

Characterization of the *NPHP1* Locus: Mutational Mechanism Involved in Deletions in Familial Juvenile Nephronophthisis

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Familial juvenile nephronophthisis is an autosomal recessive, genetically heterogeneous kidney disorder representing the most frequent inherited cause of chronic renal failure in children. A gene, *NPHP1*, responsible for ~85% of the purely renal form of nephronophthisis, has been mapped to 2q13 and characterized. The major *NPHP1* gene defect is a large homozygous deletion found in ~80% of the patients. In this study, by large-scale genomic sequencing and pulsed-field gel electrophoresis analysis, we characterized the complex organization of the *NPHP1* locus and determined the mutational mechanism that results in the large deletion observed in most patients. We showed that the deletion is 290 kb in size and that *NPHP1* is flanked by two large inverted repeats of ~330 kb. In addition, a second sequence of 45 kb located adjacent to the proximal 330-kb repeat was shown to be directly repeated 250 kb away within the distal 330-kb repeat deleting the sequence tag site (STS) 804H10R present in the proximal copy. The patients' deletion breakpoints appear to be located within the 45-kb repeat, suggesting an unequal recombination between the two homologous copies of this smaller repeat. Moreover, we demonstrated a non-pathologic rearrangement involving the two 330-kb inverted repeats found in 11 patients and, in the homozygous state, in 2 (1.3%) control individuals. This could be explained by interchromosomal mispairing of the 330-kb inverted repeat, followed by double recombination or by a prior intrachromosomal mispairing of these repeats, leading to an inversion of the *NPHP1* region, followed by an interchromosomal unequal crossover event. This complex rearrangement, as well as the common deletion found in most patients, illustrates the high level of rearrangements occurring in the centromeric region of chromosome 2.

Introduction

Familial juvenile nephronophthisis (MIM 256100) is a progressive tubulointerstitial kidney disorder with autosomal recessive inheritance. The disease is characterized by polyuria, growth retardation, and deterioration of renal function during childhood or adolescence. It accounts for 6%–10% of end-stage renal disease in children (Antignac et al. 1998). The most prominent histological changes that occur are characterized by tubular basement membrane thickening, tubular atrophy, interstitial fibrosis, and medullary cyst formation (Waldherr et al. 1982). Various extrarenal manifestations have been associated with nephronophthisis, especially Leber amaurosis (described as Senior-Løken syndrome [SLS]; Senior et al. 1961). Further abnormalities, such as cerebellar dysfunction or liver involvement, are, however, rarely

observed (reviewed in Antignac et al. 1998). A gene (*NPHP1*) responsible for the vast majority (~85%) of the purely renal form of nephronophthisis was mapped to chromosome 2q13 (Antignac et al. 1993; Medhioub et al. 1994), and it was subsequently identified by a positional cloning strategy (Hildebrandt et al. 1997; Saunier et al. 1997) by means of the knowledge of large homozygous deletions found in the majority of patients (Konrad et al. 1996). At the time, we had shown that these deletions, each covering ~250 kb, were located between two large inverted repeats of ~100 kb and that the telomeric repeat was at least partially deleted. The use of several STSs within the common deletion interval allowed the establishment of an accurate and easy diagnostic PCR-based assay (Konrad et al. 1996). Since then, different smaller deletions have been characterized, leading to the reduction of the *NPHP1* interval and the cloning of the gene (Hildebrandt et al. 1997; Saunier et al. 1997). This gene encodes a new protein, nephrocystin, which contains an Src homology 3 (SH3) domain and whose function remains to be elucidated.

In this study, by large-scale genomic sequencing of a large part of the region and by pulsed-field gel electrophoresis (PFGE), we have extensively characterized a

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Table 1
STs Mapping to the *NPHP1* Region

Name	Primer Sequence	Amplified Product Size (bp)	Annealing Temperature (°C)
47A	AAA TAC ACC CAA ACC CAC AG CCC ATC AGA GCA AAC AAG T	689	50
53A	GCC ACA AGG AGG AGG AA CGC CAA ATA CCA TAC AAA GT	615	50
57A	GGG CGG AGA CCA AGA ATA GA CGC AGG CTG ACT CAA ACC A	731	52
49.81B	TTT CTC TAT CTT TGG CGT AT TCT TTC ACC TGG CAT CTA	586	53
C40	CGA TGG ACC CAG AGC AGT TT TGG CCA TAG AGG AGA CA	598	55
R30A	CAC ACC ACC TGA GCC AAT G GTC AAG TGA GAA CGC CAC AA	510	52
C30124	GTC CAC AAC AAT CTC TAG GG GTC TCT CCA TAC TCC CTT CT	124	51
R30E	CTG GAT GGG ACG CTG TT ATT GCC ACA TGG ATT CTC TG	177	50
N123	AGA GGT GGC AGG GAA GTG AA CCT GCA ACA TCC TGG CTA AAC	149	55

genomic interval of ~900 kb covering the *NPHP1* deletion region. We have shown that this region has a very complex organization containing large inverted repeats of ~330 kb and direct repeats of 45 kb. All the deletions we have detected to date arise from the same mechanism of homologous recombination between the two 45-kb direct repeats, contrary to the suggestions of the initial data. Furthermore, we have shown that the large inverted repeats mediate a nonpathogenic rearrangement likely to arise through interchromosomal mispairing and homologous recombination.

Patients and Methods

Patients

Thirty-one unrelated patients with familial juvenile nephronophthisis, without extrarenal symptoms, and with evidence (detected by PCR) of a homozygous deletion of the *NPHP1* region were selected for PFGE analysis. Although these patients are phenotypically identical, they could be classified into three groups with regard to the STS content of their deletions, as follows.

Group A.—Group A comprised 27 unrelated patients, 7 of whom belonged to consanguineous families, with deletions encompassing markers 765F2L and 804/6 (Konrad et al. 1996). These two markers are located within the most common *NPHP1* deletion.

Group B.—Group B was made up of two unrelated patients (one from a consanguineous family) with deletions of 804H10R, which is located outside the previously described deletions (Konrad et al. 1996; Hildebrandt et al. 1997; Saunier et al. 1997).

Group C.—Group C consisted of two unrelated patients, both from consanguineous families, with deletions encompassing 765F2L, 804/6, and 804H10R.

