

# Mutations in *MAPKBP1* Cause Juvenile or Late-Onset Cilia-Independent Nephronophthisis

Maxence S. Macia,<sup>1,2</sup> Jan Halbritter,<sup>3,4,22</sup> Marion Delous,<sup>1,2,22</sup> Cecile Bredrup,<sup>5,6</sup> Arthur Gutter,<sup>1,2</sup> Emilie Filhol,<sup>1,2</sup> Anne E.C. Mellgren,<sup>5,6,7</sup> Sabine Leh,<sup>7,8</sup> Albane Bizet,<sup>1,2</sup> Daniela A. Braun,<sup>3</sup> Heon Y. Gee,<sup>3</sup> Flora Silbermann,<sup>1,2</sup> Charline Henry,<sup>1,2</sup> Pauline Krug,<sup>1,2,9</sup> Christine Bole-Feysot,<sup>2,10</sup> Patrick Nitschké,<sup>2,11</sup> Dominique Joly,<sup>12</sup> Philippe Nicoud,<sup>13</sup> André Paget,<sup>13</sup> Heidi Haugland,<sup>14</sup> Damien Brackmann,<sup>15</sup> Nayir Ahmet,<sup>16</sup> Richard Sandford,<sup>17</sup> Nurcan Cengiz,<sup>18</sup> Per M. Knappskog,<sup>6,19</sup> Helge Boman,<sup>6</sup> Bolan Linghu,<sup>20</sup> Fan Yang,<sup>20</sup> Edward J. Oakeley,<sup>21</sup> Pierre Saint Mézard,<sup>21</sup> Andreas W. Sailer,<sup>21</sup> Stefan Johansson,<sup>6,19</sup> Eyvind Rødahl,<sup>5,7,23</sup> Sophie Saunier,<sup>1,2,23,\*</sup> Friedhelm Hildebrandt,<sup>3,23,\*</sup> and Alexandre Benmerah<sup>1,2,23</sup>

Nephronophthisis (NPH), an autosomal-recessive tubulointerstitial nephritis, is the most common cause of hereditary end-stage renal disease in the first three decades of life. Since most NPH gene products (NPHP) function at the primary cilium, NPH is classified as a ciliopathy. We identified mutations in a candidate gene in eight individuals from five families presenting late-onset NPH with massive renal fibrosis. This gene encodes MAPKBP1, a poorly characterized scaffolding protein for JNK signaling. Immunofluorescence analyses showed that MAPKBP1 is not present at the primary cilium and that fibroblasts from affected individuals did not display ciliogenesis defects, indicating that MAPKBP1 may represent a new family of NPHP not involved in cilia-associated functions. Instead, MAPKBP1 is recruited to mitotic spindle poles (MSPs) during the early phases of mitosis where it colocalizes with its paralog WDR62, which plays a key role at MSP. Detected mutations compromise recruitment of MAPKBP1 to the MSP and/or its interaction with JNK2 or WDR62. Additionally, we show increased DNA damage response signaling in fibroblasts from affected individuals and upon knockdown of *Mapkbp1* in murine cell lines, a phenotype previously associated with NPH. In conclusion, we identified mutations in *MAPKBP1* as a genetic cause of juvenile or late-onset and cilia-independent NPH.

Nephronophthisis (NPH [MIM: 256100]) is an autosomal-recessive kidney disorder characterized by the development of massive interstitial fibrosis with abnormal thickness of the tubular basement membranes, atrophic and/or dilated tubules, and occasionally, formation of cysts mainly distributed at the cortico-medullary junction within normal sized or small kidneys. It is the main genetic cause of end-stage renal disease (ESRD) in the first two decades of life and three different forms have been clinically described based on the mean age at ESRD: infantile (<3 years), juvenile (mean 13 years), and late onset (mean 19 years).<sup>1</sup>

