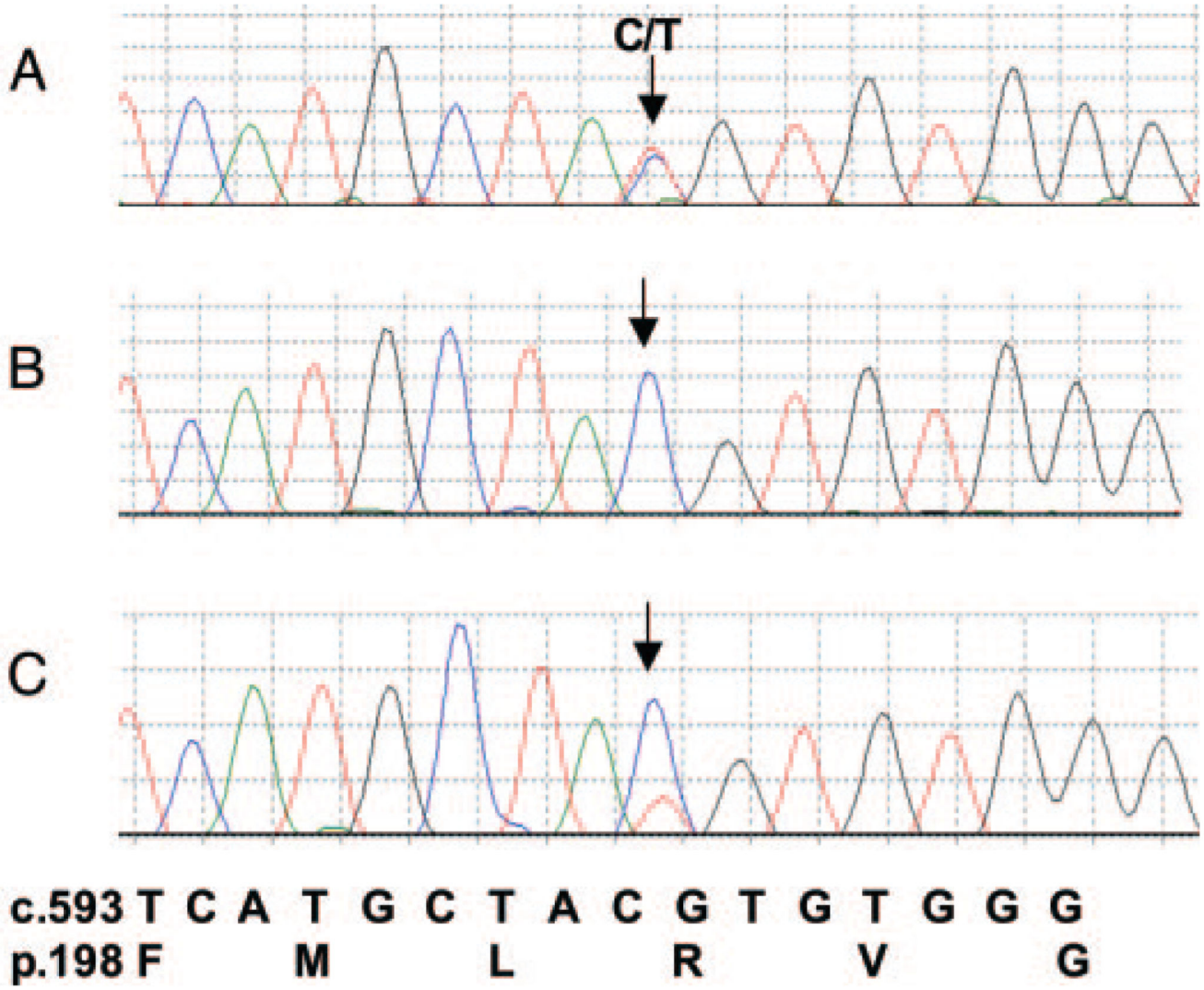


**Fig. 3.**

Analysis of the R201C mutation in somatic and germ cells. Sequence analysis of PCR product derived from leukocyte DNA from the ISPAD 115 proband in whom the R201C (c.601C→T) mutation is heterozygous (A), leukocyte DNA from the unaffected father after enrichment for the mutation (B), and semen DNA from his unaffected father after enrichment for the mutation by restriction endonuclease digestion of the normal allele (C). The position of nucleotide 601 is indicated by an *arrow*.