Large-Scale Genomic Sequencing

Four BAC clones (183K24, 187E16, 96G18, and part of 49G15) from a human BAC genomic library (Research Genetics) were sequenced as described elsewhere (Saunier et al. 1997).

PCR Analysis

Sequences derived from BAC sequencing were used to generate new STSs listed as in table 1. Primer sequences and PCR conditions were selected with the OLIGO 5.0 program (NBI) after identification and masking of repetitive sequences with the RepeatMasker program (Smit 1996). We performed PCR in a 25- μ l volume containing 50 ng of genomic DNA or one YAC colony.

Pulsed-Field Gel Electrophoresis

Agarose-embedded DNA from the patients and from YACs (879D3, 804H10, 765F2, 769G7, and 921H4) was digested with *Sfi*I and *Not*I for the patients' DNA and with *Sfi*I, *Sal*I, *Mlu*I, *Cla*I, *Sac*II, and *Not*I for the YAC DNA. Fragments were separated through 1% agarose gels in 0.5 \times Tris-borate EDTA buffer with a CHEF DRII system 5 (Biorad). Runs were performed for 24 h at 14°C and 200 V with 2–15 s of switch time for fragments of 25–250 kb and 60–120 s for fragments of 200 kb to 1.6 Mb. Fragments of 5–100 kb were separated by field inversion gel electrophoresis (FIGE Mapper;

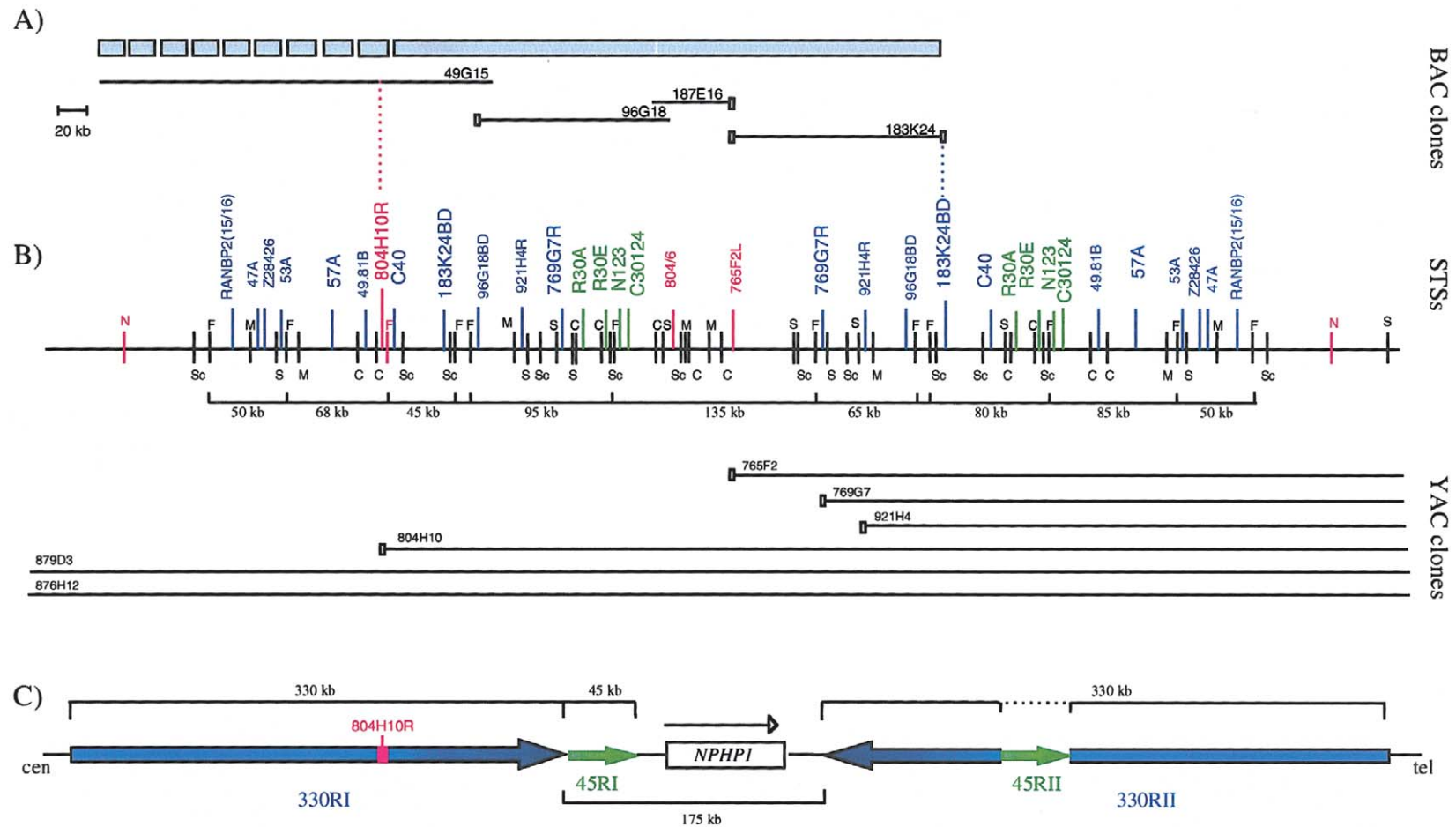


Figure 1 Characterization of the genomic region surrounding the *NPHP1* gene. *A*, Diagram of the sequenced region. The thick line represents the entirely sequenced interval, whereas the dashed thick line represents the partially sequenced region. BAC clones are represented as solid lines, and the BAC ends mapped to the region are shown as open rectangles. *B*, Restriction map of the *NPHP1* region and YAC contig spanning the region. STS positions and restriction sites (F = *Sfi*I; C = *Cl*aI; S = *Sal*I; M = *Mlu*I; Sc = *Sac*II; N = *Not*I) are indicated by vertical lines. *Not*I sites used to define the extent of the deletion are shown in red. The markers in green and blue are localized within the 45-kb direct and the 330-kb inverted repeats, respectively, and the markers in red are those initially found to be deleted by PCR analysis of individuals with nephronophthosis. *C*, Schematic representation of the genomic region surrounding the *NPHP1* gene. The shaded blue and green arrows represent, respectively, the inverted 330-kb repeats (named "330RI" and "330RII") and the direct 45-kb repeats (named "45RI" and "45RII"). The *NPHP1* gene is represented by an open box, and the direction of transcription is indicated by the small arrow above it. The red rectangle indicates the region around 804H10R in 330RI, deleted by the distal copy of the direct 45-kb repeat in 330RII.

