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Familial juvenile hyperuricaemic nephropathy (FJHN): linkage analysis in 15 families, physical and transcriptional characterisation of the FJHN critical region on chromosome 16p11.2 and the analysis of seven candidate genes

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Familial juvenile hyperuricaemic nephropathy (FJHN) is an autosomal dominant renal disease characterised by juvenile onset of hyperuricaemia, gouty arthritis, and progressive renal failure at an early age. Recent studies in four kindreds showed linkage of a gene for FJHN to the same genomic interval on chromosome 16p11.2, where the gene for the phenotypically similar medullary cystic disease type 2 (MCKD2) has been localised. In this study we performed linkage analysis in additional 15 FJHN families. Linkage of FJHN to 16p11.2 was confirmed in six families, which suggests that, in a large proportion of FJHN kindreds, the disease is likely to be caused by a gene or genes located outside of 16p11.2. Haplotype analysis of the new and previously analysed families provided two non-overlapping critical regions on 16p11.2—FJHN1, delimited by markers D16S499-D16S3036 and FJHN2, delimited by markers D16S412-D16S3116. Considering MCKD2 to be a distinct molecular entity, the analysis suggests that as many as three kidney disease genes may be located in close proximity on 16p11.2. From genomic databases we compiled integrated physical and transcription maps of whole critical genomic region in which 45 known genes and 129 predicted loci have been localised. We selected, analysed and found no pathogenic mutations in seven candidate genes. The linkage and haplotype analysis reported here demonstrates the genetic heterogeneity of FJHN. The report of integrated physical and mostly in-silico predicted transcription maps of the FJHN critical region provides a basis for precise experimental annotation of the current transcript map, which is essential for final identification of the FJHN gene(s). *European Journal of Human Genetics* (2002) 11, 145–154. doi:10.1038/sj.ejhg.5200937

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Introduction

Familial juvenile hyperuricaemic nephropathy (FJHN), (OMIM 162000), is an autosomal dominant renal disease characterised by juvenile onset of hyperuricaemia, gouty arthritis, and progressive renal failure. The disease was first noted by Duncan and Dixon in 1960.¹ More than 50 kindred originating from various ethnic groups – Caucasians (for review see²) and,^{3–11} Hungarian,¹² Japanese,^{13,14} Chinese,¹⁵ Polynesian¹⁶ and recently also Finnish¹⁷ and Chilean¹⁸ have been described so far.

The pathogenesis and molecular basis of FJHN is unknown. Evidence that hyperuricaemia associated with the urate hypoexcretion is the primary event of the nephropathy was provided by McBride *et al*,^{19,20} who found normal renal function in 42% of children biochemically proven to have FJHN. In accordance with these results, the disease seems to result from a defect of uric acid tubular transport. Candidate proteins could be either voltage-sensitive urate transporters such as hUAT1 and hUAT2,²¹ suggested urate/anion exchangers²² or other proteins which may be involved in forming, regulating, processing, trafficking, and cell surface stability of structurally functional urate channel multimeric complex. In addition to the tubular transport defect hypothesis, Puig *et al*⁷ suggested that uric acid underexcretion and hyperuricaemia may be a consequence of a primary renal hemodynamic disruption.

Furthermore, the clinical, biochemical and histological examinations observed in FJHN are similar to those observed in diseases belonging to nephronophthisis-medullary cystic kidney disease complex,²³ and it was postulated that connection between FJHN and these diseases, particularly medullary cystic kidney disease type 2 (MCKD2), may exist.^{11,24,25}

Recent studies,^{26–28} one of them long-term,²⁸ have demonstrated the beneficial effect of allopurinol in ameliorating the progression of the renal disease. However the beneficial effect of allopurinol is controversial.²⁹ Others suggest that in patients with early stage nephropathy blockade of the renin-angiotensin system may delay the progression of renal insufficiency.^{30,31} These findings underscore the importance of pre-symptomatic diagnosis, especially in children. Pre-symptomatic detection enabling diagnosis and the commencement of early treatment is vital for affected families and will also result in preventing or delaying dialysis and ultimately, renal transplantation.

Identifying the genetic defect is essential to facilitate such a diagnosis and replace the non-specific biochemical tests that depend on the accuracy of sample collection. Identifying the hypothetical defective urate transporter in FJHN may have broader relevance to a much more common disease of defective urate transport – the underexcreting gout ('primary' gout) affecting middle-aged males. It is possible that mutation and/or polymorphism in genes causing FJHN may also be implicated there.

Recently we³² and others^{11,33} localised a gene for FJHN in four kindreds to chromosome 16p11.2, and found evidence of genetic heterogeneity of the disease. Interestingly, the FJHN locus was mapped to the same genomic interval where the gene for medullary cystic disease type 2 (MCKD2) (OMIM 603860), has been localised.^{25,34} Clinical and biochemical similarities and genomic co-localisation of both loci suggest that FJHN and MCKD2 may be a single disease entity. Alignment of all critical regions detected in these studies, however, provides two non-overlapping genomic regions delimited by markers D16S499-D16S3036 and D16S403-D16S3116,¹¹ and keeps the issue of the distinctness of FJHN and MCKD loci open.

In this study we performed linkage analysis for FJHN in additional 15 families, and confirmed in six the previous observations of linkage of FJHN to 16p11.2, which suggests, that in a large proportion of FJHN kindreds, the disease is likely to be caused by a gene or genes located outside of 16p11.2. Haplotype analysis showed that two families mapped in this study might be aligned to the D16S499-D16S3036 interval only whereas the rest of the families may be aligned with both intervals. Considering MCKD2 as a distinct molecular entity, we hypothesise that as many as three genes located in close proximity on 16p11.2 may exist. From genomic databases we therefore compiled integrated physical and transcription maps of whole implicated genomic region (D16S499-D16S3116). We selected, analysed and excluded seven candidate genes. The results should facilitate efficient candidate gene selection and in combination with high throughput sequencing and functional genomic studies should help to resolve the molecular basis of FJHN/MCKD disease(s) in the future.

