IsK and KvLQT1: mutation in either of the two subunits of the slow component of the delayed rectifier potassium channel can cause Jervell and Lange-Nielsen syndrome

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The Jervell and Lange-Nielsen syndrome (JLNS) comprises profound congenital sensorineural deafness associated with syncopal episodes. These are caused by ventricular arrhythmias secondary to abnormal repolarisation, manifested by a prolonged QT interval on the electrocardiogram. Recently, in families with JLNS, Neyroud *et al.* reported homozygosity for a single mutation in *KVLQT1*, a gene which has previously been shown to be mutated in families with dominantly inherited isolated long QT syndrome [Neyroud *et al.* (1997) *Nature Genet.*, 15, 186–189]. We have analysed a group of families with JLNS and shown that the majority are consistent with mutation at this locus: five families of differing ethnic backgrounds were homozygous by descent for markers close to the *KVLQT1* gene and a further three families from the same geographical region were shown to be homozygous for a common haplotype and to have the same homozygous mutation of the *KVLQT1* gene. However, analysis of a single small consanguineous family excluded linkage to the *KVLQT1* gene, establishing genetic heterogeneity in JLNS. The affected children in this family were homozygous by descent for markers on chromosome 21, in a region containing the gene *IsK*. This codes for a transmembrane protein known to associate with *KVLQT1* to form the slow component of the delayed rectifier potassium channel. Sequencing of the affected boys showed a homozygous mutation, demonstrating that mutation in the *IsK* gene may be a rare cause of JLNS and that an indistinguishable phenotype can arise from mutations in either of the two interacting molecules.

INTRODUCTION

The cardioauditory syndrome of Jervell and Lange-Nielsen (JLNS) was first described in 1957 in a Norwegian family (1). Four of six children born to unrelated parents suffered from profound congenital deafness associated with syncope and prolongation of the QT interval on electrocardiograms. Syncope arises as a consequence of abnormal ventricular repolarisation, which triggers potentially life-threatening ventricular arrhythmias. Untreated, the syndrome has a very high mortality. Although the condition is very rare, with an estimated prevalence of up to 10 per million, a number of other cases have been reported since the original description and autosomal recessive inheritance has been confirmed (2). A hypothesis, many years ago, suggested that the connection between the auditory and the
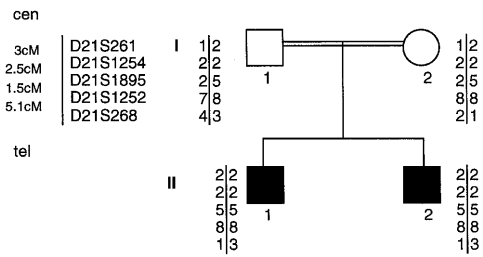
Figure 1. Family UK1S with haplotype data for chromosome 21 markers. The *IsK* gene is situated between markers D21S1254 and D21S1895 (36).

cardiac defect in JLNS may be due to transmembrane electrolyte imbalance (2).

The cardiac action potential consists of a depolarisation phase, due mainly to a large influx of sodium ions, a plateau phase dependent mainly on calcium influx and repolarisation, associated with an increase in potassium permeability. This repolarising potassium current, the delayed rectifier current, has a rapidly activating component and a slowly activating component I_{Ks}.

In the ear, endolymphatic fluid produced by the stria vascularis surrounds the sensory hair cells of the cochlea. This fluid has a high concentration of potassium and a low concentration of sodium, resulting in a resting potential of about +100 mV with respect to other parts of the cochlea (3). Developmental abnormalities of the stria vascularis in mice and in humans may result in reduction of the normal osmotic pressure in the endolymphatic duct, resulting in collapse of Reissner's membrane and features similar to those seen in post-mortem studies of humans with JLNS (4–6).