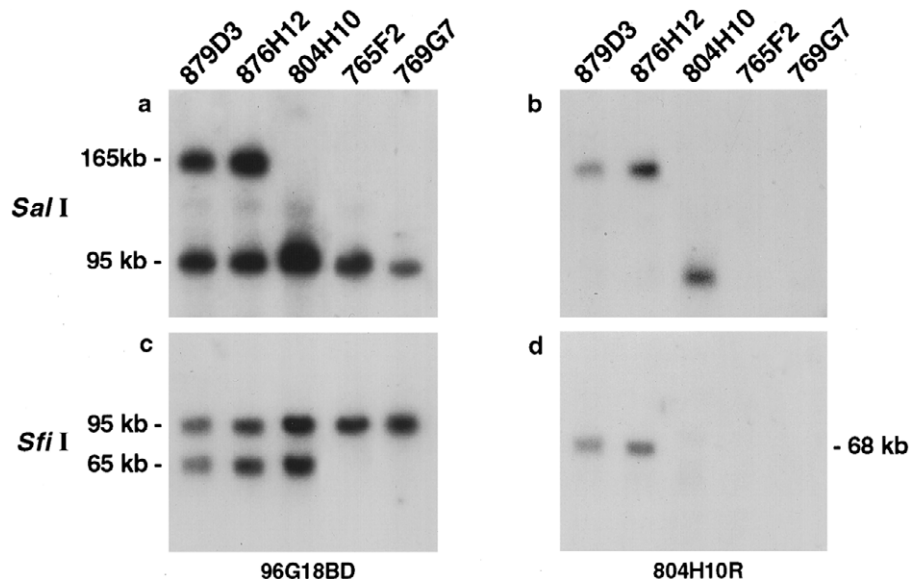


Figure 2 Southern blot hybridization of *SalI* (a, b) and *SfiI* (c, d) digested DNA from five YACs spanning the *NPHP1* region with 96G18BD (a, c) and 804H10R (b, d). Only a 165-kb *SalI* fragment and a 68-kb *SfiI* fragment were detected with the 804H10R probe in the two YACs covering the whole region (879D3 and 876H12), and no fragment was detected in the YACs covering only the distal copy of the inverted repeats (765F2 and 769G7), demonstrating that 804H10R escapes duplication.

Biorad) under the following conditions: 180 V forward, 120 V reverse, with 0.1–2-s switch time for 16 h at 20°C. Gel blotting and hybridization procedures were performed as described elsewhere (Heidet et al. 1995) with probes 804H10R, 183K24BD, and 96G18BD (Konrad et al. 1996; Saunier et al. 1997), and 47A, 53A, 49.81B, C40, R30A, R30E, and N123 (table 1).

NPHP1 Mutation Screening

NPHP1 exons were amplified by PCR with flanking intronic primers. Primers of exons 2–10, 12, 14, 15, 18, and 19 have been previously described (Saunier et al. 1997). The other primers were as follows: exon 1: 5'-GACCACCGCAAGAGAACATT-3' and 5'-GGCAAGCTCCCAGGATTAGG-3'; exon 11: 5'-AACAGAGTATTGAACTTT-3' and 5'-AGTACTGTTTAACCTGTATC-3'; exon 13: 5'-ATTCAAACCACACCAAACCC-3' and 5'-TTCCTTGTCAATAGACACAT-3'; exon 16: 5'-GCACTACTGGGTGGTATATT-3' and 5'-TGATCCCAAATTCCTGGAC-3'; exon 17: 5'-TTATAAGTTGGATTGTAGGG-3' and 5'-CAGAGTATGAAGCATTACTG-3'; and exon 20A: 5'-CGATACCTGCCAGGCACTAA-3' and 5'-GACAGTGATTTTTGGGTTCC-3'. After initial denaturation at 95°C for 3 min, PCR was performed with 30 cycles of denaturation for 1 min at 94°C, 1 min at annealing temperatures of 61°C for exon 1, 49°C for exon 17, and 50°C for exons 11, 13, 16, and 20, extension for 1 min at 72°C, and a final extension at 72°C for 10 min. PCR

products were purified with the Wizard PCR Preps DNA purification System (Promega) and directly sequenced with an Applied Biosystems DNA sequencer (model 373A) and the BigDye terminator cycle sequencing kit (PE Biosystems) according to the manufacturer's protocol. According to the recommended nomenclature system for human gene mutations (Antonarakis and the Nomenclature Working Group 1998), the nucleotide numbering of the *NPHP1* cDNA starts at the first methionine codon.

Results

Genomic Map of the Region

Three BAC clones (96G18, 187E16, and 183K24) covering about 325 kb of the *NPHP1* locus had been previously partially sequenced and characterized (Saunier et al. 1997; fig. 1A). We further extended the sequence analysis upstream to the 49G15 BAC clone and increased the percentage coverage of the aforementioned three BACs by primer walking. A total of 365 kb from STS 804H10R (i.e., the proximal end of YAC 804H10) to the distal end of BAC 183K24 (183K24BD) were almost entirely sequenced (96% coverage), and ~190 kb upstream of 804H10R (part of BAC 49G15) were partially sequenced (83% coverage). As previously shown by PFGE, within this 365-kb sequence, the 100 kb between STSs 769G7R and 183K24BD were present in two inverted copies that exhibit >97% homology (Kon-