Methods

Patients

In total, 15 FJHN families (118 individuals of which 58 were affected) were enrolled in this study. Ten families are from Great Britain (GB1–10), three from Spain (E1–3) and two from Belgium (BE1 and BE2). The diagnosis of FJHN was based on the following criteria: autosomal dominant inheritance, hyperuricaemia, gouty arthritis which progress to renal failure. Clinical and biochemical data of the GB and Spanish patients have been described in detail previously.^{2,7,20,24,27,28}

In family BE1, the first symptoms in the affected individuals were mild anaemia (Hb levels 10 g %), slowly progressive renal failure and hyperuricaemia from the age of 10 years. The patients had small, echogenic kidneys on renal echography. Terminal renal failure treated with renal transplantation developed at the age of 60 years in one of the affected males (DII.1).

In family BE2, three affected brothers developed the first symptoms of hyperuricaemia and gouty arthritis after the age of 30 years. Allopurinol treatment started at the first

symptoms. Between 45 and 50 years of age, the first symptoms of renal failure were registered (creatinin: 2.59 mg/100 ml). Renal echography showed small hyperechogenic kidneys. Between 55 and 60 years of age, symptoms of arterial hypertension and progressive preterminal renal failure (creatinin levels of 5.36 mg/ml in the proband) appeared.

Genotype analysis

Genotyping was performed as previously described,³² using a set of 13 microsatellite markers spanning the 13 cM of the critical region on chromosome 16p11.2. The marker order was as follows: cen- D16S499-D16S501-D16S3056-D16S3041-D16S3036-D16S3046-D16S403-D16S412-D16S3130-D16S417-D16S420-D16S3113-D16S401-tel and was based on the Marshfield Genetic Map.

Linkage analysis

Genotyping data were screened for errors using the PEDCHECK program.³⁵ Two point and multipoint linkage analyses, along with determination of the most likely haplotypes, were performed using the Allegro program.³⁶ The analysis was carried out under the assumption of a dominant mode of inheritance with a 95% penetrance, 5% phenocopy rate and 0.001 frequency of the disease allele. Marker allele frequencies were estimated from the families used in this study using the program Pedmanager <http://www.genome.wi.mit.edu/ftp/pub/software/pedmanag>.

Integrated genetic, physical and transcript map

Genetic and STS markers were positioned on human genomic contigs by searching in genome databases – MapViewer, builds 27–29, (<http://www.ncbi.nlm.nih.gov/>), Ensembl – V1.2.0. (<http://www.ensembl.org/>) and Human Genome Working Draft at UCSC, August 2001 freeze, (<http://genome.ucsc.edu/>). To fill the existing gaps, contig ends BAC sequences were BLASTed against the Homo sapiens genomic contig sequences, high-throughput genomic sequencing (htgs) and BAC ends sequences sections of the NCBI database. Gene content was obtained either directly from annotated contigs or using the BLAST queries of unfinished genomic sequences against dbEST and protein databases.

Candidate gene analysis

Individual genes were evaluated according to knowledge or assumption of their function, evaluation of their expression profiles, and analysis of hypothetical functional domains. Upstream promotor region sequence, genomic organisation, and genomic sequence of candidate genes were obtained using pairwise BLASTN analysis of the cDNA with corresponding BAC sequences. Genomic fragments covering promotor region (about 500 bp upstream from the most cDNA 5' end) and all of the exons and intron-exon bound-

aries were PCR amplified from genomic DNA and sequenced as previously described.³⁷ All candidate genes were analysed in one proband and one unaffected family member in each family. Genetic variations were screened against SNP and UNIGENE databases.

Results

Linkage analysis

The results of a multipoint linkage analysis are shown in Table 1 and graphically in Figure 1. We calculated the HLOD score, allowing for genetic heterogeneity by maximising the score over the parameter, representing the proportion of families in the sample demonstrating genetic linkage of the disease to the region of interest. A maximum HLOD score of 1.6137 was obtained at markers D16S3041-D16S3036 for $\alpha=0.374$. In order to determine the statistical significance of this finding, one needs to subtract 0.3 from the maximum HLOD value, as recommended by Terwilliger and Ott,³⁸ to adjust for maximisation of the score over two parameters (and the recombination fraction). It is then possible to use the well-known property of asymptotic convergence of the χ^2 distribution and two times natural logarithm of the odds ratio. After the adjustment, HLOD of 1.6137 corresponds to a χ^2 of 6.05 (one tail $P=0.007$). Thus our finding confirms the previous observations of linkage of FJHN to 16p11.2.^{11,32,33}

However, linkage to 16p11.2 may be excluded at the LOD=-2 level for families BE1 and GB4, while some of the other families also have negative LOD scores (without reaching the -2 level) throughout the candidate region. The value of $\alpha=0.374$ implies that genetic linkage between FJHN and 16p11.2 exists in only 37% of the analysed families. Thus, while confirming linkage of FJHN to the region, our analysis provides further evidence of heterogeneity and shows that a large proportion of FJHN cases are likely to be caused by genes located outside of 16p11.2.