Phenotypically, JLNS has similarities to the Romano–Ward syndrome, in which there is an isolated long QT interval without deafness, transmitted as an autosomal dominant condition. It has been hypothesised that the two conditions could be allelic (2). Three genes are known to underlie dominantly inherited isolated long QT syndrome (Romano–Ward syndrome), *HERG*, *SCN5A* and *KVLQT1*, and a fourth as yet unknown gene has been mapped to chromosome 4 (7–10). The three known genes all encode ion channels: *HERG* encodes a potassium channel gene, related to the *Drosophila ether-a-go-go* gene, which underlies I_{Kr}, the rapidly activating component of the delayed rectifier current responsible for cardiac repolarisation; *SCN5A* encodes an α subunit of a cardiac sodium channel; *KVLQT1* encodes a potassium channel. KvLQT1, the product of the *KVLQT1* gene, has been shown to associate with IsK, a small membrane-spanning glycoprotein. Together these two molecules reproduce the properties of I_{Ks}, the slowly activating component of the delayed rectifier current (11,12). Recently it has been shown at the molecular level that JLNS and Romano–Ward syndrome can be caused by mutations in the same gene, *KVLQT1*, JLNS being the homozygous form of Romano–Ward syndrome (13,14).

We analysed a single small family with JLNS and excluded the *KVLQT1* gene as the disease locus, proving that genetic heterogeneity exists in JLNS as it does in Romano–Ward syndrome. Using this family, a genome search, combined with exclusion of other candidate loci, identified a region of homozygosity on chromosome 21, in a region harbouring the candidate gene *IsK*. We demonstrate a homozygous mutation in *IsK* in this family. Haplotyping of a group of families with JLNS shows that the majority of families are consistent with mutation in *KVLQT1*, but that mutation in *IsK*, whose protein product associates with that of the *KVLQT1* gene to form the delayed rectifier potassium channel, may be a rare cause of JLNS, phenotypically indistinguishable from that caused by mutation in *KVLQT1*.

RESULTS

Chromosome 21-linked family

Haplotype analysis in family UK1S excluded linkage to the *KVLQT1* locus on chromosome 11, as the affected brothers were not homozygous by descent for any markers in this region of chromosome 11 (data not shown). In addition, all the other known long QT loci, as well as all loci for non-syndromic recessive and dominant deafness described to date, were not linked to the disease (15). Non-syndromic loci were tested on the basis of observations that syndromic and non-syndromic forms of deafness may map to the same loci and therefore could be allelic (16,17). A genome-wide search in family UK1S demonstrated a region of homozygosity by descent on chromosome 21 with D21S261 after 50 markers had been genotyped. Other markers which showed homozygosity are D21S1895 and D21S1252, but not D21S268 (D21S1254 was uninformative). Markers are in the order cen, D21S261, D21S1254, D21S1895, D21S1252, D21S268, tel. Marker haplotypes in this region are shown in
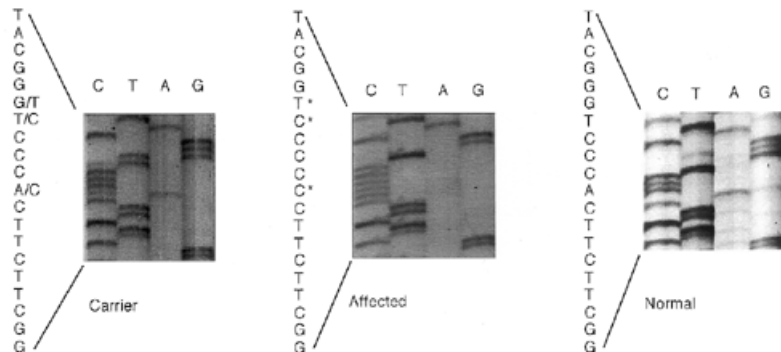