NPH can be either isolated or associated with different extra-renal manifestations (retinal dystrophy, liver fibrosis, skeleton dysplasia, etc.) in syndromic forms referred to hereafter as nephronophthisis-related ciliopathies (NPH-RCs). Indeed, the great majority of the 20 genes associated with NPH-RCs encode proteins (NPHP) that localize to

primary cilia where they play key functions in the biogenesis of this organelle as well as in cilia-dependent signaling pathways.<sup>1–3</sup> NPH has therefore been classified as a ciliopathy, in a growing family of genetic diseases linked to either primary and/or motile cilia dysfunctions.<sup>4</sup> In addition, recent evidence established that NPH-RCs can also be linked to the DNA damage response (DDR) signaling pathway. Initially identified for *NPHP14/ZNF423* (MIM: 614844) and *NPHP15/CEP164* (MIM: 614848),<sup>5</sup> enhanced DDR signaling was also associated with mutations in several other *NPHP* genes evidenced by mouse models of *NPHP6/CEP290*<sup>6</sup> (MIM: 610142), *NPHP9/NEK8*<sup>7,8</sup> (MIM: 613824), and *NPHP10/SDCCAG8*<sup>9</sup> (MIM: 613524). Altogether, these data suggest that increased DDR signaling may underlie progressive renal disease seen in NPH<sup>10</sup> as well as a possible functional link between cilia and regulation of DDR signaling which remains to be uncovered. Except for

<sup>1</sup>INSERM, UMR-1163, Laboratory of Inherited Kidney Diseases, 75015 Paris, France; <sup>2</sup>Paris Descartes - Sorbonne Paris Cité University, Imagine Institute, 75015 Paris, France; <sup>3</sup>Division of Nephrology, Department of Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA; <sup>4</sup>Division of Nephrology, Department of Internal Medicine, University Clinic Leipzig, 04103 Leipzig, Germany; <sup>5</sup>Department of Ophthalmology, Haukeland University Hospital, 5021 Bergen, Norway; <sup>6</sup>Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, 5021 Bergen, Norway; <sup>7</sup>Department of Clinical Medicine, University of Bergen, 5021 Bergen, Norway; <sup>8</sup>Department of Pathology, Haukeland University Hospital, 5021 Bergen, Norway; <sup>9</sup>Pediatric Nephrology Department, Necker-Enfants Malades Hospital, 75015 Paris, France; <sup>10</sup>Inserm UMR-1163, Genomic Core Facility, 75015 Paris, France; <sup>11</sup>Paris Descartes - Sorbonne Paris Cité University, Bioinformatics Core Facility, 75015 Paris, France; <sup>12</sup>Nephrology Department, Necker-Enfants Malades Hospital, 75015 Paris, France; <sup>13</sup>Hemodialysis-Nephrology Department, Mont-Blanc Hospitals, 74700 Sallanches, France; <sup>14</sup>Department of Ophthalmology, Førde Central Hospital, 6812 Førde, Norway; <sup>15</sup>Department of Pediatrics, Haukeland University Hospital, 5021 Bergen, Norway; <sup>16</sup>University of Istanbul, 34452 Istanbul, Turkey; <sup>17</sup>Academic Department of Medical Genetics, University of Cambridge, CB2 0QQ Cambridge, UK; <sup>18</sup>Baskent University, 06810 Ankara, Turkey; <sup>19</sup>Department of Clinical Science, University of Bergen, 5021 Bergen, Norway; <sup>20</sup>Novartis Institutes for Biomedical Research, Cambridge, MA 02139, USA; <sup>21</sup>Novartis Institutes for Biomedical Research, 4002 Basel, Switzerland