Haplotype analysis

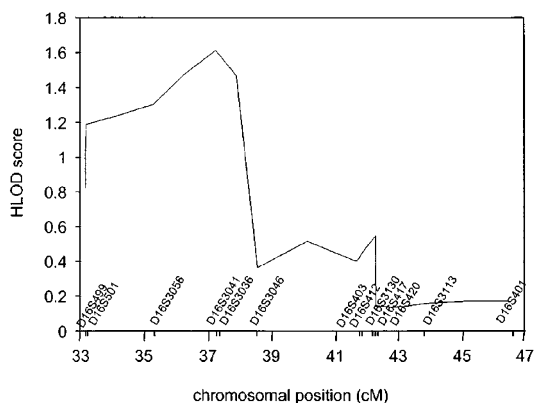
Haplotype analysis showed consistent segregation of 16p11.2 haplotypes and the FJHN phenotype in six of the families (BE2, GB2, GB3, GB8, E1, E3). Thus, six out of 15 families (40%) appear to have haplotypes consistent with linkage to FJHN, providing another estimate of the degree of genetic heterogeneity of the disease. However, the haplotypes segregating within individual families are not shared across families, nor is any single allele at any of the linked markers shared across families.

Pedigrees of these families are shown in Figure 2. We have aligned detected recombination events with critical regions delimited by haplotype analyses in all the FJHN and MCKD families linked to chromosome 16p11.2 region so far (Figure 3). This alignment provides two genetic intervals – D16S499-D16S3036 (FJHN1) and D16S412-D16S3116 (FJHN2) delimited by critical recombinations detected in Belgian¹¹ and Japanese³³ families. From eight haplotypes

Table 1 Maximum LOD scores within the D16499-D16S3046 interval for individual families

Family	Maximum multipoint LOD score
BE1	-4.9266 ^a
BE2	0.8437 ^b
GB1	-0.3521 ^c
GB2	0.2919 ^b
GB3	1.4520 ^b
GB4	-5.9092 ^a
GB5	-0.2848 ^c
GB6	0.1155 ^c
GB7	-0.4721 ^c
GB8	0.5862 ^b
GB9	0.2911 ^c
GB10	-1.1788 ^c
E1	0.5872 ^b
E2	-1.0602 ^c
E3	0.8346 ^b

^aFamilies excluded for linkage to 16p11.2. ^bFamilies segregated haplotypes fully consistent with linkage. ^cFamilies with haplotypes inconsistent with linkage.

**Figure 1** Multi-point overlapping HLOD score analysis of the markers in the linked region on chromosome 16.

defined in this and previous study,³² two haplotypes overlap with the FJHN1 region only. The remaining six haplotypes, as well as two haplotypes defined in MCKD2 studies,^{25,34} overlap with both FJHN1 and FJHN2 regions.

Integrated genetic, physical and transcript map

In silico analysis positioned all of the Marshfield genetic markers used for haplotype analysis to four genomic contigs – NT_010393, NT_024776, NT_010441 and NT_10604. Using the BAC end sequence content analysis, three BAC clones closing the gaps between individual contigs were identified. This effort, summarised in Figure 4, delimited approximately 12.6 Mb region on chromosome 16p11.2. Based on the NCBI contig assembly and annotation this region contains altogether 45 known genes and 129 predicted loci.

Candidate gene analysis

The following genes were considered and analysed as potential candidates for FJHN.

MIR16 (RGS16-INTERACTING MEMBRANE PROTEIN), UniGene Cluster Hs.107014 is an integral membrane glycoprotein with putative phosphodiesterase enzymatic activity, localised on the liver and kidney plasma membrane where it may play important roles in lipid metabolism and G protein signalling.³⁹ Sequence analysis showed heterozygous transition c1305A-G located in 3'UTR in one of the patients. This polymorphism was reported in dbSNP previously.

GPRC5B (G protein-coupled receptor) UniGene Cluster Hs.242407 is a member of type 3 GPCR family. The specific function of this protein is unknown; however, it may mediate the cellular effects of retinoic acid on the G protein signal transduction cascade.⁴⁰ Seven sequence variants were detected in GPRC5B. Three single base pair deletions were present homozygously in both patients and controls suggesting the sequencing error in genome databases. Three heterozygous single base pair substitutions: g1756A-G, g11749G-A and g1086T-C were found in non-coding regions of both patients and controls. Homozygous silent transition c804C-T was found in a control.

Ks-1 (kidney specific protein 1, Sa protein), LOC123876, is a human homologue of rat hypertension associated protein which exhibits a unique tissue-specific expression exclusively in mature kidneys and livers. A recent study identified the protein as a medium-chain acyl-CoA synthetase.⁴¹ Six sequence variants were detected in the Ks-1 gene. Two transitions, c340G-A and c428T-C, changing 114Gln-Lys and 143Leu-Ser, respectively, transversion g1166C-A, and a homozygous single base pair deletion g3385delT were found in both patients and controls. Two heterozygous transitions, silent c438G-A (146 Leu-Leu) and g3384C-T, were found in both patients and controls.

Ks-2 (kidney specific protein 2), MACS1 (Butyryl Coenzyme A synthetase 1), UniGene Cluster Hs.98732, is localised in an opposite transcriptional direction in close proximity to the Ks-1 gene. Both rat and mouse homologues showed kidney and liver specific gene expression.⁴¹ Three sequence variants, silent transitions c45T-C and c720G-A and c314insT were detected in Ks-2 gene. All of these variants were homozygous in both patients and controls.