Figure 2. Sequence analysis of the mutation in an affected individual is shown together with that of a normal and a carrier parent. Substituted bases are marked * in the affected individual. The carrier parent is clearly heterozygous for the mutation.
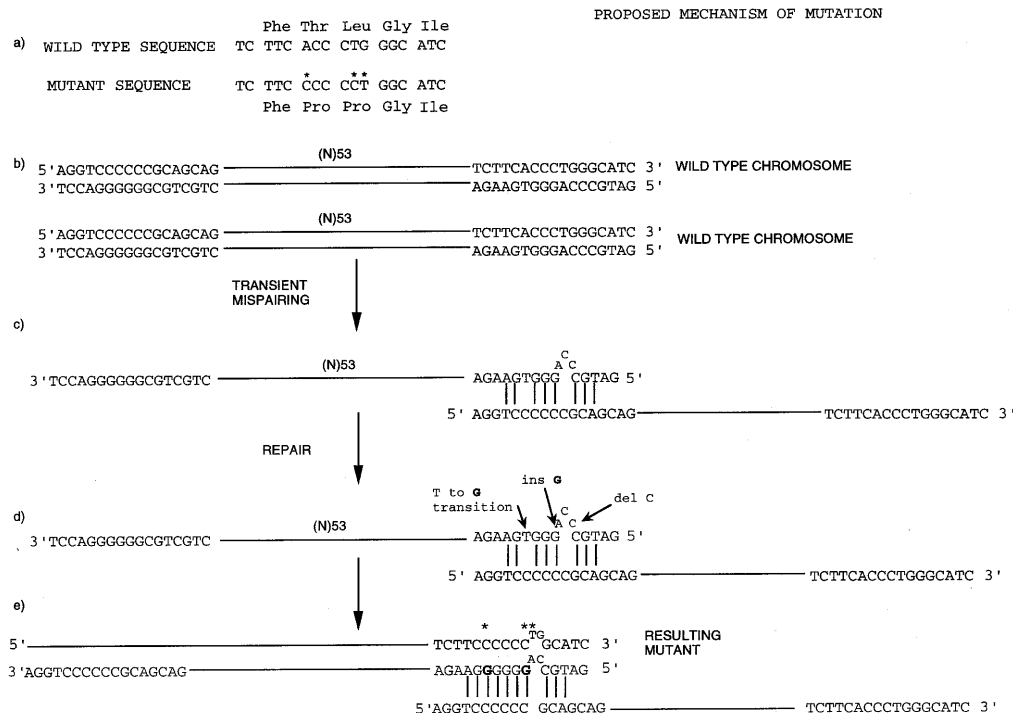
PROPOSED MECHANISM OF MUTATION

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                        Phe Thr Leu Gly Ile
a)  WILD TYPE SEQUENCE  TC TTC ACC CTG GGC ATC

                             *    **
    MUTANT SEQUENCE      TC TTC CCC CCT GGC ATC
                        Phe Pro Pro Gly Ile
```

```
                          (N)53
b)  5'AGGTCCCCCCGCAGCAG————————————TCTTCACCCTGGGCATC 3'  WILD TYPE CHROMOSOME
    3'TCCAGGGGGGCGTCGTC————————————AGAAGTGGGACCCGTAG 5'

                          (N)53
    5'AGGTCCCCCCGCAGCAG————————————TCTTCACCCTGGGCATC 3'  WILD TYPE CHROMOSOME
    3'TCCAGGGGGGCGTCGTC————————————AGAAGTGGGACCCGTAG 5'
```

TRANSIENT
MISPAIRING

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c)
                                             C
                                            A C
    3'TCCAGGGGGGCGTCGTC———(N)53———AGAAGTGGG CGTAG 5'
                                   || ||| |||
                  5' AGGTCCCCCCGCAGCAG————————————TCTTCACCCTGGGCATC 3'
```

REPAIR

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                                   T to G        ins G        del C
d)                                 transition     C
    3'TCCAGGGGGGCGTCGTC———(N)53———AGAAGTGGG A C CGTAG 5'
                                   || ||| |||
                  5' AGGTCCCCCCGCAGCAG————————————TCTTCACCCTGGGCATC 3'
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e)
                                   *    **
                                       TG
    5'————————————————————TCTTCCCCCC GCATC 3'  RESULTING MUTANT
    3'AGGTCCCCCCGCAGCAG————————————AGAAGGGGGG CGTAG 5'
                                    AC
                                   |||||||| |||
                  5'AGGTCCCCCC GCAGCAG————————————TCTTCACCCTGGGCATC 3'
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Figure 3. (**a**) The wild type and mutant sequences. There are three nucleotide substitutions which are indicated by * in the mutant sequence. (**b–d**) A proposed mechanism for the complex mutation. There are two regions of sequence similarity ~60 bases apart in the gene. Transient mispairing between these two regions is shown in (c). Attempted correction of the 3′-sequence based on the template of the 5′-sequence is shown in (d). Partial correction results in the changes observed in the mutant sequence.