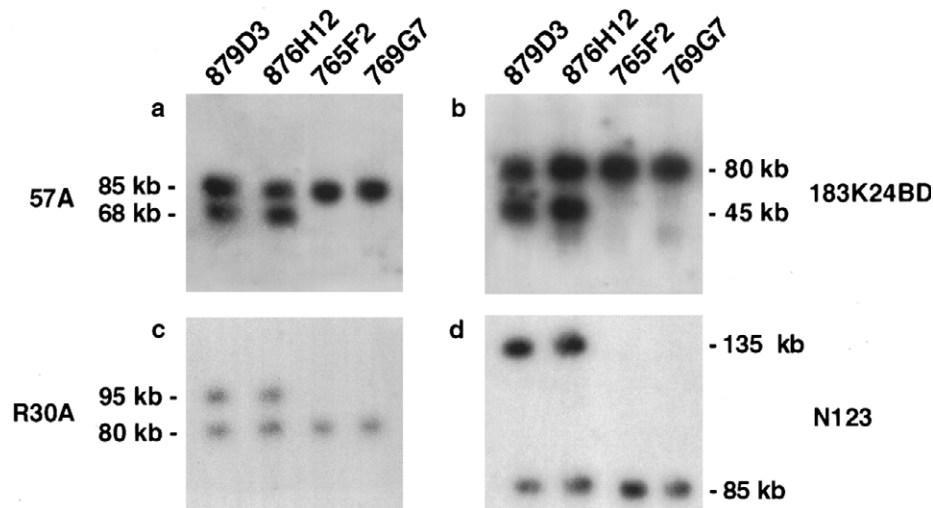


Figure 3 *Sfi*I-digested DNA of four YACs spanning the *NPHP1* region successively hybridized with probes 57A (a), 183K24BD (b), R30A (c), and N123 (d). All markers are duplicated; two *Sfi*I fragments are present in YACs covering the whole *NPHP1* region (879D3 and 876H12), whereas only one fragment is detected in YACs covering only the telomeric part of the region (765F2 and 769G7), allowing us to distinguish both copies. Note that R30A and 183K24BD detect the same 80-kb telomeric fragment, and that 57A and N123 detect the same 85-kb telomeric fragment.

rad et al. 1996; Saunier et al. 1997). To further study regions of interest around the markers 804H10R, 769G7R, and 183K24BD and to define patients' deletion breakpoints, we generated new STSs (see Patients and Methods) and checked, by PCR of YAC DNA, whether these were single copies or were also duplicated distal to the *NPHP1* region. To build a long-range restriction map of the region, some of these STSs were used as probes to hybridize YAC DNA digested with different rare-cutting restriction enzymes (fig. 1B). By combining these different approaches, we were able to show that the *NPHP1* gene is included in a 175-kb genomic region framed by two large inverted repeats of 300–330 kb (fig. 1C). We named the proximal and the distal copies 330RI and 330RII, respectively. One STS (804H10R) present in the centromeric repeat was not found in 330RII, as shown by its failure to hybridize YACs covering the distal region (fig. 2). Furthermore, we showed by PCR that four STSs—C30124, N123, R30E, and R30A—located 20–60 kb upstream of the first exon of *NPHP1*, were also duplicated downstream of the gene. By using these markers as well as STSs 57A and 183K24BD as hybridization probes (fig. 3), we were able to show that a ~45-kb sequence, located 20 kb upstream of *NPHP1* exon 1, was directly repeated 250 kb away, downstream of the gene, and within 330RII, deleting the STS 804H10R (fig. 1C) and removing a nearby *Sfi*I site (shown in red in fig. 1B). We named the proximal and the distal copies “45RI” and “45RII,” respectively.

Mechanism of Deletions In Nephronophthisis: Homologous Recombination between Direct Repeats

Twenty patients from group A, with homozygous deletions of the whole *NPHP1* gene as identified by PCR of 765F2L and 804/6 (fig. 4), were shown to have the same hybridization pattern on PFGE: hybridization of the *Not*I-digested patient DNA with 183K24BD, located within the 330-kb repeats, detected a ~510-kb fragment (fig. 5, lanes 2–10) instead of the ~800-kb fragment observed in controls (fig. 5, lane 1). Hybridization of *Sfi*I-digested DNA revealed only the proximal fragments (95 kb and 45 kb) with probes 96G18BD and 183K24BD, as the 80- and 65-kb telomeric fragments present in the control (fig. 6, lane 1) were absent (fig. 6, lanes 2–10). Additionally, hybridization with R30A and R30E probes located within the 45-kb repeats failed to detect the 80-kb telomeric *Sfi*I fragment. However, hybridization with N123 also located in the 45-kb repeats revealed a normal 85-kb telomeric fragment, but no centromeric fragment (data not shown). It thus appeared that the breakpoints in these 20 patients all fall within the 45-kb repeats. In an attempt to further refine the localization of the breakpoints, we performed Southern-blot analyses with more-frequently-cutting enzymes (*Eco*RI, *Bam*HI, *Hind*III, and *Pst*I), with probes located in the 45-kb repeats. Unfortunately, no abnormal fragment could be found (data not shown). These results are consistent with deletions arising through homologous recombination between the two 45-kb direct repeats.