CRYM (mu-crystalline homologue-NADP-regulated thyroid hormone binding protein), UniGene Cluster Hs.924, is responsible for most of the intracellular high-affinity T3 and T4 binding in the kidney and other thyroid hormone-responsive tissues. Thyroid hormone homeostasis plays an important role in kidney growth, renal haemodynamic, tubular function and electrolyte excretion, and

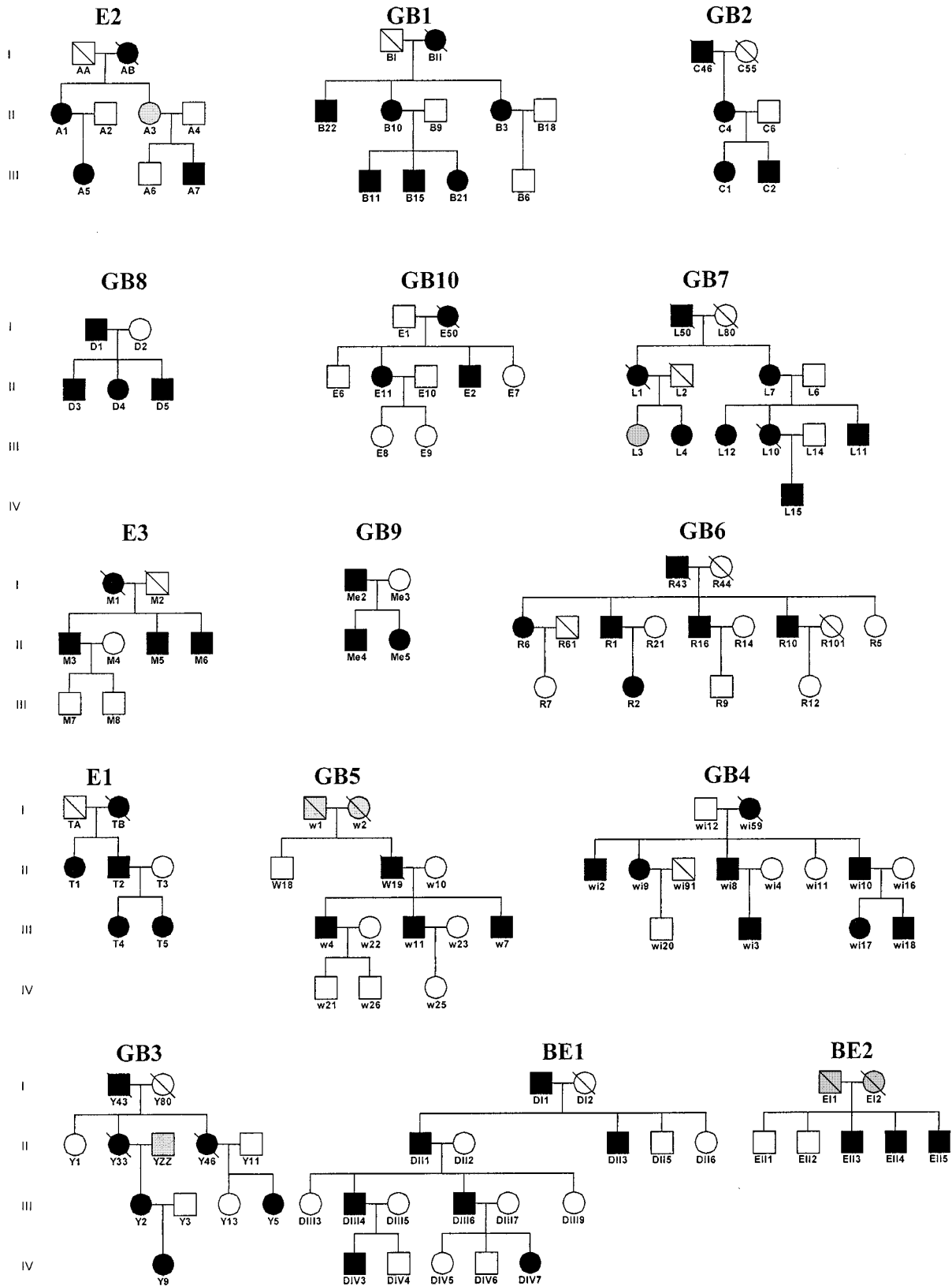


Figure 2 Pedigree diagrams of the investigated families.

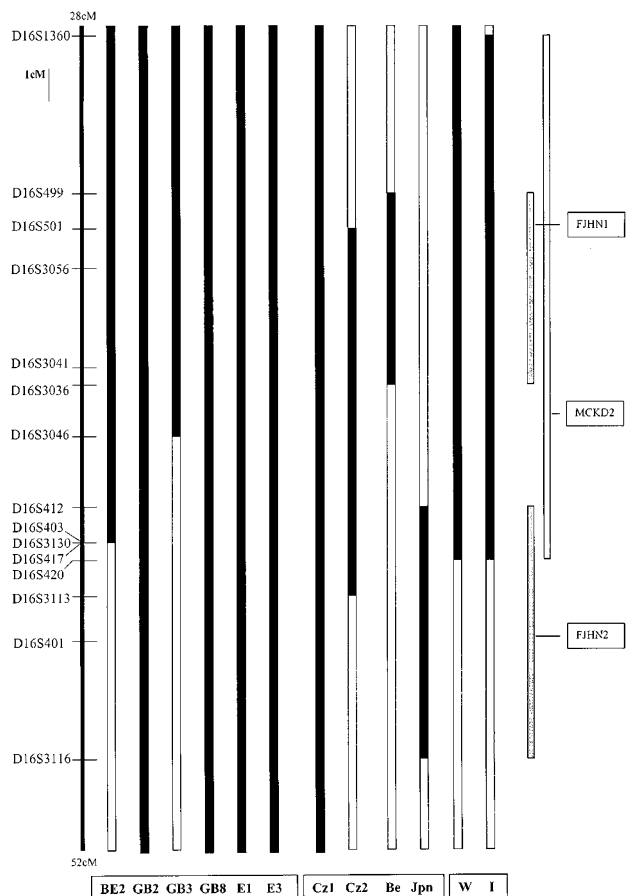


Figure 3 Genetic map of the FJHN critical region on chromosome 16p11.2 showing schematic representation of crucial recombination events (full bars) detected in six FJHN families from this study (BE2, GB2, GB3, GB8, E1 and E3), in four FJHN families (Cz1 and Cz2,³² Be¹¹ and Jpn³³ and two MCKD2 families (W²⁵ and I³⁴) from studies reported earlier. Implicated critical region FJHN1, FJHN2 and MCKD2 are shown as grey bars.

association of hyperuricaemia with both hypothyroidism⁴² and hyperthyroidism⁴³ has been reported. Three single base pair substitutions located in non-coding regions were detected in the CRYM gene. Two heterozygous substitutions, g2395C-G and g2646T-C, and one homozygous transition g16287T-C were found in both patients and controls.