Figure 1. The maximum lod scores for this small family at zero recombination were 1.47 for D21S1252 and 1.69 for D21S1895.

A candidate gene, the potassium channel *IsK* (minK) mapping to the homozygous region, was screened for mutations in family UK1S using SSCP (18). Previously described intragenic polymorphisms were detected (19,20) and shifted bands were observed in both parents in family UK1S in double-stranded DNA (possibly heteroduplexes), which were not seen in over 60 control individuals (120 chromosomes) or in any other individuals with JLNS from 11 families. Sequencing of the PCR products of the *IsK* gene showed the changes illustrated in Figures 2 and 3a, in which three separate nucleotides have been altered. The changes cause Thr59 and Leu60 each to be replaced by Pro in the transmembrane region of the predicted protein.

Chromosome 11-linked families

Haplotype data from eight families are shown in Figure 4. The offspring of families N8A and UK2T are the product of first cousin marriages. In families N1H, N3S, N5B and N10D, consanguinity or a founder effect is suspected (represented by a dashed line), as the parents originate from the same county in Norway. Parents of families N6K and N7J are not known to be related.

Homozygosity by descent was seen for all the markers typed, D11S4046, D11S1318, D11S4088 and D11S4146, in families UK2T and N3S. In family N8A and family N5B, markers distal to D11S1318 were homozygous, whereas in family N1H, homozygosity was observed for D11S1318 itself and the marker proximal to it, D11S4046. In family N10D, homozygosity was

observed for the following markers, D11S4046, D11S1318 and D11S4088, as shown in Figure 4.

SSCP screening of the *KVLQT1* gene corresponding to the S2–S3 domains of the protein (PCR primers 1F and 1R) (9) showed an aberrant conformer which is homozygous in the affected individuals in families N1H, N5B and N10D and present in the heterozygous state in their parents (not shown). Sequencing of the PCR product showed a homozygous 5 bp deletion of nt 187–191 (according to the numbering in 9) in affected individuals, which abolishes a *Hha*I restriction enzyme site (Fig. 5). This was not seen in 40 normal controls (80 chromosomes).