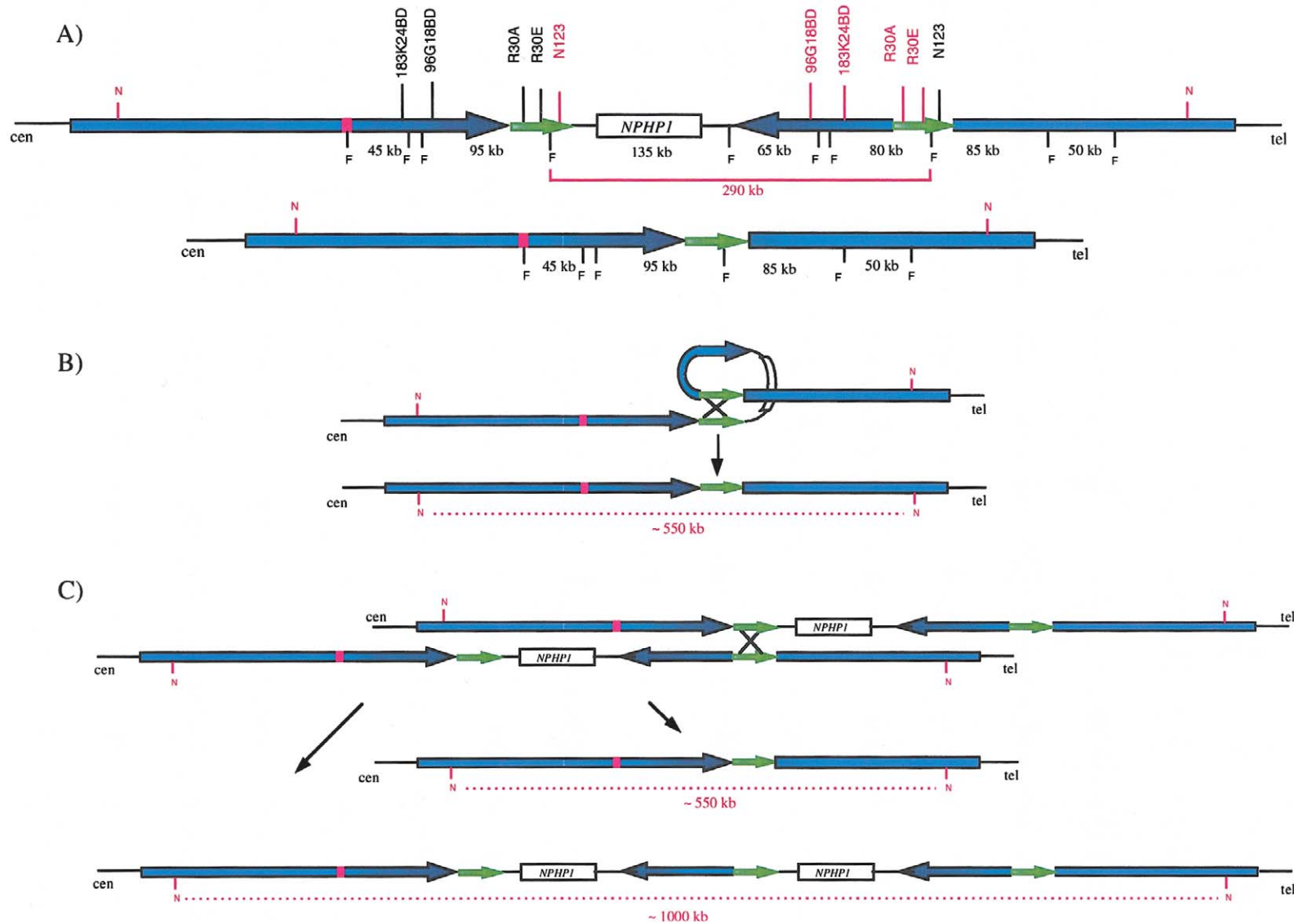


Figure 4 A, Schematic representation of the common *NPHP1* deletion involving an homologous recombination between the 45-kb direct repeats and leading to a deletion of 290 kb; *top*, normal chromosome; *bottom*, deleted chromosome. B, Model of unequal recombination by chromosome misalignment followed by formation of a loop structure. C, Model of unequal recombination by unequal crossing-over between two different chromosomes, leading in theory to either a deletion (*middle*) or duplication (*bottom*) of the *NPHP1* gene.

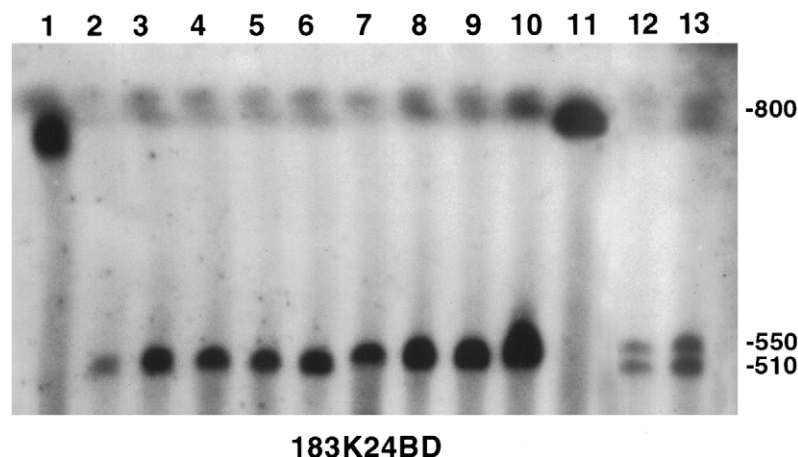


Figure 5 PFGE of *NotI*-digested DNA of 12 patients and a control individual hybridized with 183K24BD. Lane 1, control individual showing a normal ~800-kb *NotI* fragment. Lanes 2–10, patients from group A with homozygous deletion of markers 765F2L and 804/6, as detected by PCR. An abnormal ~510 kb *NotI* fragment is detected. Lane 11, patient from group C only deleted for 804H10R by PCR, with an apparently normal 800-kb *NotI* fragment. Lanes 12 and 13, patients from group A deleted for 765F2L and 804/6 with two distinct *NotI* fragments (~510 kb and ~550 kb).

Additionally, the more detailed characterization of the *NPHP1* region allowed us to determine the exact size of the deletion (previously estimated as ~250 kb) to be 290 kb.

Characterization of a Nonpathogenic Rearrangement Mediated by the 330-kb Inverted Repeats

The two patients from group B, one of whom was the product of a consanguineous marriage, were found by PCR to have a homozygous deletion of 804H10R, an STS that is located outside of the common *NPHP1* deletion interval. No fragment was detected when *EcoRI*-

digested DNA was hybridized with 804H10R, confirming the presence of a deletion encompassing the whole marker (data not shown). Furthermore, by using probes (57A and 183K24BD) that cover ~80kb around 804H10R and map within the 330-kb repeats, the 68- and 45-kb proximal *SfiI* fragments detected by these probes, respectively, were absent (data not shown). Surprisingly, an apparently normal-sized ~800-kb fragment was detected when *NotI*-digested DNA was hybridized with 183K24BD (fig. 5, lane 11), excluding the presence of a large deletion. An hypothesis to reconcile these apparently discordant results is the replacement of

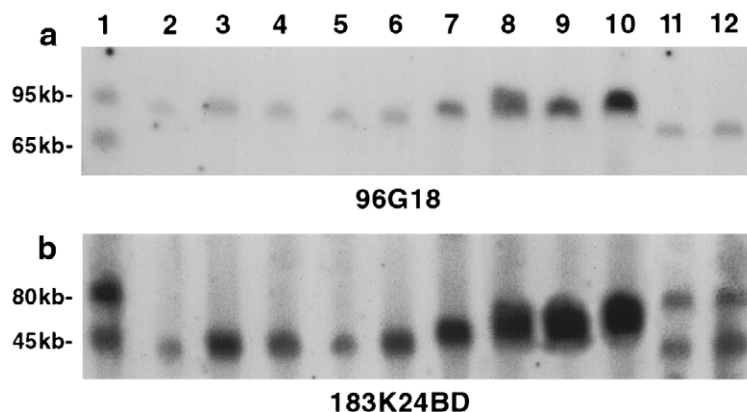


Figure 6 PFGE of *SfiI*-digested DNA of 11 patients and a control individual successively hybridized with 96G18BD and 183K24BD. Hybridization with probes 96G18BD (a) and 183K24BD (b) reveals two *SfiI* fragments in the control individual (lane 1), 95-kb and 45-kb proximal fragments, and 80- and 65-kb telomeric fragments, respectively. Lanes 2–10, patients from group A with deletion of markers 765F2L and 804/6 by PCR. The normal proximal fragments detected by both probes are missing. Lanes 11 and 12, patients from group A with two distinct abnormal *NotI* fragments (fig. 5). Hybridization with 183K24BD reveals the normal 80-kb telomeric *SfiI* fragment.