KIAA1203 UniGene Cluster Hs.16953 is expressed in kidney and contains ubiquitin carboxyl-terminal hydrolase family two domain which may function, through proteosomal pathway, as a regulator of the amiloride-sensitive epithelial Na(+) channel SCNN1,⁴⁴ whose two subunits, SCNN1B and SCNN1G are located in KIAA1203 in close proximity on 16p11.2. Three single base pair substitutions were found in the KIAA1203 gene. The transversion c1463T-G changing 488Leu-Arg was found to be both homo-

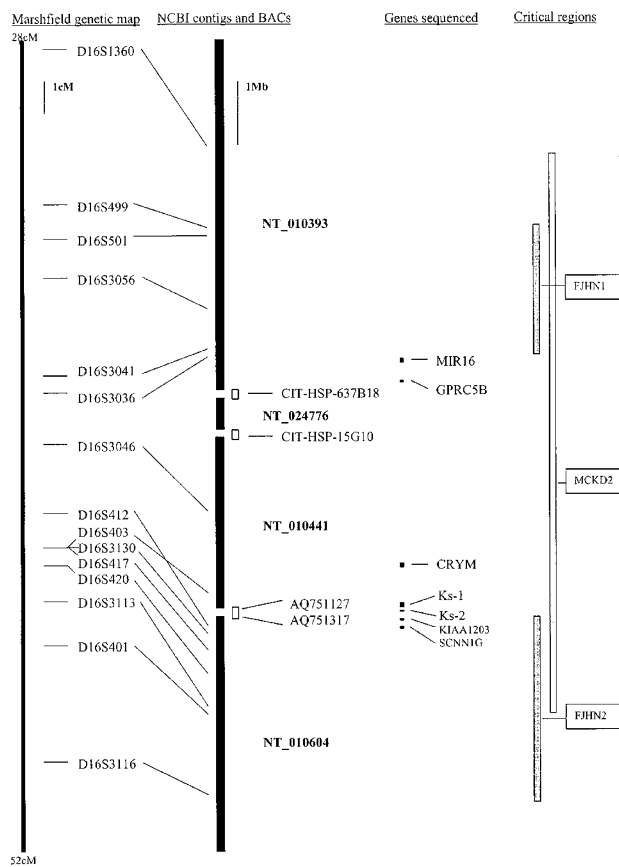


Figure 4 Integrated genetic, physical and transcription map of the FJHN/MCKD2 locus. Genomic contigs are annotated as in NCBI MapViewer. Gapping BAC clones are annotated by their names or BAC end sequence accession.

zygous and heterozygous in patients and controls. The transition c12476G-A changing 822Asp-Asn was homozygous in one of the controls. The transition g14188G-A was found to be heterozygous in both patients and controls.

SCNN1G (gamma subunit of the amiloride-sensitive epithelial sodium channel ENaC), UniGene Cluster Hs.3112, is a protein which mutations result in Liddle's syndrome or pseudohypoaldosteronism type I. It is suggested that variants in this gene may play a role in the pathogenesis of more common forms of human hypertension.⁴⁵ Twenty sequence variants were found in SCNN1G gene. Three resulted in amino acid substitution c532T-A (178Trp-Arg), c1504C-G (502Pro-Ala) and c1840T-C (614Ser-Ala), and were found to be homozygous in both patients and controls. Five heterozygous silent transitions, c387T-C, c474T-C, c549C-T, c636C-T, and a homozygous c825T-C transition were in the coding region of the gene in both patients and controls. Two homozygous substitutions, -c106C-G and -c31G-A were found in the 5'UTR in both patients and controls. Of 10 substitutions located

in non-coding region of the gene, six –173ex1G-A, –99ex3T-C, +29ex9T-C +33ex11T-G, –7ex11G-A and –49ex12A-G were heter-ozygous and four substitutions –93ex2G-C, –49ex2A-G, –4ex2G-C and +8ex4 were homozygous in both patients and controls. A summary of all the genetic variants found is shown in Table 2.

Discussion

Linkage analysis

The results of the linkage analysis confirm the already demonstrated linkage of FJHN to chromosome 16p11.2. However, the results also show that there is significant heterogeneity underlying the disease. On the basis of the

Table 2 Summary of all the genetic variants found in selected candidate genes

Gene	Promotor region ^a	# Exons	Variations				Amino acid substitution	dbSNP accession
			Substitutions		Ins/Del			
			gDNA	cDNA	gDNA	cDNA		
MIR16	–510	6	gi 2951946 18802 A→G	gi 19923804 1305 A→G			rs7135	
GPRC5B	–12030/–11530	4	gi 3342217	gi 7706450	–11859delA –11840delT –11774delT			
KS1	–461	7	–11756 A→G –11749 G→A 1086 T→C 804 C→T gi 3219338 1166 C→A 3384 C→T	804 C→T gi 20561512				
KS2	–459	7	3482 G→A 5706 T→C 5716 G→A gi 3219338 46 T→C	340 G→A 428 T→C 438 G→A gi 16418448 45 T→C	3385delT	114 Gln→Lys 143 Leu→Ser		
CRYM	–724	8	13944 G→A gi 2335061 16287 T→C 2646 T→C 2395 C→G	720 G→A gi 2555014	10142insT	314 insT		
KIAA1203	–100	9	gi 22069010 21396 T→G 14188 G→A 19459 G→A	gi 220688 1463 T→G			488 Leu→Arg	
SCNN1G	–321	13	gi 20562397	2476 G→A gi 18585683 –106 C→G –31 G→A 387 T→C 474 T→C 532 T→A 549 C→T 636 C→T 825 T→C 1504 C→G 1840 T→C			826 Asp→Asn	
			–173(ex1) G→A –93(ex2) G→C –49(ex2) A→G –4(ex2) G→C –99(ex3) T→C +8(ex4) A→G +29(ex9) T→C +33(ex11) T→G –7(ex11) G→A –49(ex12) A→G				rs5732 rs5735 rs5737 rs5731	