DISCUSSION

We provide evidence that mutation in the gene *IsK* can cause JLNS, which appears to be clinically indistinguishable from cases caused by mutation in *KVLQT1*. The biological interaction of the two molecules supports this observation. The product of the *KVLQT1* gene has been shown to associate with the product of the *IsK* gene to form a channel with properties of the slow component of the delayed rectifier current in the heart (11,12). In the ear, IsK appears to have a role in the transport of high concentrations of K^+ into the extracellular endolymph surrounding the hair cells (21). Mice homozygous for a complete targeted disruption of the *IsK* gene show shaker/waltzer behaviour characteristic of inner ear dysfunction and inner ear pathology closely resembling that seen in human subjects who have died from JLNS (21). Combined with the mapping data in family UK1S, this rendered
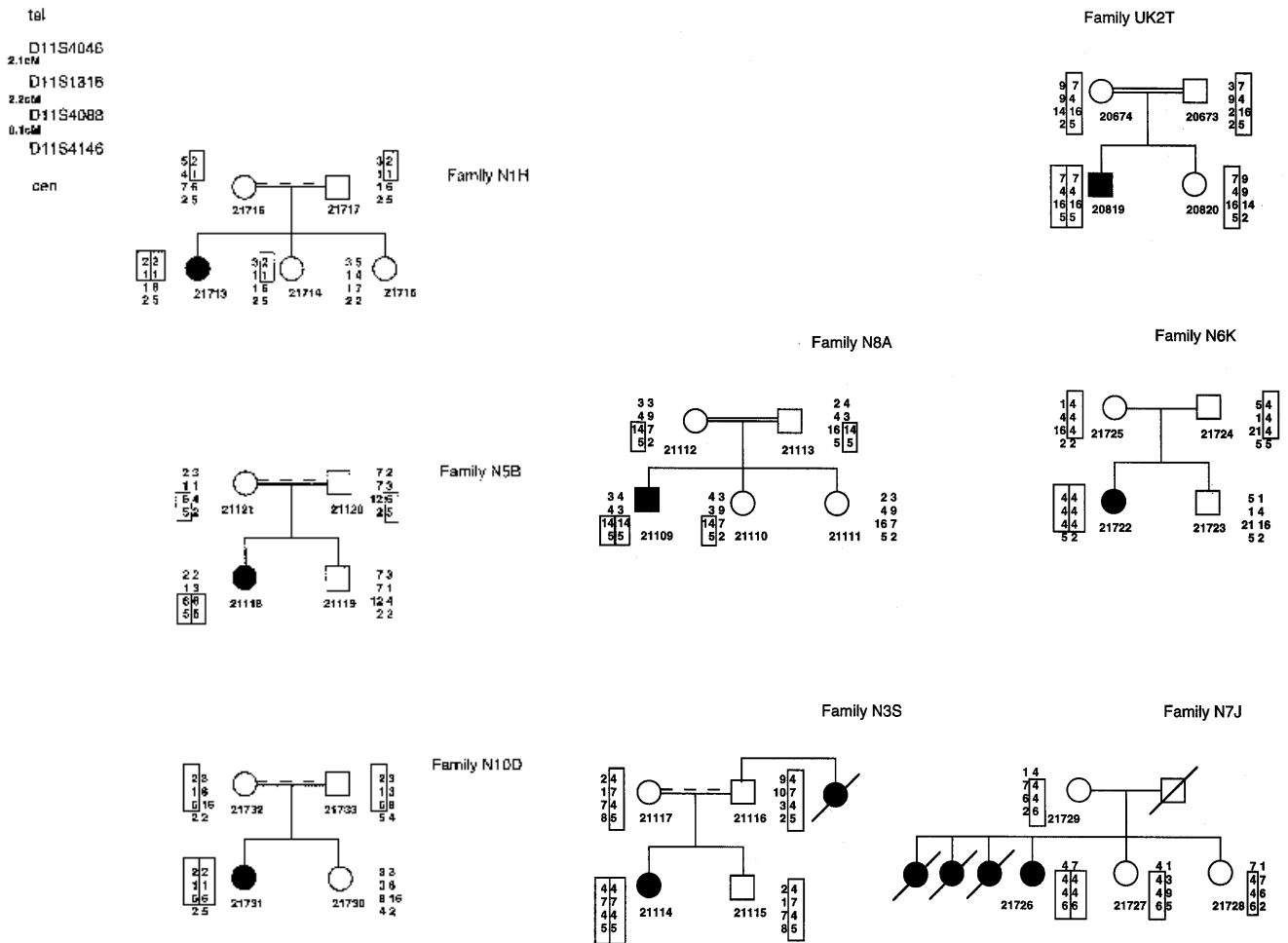
Figure 4. Haplotype analysis of eight families. Dashed line indicates suspected consanguinity (see text). Families UK2T and N3S are homozygous by descent for all the markers typed, D11S4046, D11S1318, D11S4088 and D11S4146. In families N8A and N5B markers distal to D11S1318 are homozygous, whereas in family N1H homozygosity is seen for D11S1318 itself and the marker proximal to it, D11S4046. Order of markers is as indicated in the key.

IsK an excellent candidate gene for JLNS in a family in whom the *KVLQT1* gene had been excluded.

The minK protein (product of the *IsK* gene) has an unusually simple structure for an ion channel, consisting of only 130 amino acids. The molecule has an extracellular portion, a transmembrane α-helical region and an intracellular region (22). Sequencing of the PCR products of the *IsK* gene in the affected boys in family UK1S showed the changes illustrated in Figures 2 and 3a in which three separate nucleotides have been altered. The changes cause Thr59 and Leu60 each to be replaced by Pro in the transmembrane region of the predicted protein.