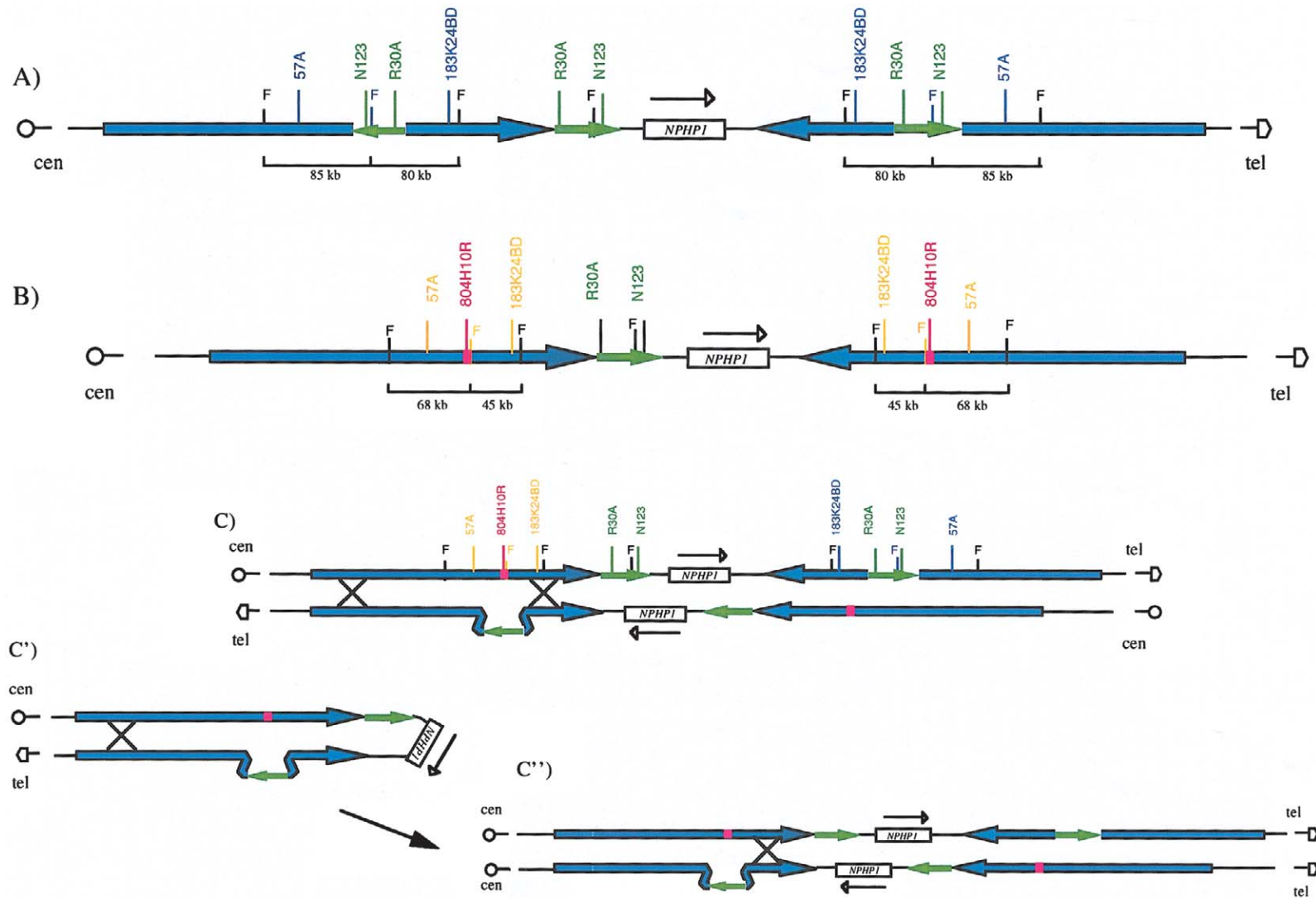


Figure 7 A, Genomic *Sfi*I map of the *NPHP1* region when 804H10R is missing and replaced by an additional copy of 45R1I. B, Reciprocal rearrangement leading to the duplication of 804H10R. C, Models for nonpathogenic DNA rearrangements mediated by mispairing of the inverted repeats and double homologous recombination (C) or by a crossing-over between the inverted repeats leading to an inversion of the whole *NPHP1* region (C'), followed by a crossing-over, localized telomeric of the 804H10R marker (C''). Centromeric and telomeric copies of 57A and 183K24BD are indicated in orange and blue, respectively.

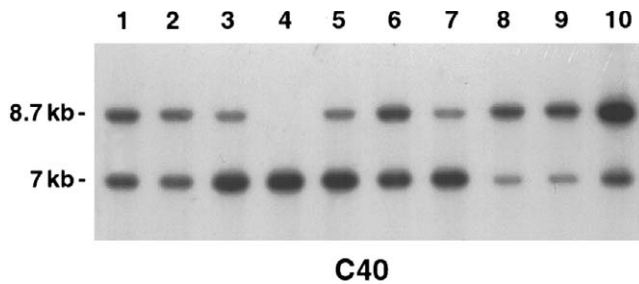


Figure 8 Southern blot analysis of *Pst*I-digested DNA of control individuals hybridized with probe C40. Lanes 3, 5, and 7 show that the 7-kb fragment is more intense than the 8.7-kb fragment, suggesting that each individual has only one copy of 804H10R and three copies of 45R1I. Lanes 8–10 show that the 8.7-kb fragment is more intense than the 7-kb fragment, suggesting the occurrence of the reciprocal event (i.e., three copies of 804H10R and one copy of 45R1I) in these individuals. Lane 4 shows a control individual carrying a homozygous deletion of 804H10R as detected by PCR. Lanes 1, 2, and 6 depict control individuals showing two bands of the same intensity; therefore, they are not carrying a nonpathogenic rearrangement.