Promotor regions correspond to number of base pairs or genomic region sequenced upstream from the presumed 1st ATG. Annotation of sequence variants is based on their positions respective to the presumed 1st ATG in indicated GenBank sequences.

maximum HLOD score, we estimate that the proportion (α) of families segregating to a gene linked to 16p11.2 is 0.37. However, the parameter α maximised under the hypothesis of heterogeneity should be interpreted as the proportion of individuals in the overall sample that belong in families segregating the linked gene, rather than simply the proportion of families segregating the gene. Hence, the estimate will be influenced by the relative size of families. In our sample, the biggest families (BE1, GB4 and GB6) do not support linkage to 16p11.2. Effectively, the value of $\alpha=0.37$ is likely to be an underestimate.

Haplotype analysis

As an alternative approach, investigation of haplotypes segregating within individual families reveals that 40% of the families have haplotypes consistent with linkage to the investigated region. This value, in turn, is likely to be an overestimate, since some of the families are small and are likely to support linkage by random chance. Nevertheless, by combining the above approaches we are able to estimate that, within our sample, between 37 and 40% of FJHN cases are explained by linkage to 16p11.2. In an earlier study³² we found that two out of three FJHN families of Czech origin showed linkage of the disease phenotype to 16p11.2. The current results demonstrate that genetic heterogeneity of FJHN is also prevalent in other European populations. Furthermore, our haplotype analysis shows that there is no common haplotype or allele segregating with the disease across families. This suggests that the disease may be the result of several independent *de novo* mutations, or that the underlying mutation is old and shows no linkage disequilibrium with a particular marker locus. However, since the distance between markers is of the order of 1 cM, it is likely that linkage disequilibrium between the causative mutation and the markers may have decayed even for a reasonably young mutation. A much finer marker map would be required to determine which of the above hypotheses is correct.

In addition, we present an up to date synopsis of FJHN linkage results in the context of recently published FJHN/MCKD2 studies.^{11,25,32–34} The alignment of detected FJHN haplotypes provided two non-overlapping critical regions – *FJHN1* delimited by markers D16S499-D16S3036 and *FJHN2* delimited by markers D16S412-D16S3116. Out of ten FJHN and two MCKD2 families mapped to 16p11.2 so far, three FJHN families are linked only to the *FJHN1* and one FJHN family only to the *FJHN2* regions. The remaining six FJHN and both MCKD2 families reported may be linked to both regions. If FJHN and MCKD2 are two different disease entities then it may be postulated that up to three different genes *FJHN1*, *MCKD2* and *FJHN2* may exist in close proximity on chromosome 16. Considering FJHN and MCKD2 as a single disease entity, two loci *FJHN1* and *FJHN2* may exist. One locus is probable when we omit the results of the Japanese study on the basis of possible

inconsistencies in current human genome maps, genotyping errors or existence of phenocopy.

Integrated genetic, physical and transcript map

Since neither of the hypotheses can be excluded, we focused our attention on the genomic region delimited by markers D16S499 and D16S3116. We have placed all of the Marshfield genetic markers located within the critical region on four genomic contigs. Using BLAST searches we have identified three gap closing BAC clones and obtained thus a contiguous stretch of 7.5 Mbp sequence along which, according to the NCBI annotation, 45 genes and 129 gene-like features are localised.

We have tried to place hypothesis of multiple loci in close proximity associated with similar phenotypes into the context of the critical region gene content. In general, a model of several loci in close proximity can be explained by defects of duplicated genes, genetic defect of proteins comprising multimeric complex (channel, receptor, signalling pathway) and genes responsible for such a complex regulation, processing, trafficking and cell surface stability. Evaluating the genomic structure and gene content, justification for the above hypothesis may be seen in the following features of the critical region; (1) presence of duplicated sequence tracts distributed throughout the p-arm of chromosome 16;⁴⁶ (2) co-localisation of the amiloride-sensitive epithelial sodium channel (ENaC) subunits *SCNN1B* and *SCNN1G* and of the ENaC possible regulators such as ubiquitin carboxyl-terminal hydrolase *KIAA1203*, elongation factor-2 kinase and protein kinase *PRKCB1*; (3) presence of several genes involved in kidney G protein-coupled receptor signalling and microtubule-dependent vesicle transport such as *GPRC5B*, *MIR16*, *LOC124724*, *ARL6IP*, *COG7*, *GGA*, *LOC90404*, *dynein*, *ERN* and *RICH1*; (4) presence of genes involved in regulated protein degradation such as polo-like kinase *PLK*, *KIAA1203* or *LOC123803*; and (5) presence of genes involved in podocyte formation – *GGA* and *ARL6IP*.

The gene content analysis showed that the delimited chromosomal region contains a number of genes, which may contribute important kidney specific structural units or regulatory functions. Since we have not found evidence for the presence of the urate transporter, we focused on some of the genes which might be involved in G-protein signalling, regulated protein degradation, thyroid hormone homeostasis or which were found to be associated with hypertension.

Candidate gene analyses

Following the sequence analysis of two genes localised within the critical region – uromodulin and *SAH*,²⁴ we have analysed seven more candidate genes. In all of the genes no 'classic' deleterious mutations were detected within the promotor regions, exon/intron junctions and coding sequences. This finding however cannot completely rule

out the involvement of those genes in the disease development. The transcription of any of those genes can be greatly affected by mutations in distant promotor regions or by intronic mutations affecting mRNA processing. The cDNA studies, which would be very useful in this respect, could not be performed since we have no affected kidney tissue biopsies available.