The cyclical structure of proline is expected to influence the resulting protein structure, since it causes bending of folded protein chains (23). Indeed, observations of the relative occurrence of amino acid residues in α-helices show that proline is the least likely of all the amino acids to be found in this secondary structure (23). Site-directed mutagenesis of the *IsK* gene has also contributed insight into the functional domains of this small protein. Amino acid substitutions at a number of positions within and outside the transmembrane region were found to have varying effects on the functional activity of the channel. Mutation of Thr59 to a structurally related amino acid, valine, was shown to cause a reduction in channel activity as judged by electrophysiological

measurements in oocyte systems (22). The mutation in family UK1S alters both this amino acid, Thr59 and the one adjacent to it, Leu60, and is therefore very likely to be of functional significance and disease causing. *In vitro* studies in oocyte systems should confirm the functional significance of this mutation.

A recent survey of published databases has indicated that complex changes such as the one described here are very rare (24). It is interesting to speculate how such a mutation may have arisen and a proposed mechanism is outlined in Figure 3. Approximately 60 bases upstream of the mutation is a small region of sequence similarity sufficient to allow transient mispairing of the two regions in the germline of an ancestor (Fig. 3c). This would be followed by attempted correction of the 3′-sequence, based on the template of the upstream 5′-sequence. Certainly the base changes that have arisen as a result of the mutation create greater homology between these two regions. These changes are present in carriers and in both affected individuals.

In family UK1S, the following are evidence that mutation in the *IsK* gene causes JLNS: mutation at *KVLQT1* has been excluded in this family due to the absence of homozygosity by descent for markers in this region, but homozygosity for markers flanking the *IsK* gene is demonstrated. The chromosome 21 markers typed are highly polymorphic, with D21S1895 having 10 alleles and
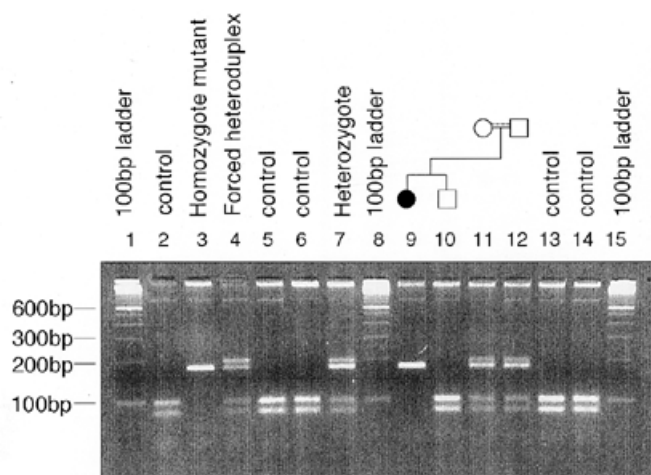
Figure 5. PCR amplification products from the S2–S3 domains of the *KVLQT1* gene (9) after digestion with the enzyme *Hha*I. In normal controls, digestion yields fragments of 98 and ~80 bp (lanes 2, 5, 6, 13 and 14). In the homozygous mutant, the 5 bp deletion abolishes the cut site for the enzyme, resulting in a single fragment of ~180 bp (lanes 3 and 9). In heterozygotes after *Hha*I digestion there are fragments of ~180, 98 and ~80 bp and an additional fainter band at 200 bp (lanes 7, 11 and 12). This faint band at 200 bp probably represents heteroduplexes, partially resistant to *Hha*I digestion. This band, which is not present in normals or homozygous mutants, can be created by mixing PCR products from a normal and a homozygous mutant and then digesting with *Hha*I (lane 4).

heterozygosity of 82%. The sequence changes in *IsK* in the affected boys of family UK1S are not seen on SSCP analysis of 120 normal chromosomes. This mutation results in substitution of two proline residues, predicted to cause severe disruption to the helical structure of the transmembrane region of the molecule. Functional studies on one of the mutated residues has previously shown this to be important for channel activity. Interaction of the *IsK* gene product with that of the *KVLQT1* gene, already known to cause JLNS, provides a plausible biological mechanism for the disease pathology. The rarity of mutations in the *IsK* gene in JLNS is accounted for by demonstrating that the majority of cases of this rare condition are very likely to be caused by mutation in *KVLQT1*. The identification of *IsK* as a gene underlying JLNS in humans is strongly supported by observations in *Isk* knockout mice.