804H10R by a third copy of the 45-kb repeat in the opposite orientation. The genomic map of these two patients obtained by hybridizing their *Sfi*I-digested DNA with probes 57A, 183K24BD, 96G18BD, R30A, and N123 is in agreement with this hypothesis. The absent 68-kb (probes 57A and N123) and 45-kb (183K24BD and R30A) proximal *Sfi*I fragments are replaced by the duplication of the 85- and 80-kb distal fragments respectively. A summary of these data is schematically represented in figure 7A.

This rearrangement does not actually involve the *NPHP1* gene and thus is unlikely to explain the phenotype in patients from group B. The absence of the 804H10R PCR product upon amplification of DNA from 2 of 152 control individuals (1.3%) supported the hypothesis that this rearrangement was nonpathogenic. This result was further confirmed by performing Southern blotting analysis by using the probe C40, which is 5 kb from 804H10R and located within the 330-kb repeats (fig. 1B). Hybridization with this probe, which normally detects the same 8.7-kb *Pst*I fragment as 804H10R as well as a telomeric 7-kb *Pst*I fragment located within 330R1I, resulted in the detection of only the 7-kb fragment for the DNA found to be deleted for 804H10R by PCR (fig. 8) of the two patients and the two control individuals. Careful analysis of Southern blots containing DNA from 152 controls allowed us to detect individuals heterozygous for this nonpathogenic rearrangement: individuals heterozygous for the deletion of 804H10R have a more intense 7-kb *Pst*I fragment in comparison with the 8.7-kb fragment (21%), and individuals heterozygous for the reciprocal event leading to a duplication of 804H10R (fig. 7B) present with a stronger 8.7-kb fragment (13%; fig. 8). To determine the

cause of nephronophthisis in the two group B patients, we searched for point mutations in the *NPHP1* gene by PCR and direct sequencing of all the 20 exons of the gene. In one patient, we detected an homozygous single-base deletion at position 639 of exon 7, causing a frame shift and generating a stop codon leading to a putative truncated polypeptide. In the other patient, two heterozygous missense mutations in exon 6 (a T→C substitution at position 539 leading to the substitution of a leucine by a proline at position 180 in the SH3 domain) and exon 8 (an A→G substitution at position 859, changing an arginine to a serine at position 287). Neither of these two missense mutations was detected in a panel of 50 unrelated control individuals who were also screened.

Two additional unrelated patients from group C who originated from consanguineous marriages had no PCR products with markers 765F2L, 804/6 (located in the common deletion), or 804H10R involved in the nonpathogenic rearrangement. The 183K24BD probe, hybridized to *Not*I-digested DNA of these two patients, detected a ~550-kb fragment (data not shown), a fragment slightly larger than the fragment detected in patients without PCR products for only 765F2L and 804/6 (fig. 5, lanes 2–10). In these two patients, *Sfi*I-digested DNA hybridized to 183K24BD demonstrated the presence of the distal fragment, but not the proximal one (data not shown). The pattern observed in these two patients is consistent with the homozygous pathogenic deletions occurring on chromosomes that also carry the nonpathogenic rearrangement.

Finally, seven patients from group A—all from non-consanguineous marriages and homozygously deleted for 765F2L and 804/6 but not for 804H10R—have two distinct *Not*I fragments, ~510 and ~550 kb, detected by 183K24BD (fig. 5, lanes 12 and 13). The 183K24BD probe hybridized to *Sfi*I-digested DNA detected in all these patients the normal 45-kb proximal and 80-kb distal *Sfi*I fragments (fig. 6b, lanes 11 and 12), which initially suggested that they might have a smaller deletion (Saunier et al. 1997). However, these additional data indicated that these patients are heterozygous for the nonpathogenic rearrangement involving 804H10R and the *NPHP1* deletions, mediated by the same mechanism of homologous recombination, had occurred on two different chromosomes 2, explaining the two distinct *Not*I fragments. This was confirmed by the pattern obtained with hybridization of *Not*I-digested DNA from the patients' parents with 804H10R, since the ~550kb *Not*I fragment corresponding to the deleted allele was completely absent in one of each parent (fig. 9).

Discussion

The most frequent mutation observed in familial juvenile nephronophthisis is a large homozygous deletion en-

comprising the whole *NPHP1* gene in the centromeric region of chromosome 2 (Konrad et al. 1996; Hildebrandt et al. 1997; Saunier et al. 1997). In this report, the high level of genomic detail obtained by sequencing and PFGE analysis allowed us to characterize the very complex organization of the *NPHP1* region; it also allowed us to demonstrate the mechanism leading to this common *NPHP1* gene deletion mutation. We showed that the *NPHP1* gene is flanked by two large inverted repeats of ~330 kb, the distal one interrupted by a ~45-kb sequence, which is in turn directly repeated upstream of the *NPHP1* gene. The patients' deletion breakpoints appear to be located within the 45-kb repeat. This would suggest an unequal recombination event between two homologous but nonallelic copies of 45-kb repeats, which leads to the 290-kb deletion. However, the extremely high sequence homology that exists between the two repeats, as suggested by the conservation of all the restriction sites in both copies, precluded easy identification of the exact position of the deletion breakpoints. More refined studies, such as those performed by Reiter et al. (1997), might allow us to define the breakpoints. Low-copy number repeats have been implicated in mediating rearrangements of other human disorders, as reviewed in Lupski (1998). Large rearrangements have been described between repeats on the X chromosome, leading to steroid sulfatase gene deletions (Li et al. 1992), and reciprocal duplication and deletion products of unequal crossing-over events on chromosomal band 17p11.2 are responsible for Charcot-Marie-Tooth disease type 1A (Pentao et al. 1992) and hereditary neuropathy with liability to pressure palsies (Chance et al. 1994), respectively. In these cases, rearrangements arise from unequal crossing-over and homologous recombi-

nation between 24-kb repeats that are 1.5 Mb apart (Reiter et al. 1997). Similar mechanisms have also been reported for deletion formation in Smith-Magenis syndrome (Chen et al. 1997) and velocardiofacial syndrome (Edelmann et al. 1999). In the case of *NPHP1* deletion, the unequal recombination between two homologous but nonallelic 45-kb repeats may occur either by chromosome misalignment, followed by an unequal crossing-over, or by the formation of a loop structure on a single chromosome, as illustrated in figure 4B and 4C. We did not detect any de novo deletions, although haplotype analyses in *NPHP1* families strongly suggest that the deletions are not due to a founder effect (Konrad et al. 1996). We found no evidence of the expected reciprocal event of the deletion: a duplication of *NPHP1* region (the ~1,000 kb *NotI* fragment as illustrated in fig. 4C) in patients or control individuals. However, such a duplication could lead to a distinct disease phenotype as shown in Charcot-Marie-Tooth disease type 1A (Pentao et al. 1992) and may therefore not be present in normal controls. Thus, our results did not allow us to determine whether the *NPHP1* deletion are mediated by inter- or intrachromosomal recombination.