In summary, FJHN is a genetically heterogeneous disease. To elucidate its molecular basis, further efforts must be aimed at better clinical characterisation of FJHN/MCKD patients. Affected families must be re-evaluated and followed to determine whether the disease is manageable by allopurinol and whether and when cysts occurred. Importantly, members of all three GB kindreds showing linkage in this report and identified prior to the onset of severe renal disease have been treated successfully with allopurinol – in the case of GB2 and three for from 20 to 37 years.^{27,28} Further gene mapping efforts must be focused on both refining the 16p11.2 locus and discover other FJHN loci in families not linked to 16p11.2. In this regard, it is of interest that kindred GB4, with a history of cysts at biopsy and phenotypically identical with MCKD2, has been found recently (following the onset of diabetes type 2) to have a defect on 17q involving the hepatocyte nuclear factor 1b gene (Bingham *et al*, personal communication).

Further studies must also be focused on experimentally confined completion of physical and transcript maps of FJHN critical regions and complementary genomic and proteomic approaches. Expression profiling using the microarray technology or 2D gel protein analysis may provide useful information on candidate gene selection and pathogenesis of the disease, and attempts to obtain access to sufficient amount of affected tissues must be undertaken.

The linkage and haplotype analysis reported here further demonstrate the genetic heterogeneity of FJHN and imply the hypothesis of several loci located in close proximity on 16p11.2 as well. This situation greatly hampers the positional cloning effort and indicates that better clinical characterisation and functional approaches must be undertaken. The report of integrated physical and mostly in-silico predicted transcription maps of the FJHN critical region provides basis for precise experimental annotation of the current transcript map, which is essential for final identification of the FJHN gene(s).

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References

- 1 Duncan H, Dixon A: Gout, familial hyperuricaemia and renal disease. *Q J Med* 1960; **29**: 127–136.
- 2 Cameron JS, Moro F, Simmonds HA: Gout, uric acid and purine metabolism in paediatric nephrology. *Pediatr Nephrol* 1993; **7**: 105–118.
- 3 Van Goor W, Kooiker CJ, Mees EJ: An unusual form of renal disease associated with gout and hypertension. *J Clin Pathol* 1971; **24**: 354–359.
- 4 Massari PU, Hsu CH, Barnes RV, Fox IH, Gikas PW, Weller JM: Familial hyperuricaemia and renal disease. *Arch Intern Med* 1980; **140**: 680–684.
- 5 Richmond JM, Kincaid-Smith P, Whitworth JA, Becker GJ: Familial urate nephropathy. *Clin Nephrol* 1981; **16**: 163–168.
- 6 Leumann EP, Wegmann W: Familial nephropathy with hyperuricaemia and gout. *Nephron* 1983; **34**: 51–57.
- 7 Puig JG, Miranda ME, Mateos FA *et al*: Hereditary nephropathy associated with hyperuricaemia and gout. *Arch Intern Med* 1993; **153**: 357–365.
- 8 Sebesta I, Krijt J, Pavelka K, Maly J, Simmonds HA, McBride MB: Familial juvenile hyperuricaemic nephropathy in adolescents. *Adv Exp Med Biol* 1994; **370**: 73–76.
- 9 Pavelka Jr K, Sebesta I, Blovska J, Maly J, Chadimova M: [Familial juvenile gouty nephropathy]. *Cas Lek Cesk* 1996; **135**: 668–671.
- 10 Lhotta K, Gruber J, Sgonc R, Fend F, Konig P: Apoptosis of tubular epithelial cells in familial juvenile gouty nephropathy. *Nephron* 1998; **79**: 340–344.
- 11 Dahan K, Fuchshuber A, Adamis S *et al*: Familial juvenile hyperuricaemic nephropathy and autosomal dominant medullary cystic kidney disease type 2: two facets of the same disease? *J Am Soc Nephrol* 2001; **12**: 2348–2357.
- 12 Korom I, Szabo L, Laszlo A, Dallmann L, Ormos J: [Infantile familial hyperuricaemia with gouty manifestations]. *Orv Hetil* 1979; **120**: 589–592.
- 13 Murakami T, Kawakami H, Nakatsuka K, Jojima K, Nohno H, Matsuzaki H: Underexcretory-type hyperuricaemia, disproportionate to the reduced glomerular filtration rate, in two boys with mild proteinuria. *Nephron* 1990; **56**: 439–442.
- 14 Saeki A, Hosoya T, Okabe H *et al*: Newly discovered familial juvenile gouty nephropathy in a Japanese family. *Nephron* 1995; **70**: 359–366.
- 15 Lam PW, Peh WC: Clinics in diagnostic imaging (2). Juvenile gouty arthropathy with associated nephropathy. *Singapore Med J* 1995; **36**: 85–87.
- 16 Reiter L, Brown MA, Edmonds J: Familial hyperuricaemic nephropathy. *Am J Kidney Dis* 1995; **25**: 235–241.
- 17 Auranen M, Ala-Mello S, Turunen JA, Jarvela I: Further evidence for linkage of autosomal-dominant medullary cystic kidney disease on chromosome 1q21. *Kidney Int* 2001; **60**: 1225–1232.
- 18 Badilla A, Rojas C: [Familial gout and nephropathy in a young woman. Report of one case]. *Rev Med Chil* 2001; **129**: 666–670.
- 19 McBride MB, Simmonds HA, Moro F: Familial renal disease or familial juvenile hyperuricaemic nephropathy? *J Inherit Metab Dis* 1997; **20**: 351–353.
- 20 McBride MB, Rigden S, Haycock GB *et al*: Presymptomatic detection of familial juvenile hyperuricaemic nephropathy in children. *Pediatr Nephrol* 1998; **12**: 357–364.
- 21 Lipkowitz MS, Leal-Pinto E, Rappoport JZ, Najfeld V, Abramson RG: Functional reconstitution, membrane targeting, genomic structure, and chromosomal localization of a human urate transporter. *J Clin Invest* 2001; **107**: 1103–1115.
- 22 Krick W, Wolff NA, Burckhardt G: Voltage-driven p-aminohippurate, chloride, and urate transport in porcine renal brush-border membrane vesicles. *Pflugers Arch* 2000; **441**: 125–132.
- 23 Hildebrandt F, Omram H: New insights: nephronophthisis-medullary cystic kidney disease. *Pediatr Nephrol* 2001; **16**: 168–176.