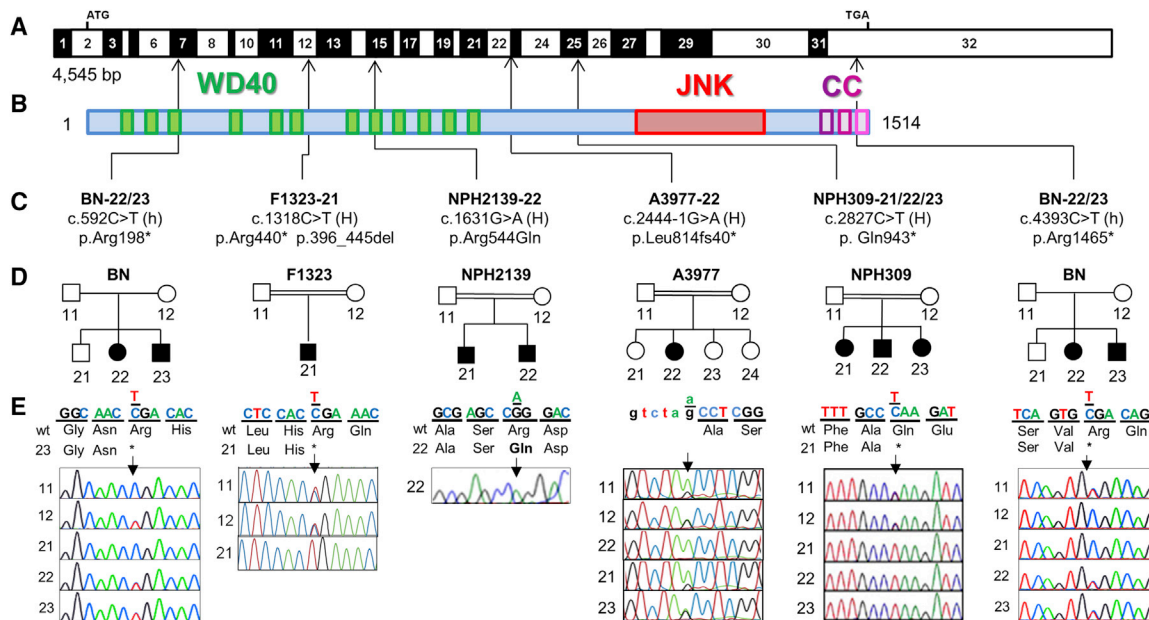
<sup>22</sup>These authors contributed equally to this work

<sup>23</sup>These authors contributed equally to this work

\*Correspondence: [sophie.saunier@inserm.fr](mailto:sophie.saunier@inserm.fr) (S.S.), [friedhelm.hildebrandt@childrens.harvard.edu](mailto:friedhelm.hildebrandt@childrens.harvard.edu) (F.H.)

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**Figure 1. Biallelic Mutations in *MAPKBP1* Cause Nephronophthisis**

(A) Exon structure of *MAPKBP1* cDNA. Positions of start codon (ATG) and stop codon (TGA) are indicated.

(B) Domain structure of the protein. *MAPKBP1* contains 12 WD repeats (green), located at the N terminus, a JNK-binding region located in the C-terminal part (red), and conserved coiled-coil domains at the C terminus (CC, purple).

(C) Relation of four homozygous (H) and two compound heterozygous mutations (h) to exons and protein domains is indicated by black arrows.

(D and E) Pedigrees (D) and chromatograms and segregation (E). Mutated nucleotides are shown above wild-type. In addition, individuals BN-21 and BN-23 also presented with retinitis pigmentosa (RP) linked to a homozygous mutation in *PDE6A*.

See Figure S5. Segregation of the *MAPKBP1* mutations was not examined in family NPH2139 due to the unavailability of the DNA of the affected brother and the parents.

ZNF423 (MIM: 604557), which was not implicated in direct ciliary function, all NPHPs that have been involved in DDR signaling do also play a crucial role at primary cilia.<sup>1,10</sup>

In order to identify additional genes mutated in NPH, we independently performed homozygosity mapping and/or whole-exome sequencing (WES) on affected individuals from our cohorts (Figure S1, Table S1). Blood samples and pedigrees were obtained from individuals with diagnosed NPH-RCs. Written informed consent was obtained from all individuals enrolled in this study and/or from parents and approved by the Comité de Protection des Personnes pour la Recherche Biomédicale Ile de France II, the Institutional Review Board (IRB) at the Boston Children's Hospital (Boston), or the Regional Committee for Medical and Research Ethics, Western Norway (IRB no. 00001872). Affected individuals were included in WES upon either parental consanguinity or as member of a multiplex family. In total, WES was performed in 141 families, 85 with isolated NPH and 56 with syndromic NPH. Compound heterozygous nonsense (c.592C>T [p.Arg198\*]; c.4393C>T [p.Arg1465\*] in BN-23), homozygous nonsense (c.1318C>T [p.Arg440\*] in F1323-21; c.2827C>T [p.Gln943\*] in NPH309-21), as well as homozygous splice site mutations (c.2444-1G>A [p.Leu814fs40\*] in A3977-22) were identified in *MAPKBP1* (GenBank: NM\_001128608.1) in four affected individuals from four independent families (Figure 1 and Table 1). Additional