Although 11 unrelated individuals/families with JLNS were screened for mutations in the *IsK* gene, none were found. This is not surprising, as haplotyping of eight of the families, presented here, together with the mutation described in *KVLQT1*, provide good evidence that the majority of families map to the *KVLQT1* locus. Of the remaining three families, all are non-consanguineous and provide very little mapping information (pedigrees not shown). However, two of these three non-consanguineous families are heterozygous for the 5 bp deletion characterised here and the third shows a heterozygous SSCP shift in the *KVLQT1* gene, indicating that they may also be accounted for by mutation at the major locus, *KVLQT1* (data not shown). Thus all individuals with JLNS available to us for study are accounted for.

Since families N1H, N5B and N10D originate from the same part of Norway, a founder effect is likely to account for the mutation in these families and, indeed, a common haplotype is seen in these three families, suggestive of linkage disequilibrium.

The 5 bp deletion in the *KVLQT1* gene encoding the S2–S3 membrane-spanning segment of the predicted channel will cause a frameshift and is highly likely to be disease causing.

The results shown in Figure 4 refine previous combined mapping data on the location of the *KVLQT1* gene, probably because a large number of ancestral meioses have been sampled. Previous analysis of recombinants in families with JLNS had indicated that the *KVLQT1* gene mapped between D11S922 and D11S4146 (13), with the presumed order being D11S922, D11S4046, D11S1318, (D11S4088), D11S4146. The mapping of 12 recombinants in eight French families with dominant long QT syndrome placed the gene distal to D11S1318 (25). Our data indicate that the mutant gene is located between the markers D11S1318 and D11S4088, but given that the *KVLQT1* gene covers a large genomic region (at least 300 kb), it is possible that some of these markers may lie within the gene itself (26).

Not all families of Norwegian origin share the common haplotype or demonstrate the SSCP shift seen in families N1H, N5B and N10D. Families N6K and N7J share a haplotype which differs from that seen in families N1H, N5B and N10D, suggesting that more than one mutation in the *KVLQT1* gene must be present in the Norwegian population. However, this group of eight families shown in Figure 4 originates from several different ethnic backgrounds, including Turkey, Pakistan and Norway. This indicates that the majority of cases of JLNS can be accounted for by mutation in the *KVLQT1* gene.

Now that it has been shown that homozygous mutations in either *KVLQT1* or *IsK* can cause JLNS in humans, it remains to be seen whether JLNS can result from compound heterozygosity for mutations in both genes, so-called digenic inheritance (27), or whether the resulting phenotype may differ. Certainly, families have been reported in which there is both deafness and a long QT interval but in whom the pattern of inheritance is not completely consistent with an autosomal recessive mode of transmission (28,29).

MATERIALS AND METHODS

Family data

Individuals were considered to have JLNS if they had profound sensorineural deafness and a prolonged QT$_c$ interval. Most individuals were ascertained following syncopal episodes (although families N5B and N8A were ascertained following ECG screening of deaf children). QT$_c$ intervals were calculated using Bazett's formula and a QT$_c$ interval of >440 ms is generally considered to be prolonged (30). Some gene carriers fulfil the criteria for a prolonged QT interval.

Family UK1S is a British family. II$_1$ and II$_2$ have congenital profound sensorineural hearing loss with absent vestibular function. II$_2$ had three episodes of syncope on exertion prior to diagnosis and treatment, one of which required resuscitation. II$_2$ has a QT$_c$ interval of 488 ms and II$_1$ a QT$_c$ interval of 478 ms. QT$_c$ intervals are 414 ms in the father and 418 ms in the mother (30). II$_2$ has retinal pigmentation of unknown origin but both boys have normal electroretinograms, excluding a diagnosis of Usher syndrome.