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## Table 1
## Allele-specific primers for molecular haplotyping

| Primer | Primer sequence (5'–3') | Genotype of single allele |
|---|---|---|
| 1 | CGCCTGGCAGAGGACCCTGCT *G* | E23K (GAG)-F |
| | CTGGATGAGCAGCAGGGGGAG | E23K (GAG)-R |
| 2 | CGCCTGGCAGAGGACCCTGCT *A* | E23K (AAG)-F |
| | CTGGATGAGCAGCAGGGGGAG | E23K (AAG)-R |
| 3 | CAAGCATGCGGTGATCGC *C* | A190A (GCC)-F |
| | ACGCCTTCCAGGATGACGAT | A190A (GCC)-R |
| 4 | CAAGCATGCGGTGATCGC *T* | A190A (GCT)-F |
| | ACGCCTTCCAGGATGACGAT | A190A (GCT)-R |
| 8 | CCGCTTCCTGCAGGACG *G* | V59 (GTG)-F |
| | CTGTGGTCCTCATCAAGC TG | V59 (GTG)-R |
| 11 | AAGCAGAGGCGGCCGTGGCGCAG *G* | A190A (GCC)-F |
| | CCGAGAGGACTCTGCAGTGA | A190A (GCC)-R |
| 12 | GAGTGGTGTGGGCACTTTGC *C* | I337V (GTC)-F |
| | TCAGCAAGCATGCGGTGATC | I337V (GTC)-R |
| 13 | GAGTGGTGTGGGCACTTTGC *T* | I337V (ATC)-F |
| | TCAGCAAGCATGCGGTGATC | I337V (ATC)-R |
| 14 | CTTGCGGAGGTCACCCACG *C* | R201H (CGT)-F |
| | TCACCGGAGCCATGCTGTC | R201H (CGT)-R |
| 15 | CTTGCGGAGGTCACCCACG *T* | R201H(CAT)-F |
| | TCACCGGAGCCATGCTGTC | R201H (CAT)-R |

The *italicized* 3'-terminal nucleotide is allele specific. The *underlined* nucleotides are mismatched from the normal sequence to enhance allelic discrimination. F, Forward; R, reverse.

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## Table 2

### Origin of *de novo KCNJ11* mutations

| ISPAD family ID | *KCNJ11* mutation | PNDM/ TNDM | Technique for determining chromosome of origin | Parental origin | Germline/ somatic mosaicism | Age of parent at conception (yr) | Country of origin | Ref. |
|---|---|---|---|---|---|---|---|---|
| 137 | H46Y | PNDM | Allele-specific PCR | Maternal | Not tested | 19 | Slovakia | 12 |
| 79 | G53R | TNDM | Allele-specific PCR | Paternal | Not detected | 31 | UK | 6 |
| 138 | V59M | PNDM | Allele-specific PCR | Maternal | Not detected | 25 | UK | 12 |
| 25 | V59G | PNDM | Allele-specific PCR | Paternal | Not tested | 30 | Czech Republic | 4 |
| 50 | I182V | TNDM | Allele-specific PCR | Maternal | Somatic and germline | 39 | UK | 6 |
| 22 | R201H | PNDM | Allele-specific PCR | Paternal | Not detected | 20 | Australia | 4 |
| 110 | R201H | PNDM | Allele-specific PCR | Paternal | Not detected | Not known | Holland | 12 |
| 19 | R201H | PNDM | Microsatellite analysis | Paternal | Not detected | 24 | Holland | 4 |
| NECKER4 | R201H | PNDM | Allele-specific PCR | Paternal | Not tested | Not known | France | 20 |
| 113 | R201H | PNDM | Microsatellite analysis | Maternal | Not detected | 31 | Canada | 12 |
| 139 | R201H | PNDM | Allele-specific PCR | Paternal | Not detected | 45 | Canada | Not published |
| 140 | R201H | PNDM | Allele-specific PCR | Paternal | Not detected | 19 | Slovakia | 12 |
| 41 | R201C | PNDM | Allele-specific PCR | Paternal | Not detected | 31 | Holland | 4 |
| 115 | R210C | PNDM | Pedigree analysis | Paternal | Germline mosaic | 49 | Bulgaria | Not published |
| 58 | R201C | PNDM | Pedigree analysis | Paternal | Inferred germline mosaic | 34 | Canada | 10 |
| 57 | E227K | TNDM | Microsatellite analysis | Maternal | Not detected | 20 | German | Not published |
| 114 | E227K | TNDM | Microsatellite analysis | Paternal | Not detected | 28 | Canada | Not published |
| 10 | E229K | TNDM | Allele-specific PCR | Paternal | Not tested | Not known | UK | Not published |