competing biallelic missense variants were detected, but none of them are thought to possess an equally strong impact on the protein level, for either mild in silico prediction, occurrence in SNP databases (e.g., ExAC), or little evolutionary amino acid conservation (Table S2).

Additional screening of 342 unrelated affected individuals (108 with isolated NPH and 234 with syndromic NPH) using targeted exome sequencing<sup>2,11</sup> led to the identification in an affected individual (NPH2139-22) presenting late-onset isolated NPH of a homozygous missense variant (c.1631G>A [p.Arg544Gln]) never reported in ExAC database and predicted as damaging (PolyPhen-2 score, 1; SIFT, deleterious/score: 0; MutationTaster, disease causing). Interestingly, the Arg544 is part of a stretch of 12 aa that is highly conserved (almost identical) in both *MAPKBP1* and *WDR62* proteins from humans to *Drosophila* (Figure S2A). Furthermore, 3D structure analysis on equivalent residues in other WD40 domain-containing proteins indicates that Arg544 is located in a conserved site involved in the interaction of the WD40 domain with its partner(s) (Figures S2B and S2C), suggesting that the p.Arg544Gln variation may alter the binding of *MAPKBP1* with potential and not yet identified partners.

Segregation of the identified mutations could be examined by Sanger sequencing for all the families except NPH2139. It showed that parents and unaffected siblings were heterozygous for the identified mutations (BN,

**Table 1. Mutations of MAPKBP1 in Five Families with Nephronophthisis**

Individuals	Ethnic Origin	Nucleotide Alterations <sup>a</sup>	Deduced Protein Change	Exon, Zygosity	Parental Consanguinity	ESRD Years	RTX Years	Extrarenal Clinical Features
BN-22	Norway	c.592C>T; c.4393C>T	p.Arg198*, p.Arg146S*	7, 32, Het	no	no (25)	-	loose patella, long fingers and feet
BN-23	Norway	c.592C>T; c.4393C>T	p.Arg198*, p.Arg146S*	7, 32, Het	no	no (27)	-	RP, <sup>b</sup> CHD, severe meningomyelocele, statural growth delay
F1323-21	Turkey	c.1318C>T <sup>c</sup> ; exon 12 skipping	p.Arg440*, p.396_445del	12, Hom	yes	12	15	facial dysmorphism
NPH2139-22	Portugal	c.1631C>A	p.Arg544Gln <sup>d</sup>	15, Hom	yes	no (23)	-	none
A3977-22	Turkey	c.2444-1G>A, 5' splice site	5' splice site	22, Hom	yes	15	-	short stature
NPH309-21	Italy	c.2827C>T	p.Gln943*	25, Hom	yes	22	22, 39	scoliosis
NPH309-22	Italy	c.2827C>T	p.Gln943*	25, Hom	yes	25	27, 38	scoliosis, vesiculo-ureteral reflux, cholesteatoma, amyloid angiopathy
NPH309-23	Italy	c.2827C>T	p.Gln943*	25, Hom	yes	20	21	scoliosis, mild mental retardation, short stature, palate cleft

Abbreviations are as follows: ESRD, end-stage renal disease; CHD, congenital heart disease; Het, heterozygous; Hom, homozygous; NPH, nephronophthisis; RP, retinitis pigmentosa; RTX, renal transplantation.

<sup>a</sup>cDNA mutations are numbered according to human cDNA reference sequence GenBank: NM\_001128608.1, isoform b (*MAPKBP1*), where +1 corresponds to the A of ATG start translation codon.