Family UK2T comes from Turkey but is resident in the UK. Parents are first cousins. The proband has prelingual deafness and

suffered from syncope. His QT_c is 480 ms and that of his sister is 374 ms.

Family N1H originates from Norway and has been reported previously (case 1, 31). The mother has a QT_c of 430 ms and the father's is 400 ms.

Family N3S originates from Norway. The proband is congenitally deaf and suffered from syncopal episodes elicited by stress. Her QT interval is 600 ms, but no other information is available.

Family N5B originates from Norway and has not been reported previously. The proband is congenitally deaf and the diagnosis was made on ECG examination (customary practice for all deaf children in Norway). The affected girl's QT_c is 540 ms, her father's is 410 ms, her brother's is 370 ms and that of her mother is 470 ms at rest, increasing to 540 ms on exercise.

The proband in Family N6K is congenitally deaf and comes from the same geographical region in Norway as family N7J. No QT_c intervals are available.

Family N7J has been reported previously (1) and formed the basis of the original description of this condition by Jervell and Lange-Nielsen.

Family N8A comes from Pakistan but is resident in Norway. The parents are first cousins. The diagnosis was made neonatally based on the family history and finding of a QT_c of 650 ms in the proband. He is congenitally deaf. The QT_c of the mother is 450 ms, that of the father is 410 ms, that of the proband's older sibling (21727) is 430 ms and that of the younger sister (21728) is 400 ms.

Family N10D originates from Norway. The proband is congenitally deaf and has suffered from episodes of syncope. The proband has a QT_c of 500 ms, that of her father is 470 ms, her mother's is 430 ms and her sister's is 360 ms.

PCR analysis

DNA was prepared from blood using standard protocols or from buccal swabs (32). Information on $(CA)_n$ microsatellite markers were provided by Généthon human genetic linkage maps (33), the Hereditary Hearing Loss Homepage (15) and published references (7–10,25). All genotyping was performed in 96-well Omniplates (Hybaid) under oil. PCR was performed with 40 ng genomic DNA in a 10 µl volume containing 10 pmol 3′-primer, 1× Bioline buffer (Bioline), 1.5 mM Mg^{2+}, 0.2 mM dGTP, dATP, dTTP and dCTP and 0.2 U Taq polymerase (Bioline) under standard conditions. Prior to amplification, 10 pmol 5′-primer per 10 µl reaction were end-labelled with 0.5 µl [γ-^{32}P]dATP(3000 Ci/mmol) with 0.5 U polynucleotide kinase (Promega) for 30 min. Alleles were separated as described previously (34) and sizes were determined by comparing migration relative to an M13 sequencing ladder.

Linkage analysis was performed using the MLINK programme of LINKAGE. Marker allele frequencies were obtained from Généthon (33). Penetrance was assumed to be 100% and gene frequency 0.00001.

SSCP analysis

SSCP analysis of the *KVLQT1* gene was performed using published primer sequences (10). The coding sequence of the *IsK* gene was amplified by PCR using primer pairs at 56°C according to Tesson *et al.* (20). Non-radioactive PCR products were analysed on 1× Mutation Detection Enhancement gel (FMC Bioproducts) at 4°C or room temperature, with or without 10%

glycerol, at 45 W, 0.5× TBE, for 4 h. This allowed visualisation of both single and double-stranded DNA. The gels were stained with 0.012 M silver nitrate as described previously (35).

Sequence analysis of *IsK*

Biotinylated PCR products were purified using Dynabeads (Dynal, UK) and single-stranded products sequenced using the USB Sequenase v.2.0 kit, according to the manufacturer's instructions.

Sequence analysis of *KVLQT1*

PCR products demonstrating SSCP variants were sequenced directly on both strands using the fluorescent dideoxy terminator method and analysed using an ABI 377 DNA sequencer.

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