We also detected a more common nonpathogenic DNA rearrangement involving the two 330-kb inverted repeats flanking the *NPHP1* gene. This rearrangement can be explained by various mechanisms. One such mechanism is an interchromosomal mispairing of the two copies of the inverted repeats followed by a double recombination, leading to the replacement of a short genomic sequence containing the 804H10R STS by an additional copy of the 45-kb repeat (fig. 7C). Another such mechanism is a prior intrachromosomal mispairing of the inverted repeats and an unequal crossover leading

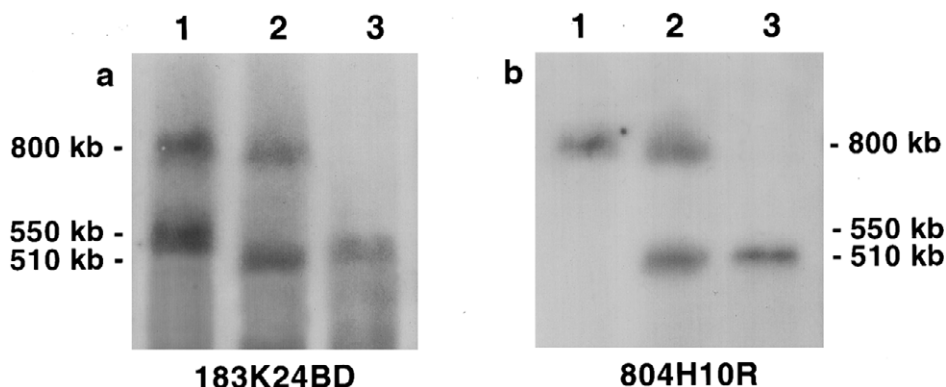


Figure 9 PFGE of *NotI*-digested DNA of a patient and her parents successively hybridized with 183K24BD (a) and 804H10R (b). Lane 3 depicts an affected individual. Lanes 1 and 2 depict the father and the mother of the patient, respectively. Each parent is heterozygous for the *NPHP1* deletion, as shown by hybridization with 183K24BD, but the father lacks the 804H10R marker on the deleted allele. Furthermore, the abnormal *NotI* fragment detected by 183K24BD in the father (~550 kb) is slightly larger than the abnormal fragment detected in the mother (~510 kb), in agreement with the occurrence of a 290-kb deletion on a chromosome carrying the nonpathogenic rearrangement and, thus, two copies of the 45-kb repeat. This proves that the child bears the nonpathogenic rearrangement on her paternal allele in addition to the 290-kb deletion on both alleles, as was suggested by the *SfiI* pattern.

to the inversion of the whole *NPHP1* region (fig. 7C'), followed by recombination between two different chromosomes (fig. 7C''), leading to the same rearrangement as in the first mechanism described.

We did not obtain evidence that led us to favor one hypothesis over the other. However, inversion events involving two inverted repeats on the same chromosome have been described in several disorders. For example, inversions in the factor VIII gene causing severe hemophilia A involve a recombination event between two inverted repeats located in intron 22 and located upstream of the gene (Lakich et al. 1993). Additionally, nonpathogenic rearrangements involving mispairing of inverted repeats leading to the maintenance of repeat sequence homogeneity and inversion of the region have been previously reported (Small et al. 1997) around the emerin locus at Xq28 and were found in 33% of women in the heterozygous state. In this study, the replacement of 804H10R by the 45-kb repeat was found in 1.3% and in 21% of controls in the homozygous and heterozygous states, respectively. We were also able to detect the reciprocal event in the heterozygous state. This represents a key element for the validation of our hypothesis concerning the mechanism of the origin of this complex rearrangement. This could also explain the results of Nothwang et al. (1998b), who found a duplication of 804H10R but no duplication of two markers (57N19T and 278P6T) located in 45 RI upstream of the *NPHP1* gene when they built a PAC contig of the *NPHP1* region. It can be supposed that the human DNA used to establish this PAC library (Genome System) bears the predicted duplication of 804H10R and the deletion of the 45RII copy.

These repetitive structures appear to predispose the human genome to frequent deletion and duplication. Their persistence through evolution may thus make particular regions of the genome more susceptible to rearrangement, consequently leading to genetic disorders (Lupski 1998). The human chromosome 2 has arisen from the fusion of two ancestral ape chromosomes (Yunis and Prakash 1982), the telomere-telomere fusion point being located at band 2q13 (Ijdo et al. 1991), telomeric to the *NPHP1* locus (Hildebrandt et al. 1996). Telomeric regions are known to contain several repeat sequences (Allshire et al. 1988). The close proximity of these identical telomeres at the centromere of chromosome 2 would be expected to make this region prone to rearrangements. Indeed, this has been illustrated by centromeric inversions found in 0.1% of controls (Vejerlev and Friedrich 1984; MacDonald and Cox 1985; Djalali et al. 1986). Additionally, several genes have been found to be duplicated within the 2q13 region, *BENE* (Saunier et al. 1997) and *RANBP2L1* (Nothwang et al. 1998a), with some on either side of the centromere

such as *IGO* (Huber et al. 1990) and *LIS2* (Reiner et al. 1995).

The elucidation of the complex structure of the *NPHP1* region allowed us to show that all the patients with nephronophthisis without extrarenal symptoms that we have studied to date do indeed have the same deletion, although this deletion occurs on different ancestral chromosomes. The detailed genomic organization of the *NPHP1* region will also help us to characterize the rearrangements involved in patients with nephronophthisis associated with extrarenal symptoms who also have deletions in the same region (Caridi et al. 1998).

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for familial juvenile nephronophthisis [MIM 256100])
RepeatMasker program, <http://ftp.genome.washington.edu/cgi-bin/RepeatMasker>

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