- 24 Pirulli D, Puzzer D, De Fusco M *et al*: Molecular analysis of uromodulin and SAH genes, positional candidates for autosomal dominant medullary cystic kidney disease linked to 16p12. *J Nephrol* 2001; **14**: 392–396.
- 25 Hateboer N, Gumbs C, Teare MD *et al*: Confirmation of a gene locus for medullary cystic kidney disease (MCKD2) on chromosome 16p12. *Kidney Int* 2001; **60**: 1233–1239.
- 26 Moro F, Simmonds HA, Cameron JS *et al*: Does allopurinol affect the progression of familial juvenile gouty nephropathy? *Adv Exp Med Biol* 1991; **309A**: 199–202.
- 27 McBride MB, Simmonds HA, Ogg CS *et al*: Efficacy of allopurinol in ameliorating the progressive renal disease in familial juvenile hyperuricaemic nephropathy (FJHN). A six-year update. *Adv Exp Med Biol* 1998; **431**: 7–11.
- 28 Fairbanks L, Cameron J, Venkat-Raman G *et al*: Early treatment with allopurinol in familial juvenile hyperuricaemic nephropathy (FJHN) ameliorates progression of renal disease in long-term studies. *QJM* 2002; **95**: 597–607.
- 29 Miranda ME: The influence of allopurinol on renal deterioration in familial nephropathy associated with hyperuricaemia (FNAH). The Spanish Group for the Study of FNAH. *Adv Exp Med Biol* 1994; **370**: 61–64.
- 30 Lewis EJ, Hunsicker LG, Clarke WR *et al*: Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 2001; **345**: 851–860.
- 31 Brenner BM, Cooper ME, de Zeeuw D *et al*: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001; **345**: 861–869.
- 32 Stibůrková B, Majewski J, Sebesta I, Zhang W, Ott J, Kmoch S: Familial juvenile hyperuricaemic nephropathy: localization of the gene on chromosome 16p11.2 and evidence for genetic heterogeneity. *Am J Hum Genet* 2000; **66**: 1989–1994.
- 33 Kamatani N, Moritani M, Yamanaka H, Takeuchi F, Hosoya T, Itakura M: Localization of a gene for familial juvenile hyperuricaemic nephropathy causing underexcretion-type gout to 16p12 by genome-wide linkage analysis of a large family. *Arthritis Rheum* 2000; **43**: 925–929.
- 34 Scolari F, Puzzer D, Amoroso A *et al*: Identification of a new locus for medullary cystic disease, on chromosome 16p12. *Am J Hum Genet* 1999; **64**: 1655–1660.
- 35 O'Connell JR, Weeks DE: PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 1998; **63**: 259–266.
- 36 Gudbjartsson DF, Jonasson K, Frigge ML, Kong A: Allegro, a new computer program for multipoint linkage analysis. *Nat Genet* 2000; **25**: 12–13.
- 37 Kmoch S, Hartmannova H, Stibůrková B, Krijt J, Zikanova M, Sebesta I: Human adenylosuccinate lyase (ADSL), cloning and characterization of full-length cDNA and its isoform, gene structure and molecular basis for ADSL deficiency in six patients. *Hum Mol Genet* 2000; **9**: 1501–1513.
- 38 Terwilliger J, Ott J: *Handbook of human genetic linkage*. Baltimore, London: Johns Hopkins University Press, 1994.
- 39 Zheng B, Chen D, Farquhar MG: MIR16, a putative membrane glycerophosphodiester phosphodiesterase, interacts with RGS16. *Proc Natl Acad Sci USA* 2000; **97**: 3999–4004.
- 40 Robbins MJ, Michalovich D, Hill J *et al*: Molecular cloning and characterization of two novel retinoic acid-inducible orphan G-protein-coupled receptors (GPC5B and GPC5C). *Genomics* 2000; **67**: 8–18.
- 41 Fujino T, Takei YA, Sone H *et al*: Molecular identification and characterization of two medium-chain acyl-CoA synthetases, MACS1 and the Sa gene product. *J Biol Chem* 2001; **276**: 35961–35966.
- 42 Klein I, Levey GS: Unusual manifestations of hypothyroidism. *Arch Intern Med* 1984; **144**: 123–128.
- 43 Sato A, Shirota T, Shinoda T *et al*: Hyperuricaemia in patients with hyperthyroidism due to Graves' disease. *Metabolism* 1995; **44**: 207–211.
- 44 Malik B, Schlanger L, Al-Khalili O *et al*: Enac degradation in A6 cells by the ubiquitin-proteasome proteolytic pathway. *J Biol Chem* 2001; **276**: 12903–12910.
- 45 Iwai N, Baba S, Mannami T *et al*: Association of sodium channel gamma-subunit promoter variant with blood pressure. *Hypertension* 2001; **38**: 86–89.
- 46 Loftus BJ, Kim UJ, Sneddon VP *et al*: Genome duplications and other features in 12 Mb of DNA sequence from human chromosome 16p and 16q. *Genomics* 1999; **60**: 295–308.