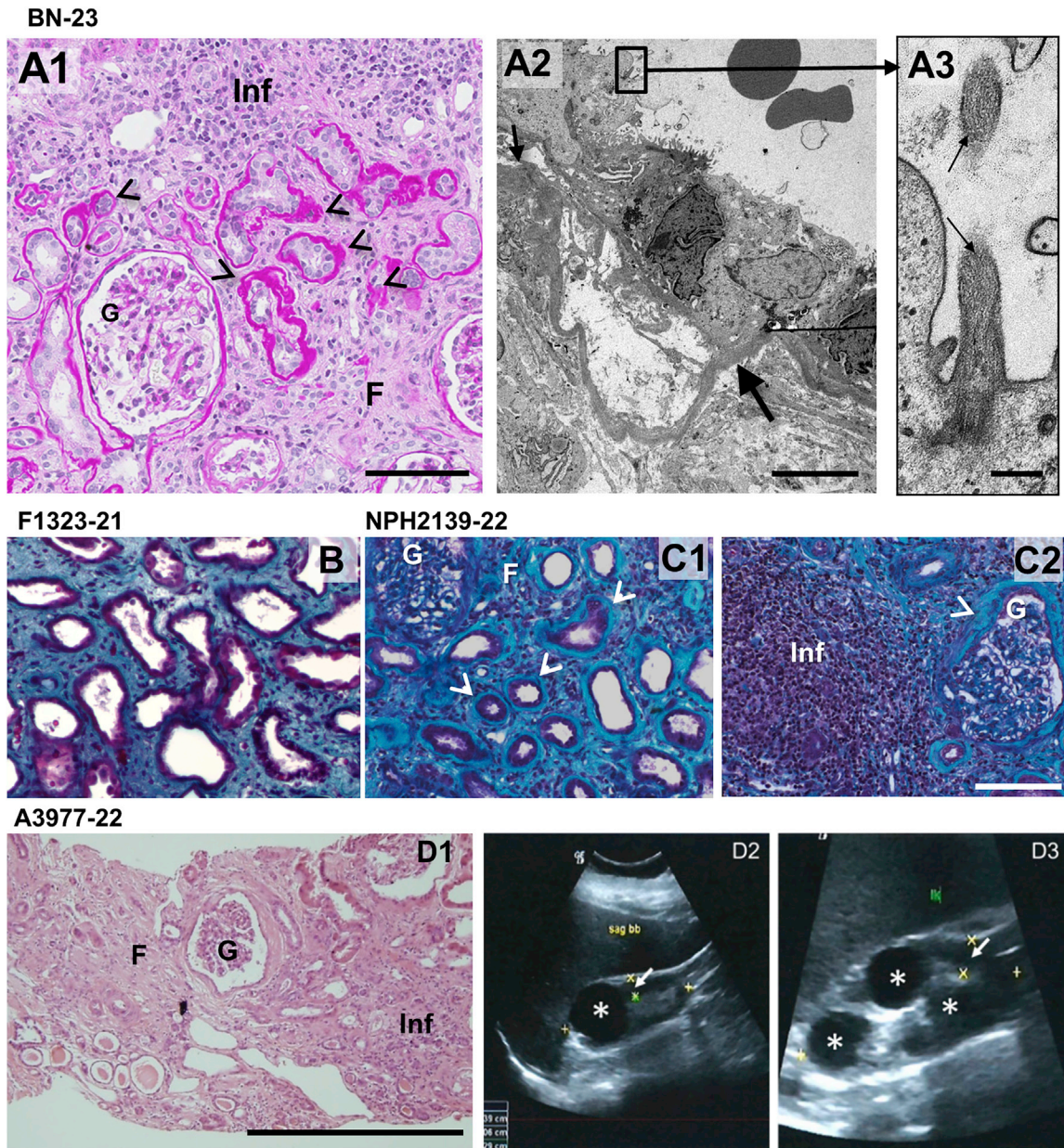
<sup>b</sup>Individuals BN-23 and BN-21 also present with RP linked to homozygous mutation in *PDE6A* (Figure S5).

<sup>c</sup>r3202001274: variant is listed in ExAC database, 2 of 121,258 are heterozygote. None of the others are currently listed.

<sup>d</sup>PolyPhen-2 score, 1; MutationTaster, disease causing; SIFT deleterious/score: 0.

F1323, NPH309, A3977) and that affected siblings were also presenting corresponding biallelic mutations in *MAPKBP1* (BN, NPH309; Figure 1 and Table 1). We consequently identified mutations in *MAPKBP1* in eight individuals from five families. They almost all presented with late-onset NPH (ESRD from 15 to >27 years) or juvenile NPH (for individual F1323-21 who presented ESRD at 12 years) (Table 1). Renal biopsies and ultrasounds from affected individuals (Figure 2) showed classical features of NPH characterized by atrophic tubules with thickening of the basement membranes, massive interstitial fibrosis, as well as the presence of interstitial infiltrate (BN-23 shown in Figure 2A, panels 1 and 2; NPH2139-21 shown in Figure 2C, panel 2; A3977-22 shown in Figure 2D, panel 1) as well as increased echogenicity and cysts (Figure 2D, panels 2 and 3).

Among the three nonsense mutations, the homozygous c.1318C>T (p.Arg440\*) mutation in individual F1323-21 was investigated more closely. Indeed, in silico analysis of *MAPKBP1* transcripts revealed an alternative splicing leading to the skipping of exon 12 (NCBI; GenBank: XM\_011521383.1). This alternative transcript is predicted to lead to an in-frame deletion and a 1,475 amino acid protein (versus 1,514 for full-length *MAPKBP1*). Because c.1318C>T (p.Arg440\*) was located in the exon 12 (Figure S3A), we next analyzed the expression and ratio of the various transcripts in individual F1323-21. Amplification of the exon 11–13 region by RT-PCR from F1323-21 fibroblasts revealed the presence of two products: one product also detected in the unrelated affected and control individuals (BN-22 and BN-12) and a shorter product (Figure S3B). Sanger sequencing analysis confirmed that the longer product corresponds to the full-length exon 12 containing transcript presenting the c.1318C>T nonsense mutation while the shorter band corresponds to a transcript that effectively lacks exon 12 (Figures S2B and S2C). The high amount of transcript lacking exon 12 in individual F1323-21 may possibly be explained by the fact that the c.1318C>T mutation is predicted to affect the binding of serine-arginine-rich protein SF2/ASF to putative exonic splicing enhancers (ESEfinder 3.0;<sup>12</sup> Figures S4A and S4B), which is then expected to affect the recruitment of splicing factors to splice sites and to lead to the skipping of exon 12 (Figure S4C). Skipping of exon 12 results in an in-frame deletion of 49 amino acids (aa 396 to 445) encompassing the sixth WD repeat out of the seven predicted to form a WD domain (Figure S3D). The resulting Δexon12 V5-tagged construct was poorly expressed upon transient transfection, with a migration profile in western blot experiments different from what was expected (Figure S3E, arrow), suggesting that the lack of exon 12 likely leads to misfolding of the N-terminal WD domain and decreased stability of the resulting protein. In conclusion, individual F1323-21 expressed two *MAPKBP1* transcripts, one coding for the expected p.Arg440\* protein and a shorter spliced form lacking exon 12, both of these proteins being likely non- or poorly functional (see below).



**Figure 2. Histological and Sonographic Kidney Lesions in Individuals with *MAPKBP1* Mutations**

(A1) Periodic acid-Schiff staining of a kidney biopsy from individual BN-23 showing tubular atrophy (arrowheads), interstitial fibrosis (F), focal interstitial infiltration (Inf), and normal glomerulus (G). Scale bar represents 100  $\mu$ m.

(A2) Ultrastructure (transmission electron microscopy picture) from a tubule with abrupt transition from thin to thickened and disorganized basement membrane (arrow). Scale bar represents 5  $\mu$ m.

(A3) Example of a primary cilium found at the apical membrane of a tubular epithelial cell (arrows). Scale bar represents 250  $\mu$ m.

(B) Trichrome staining of a kidney biopsy from individual F1323-21 showing fibrosis, thickened basal membrane, as well as atrophic and dilated tubules.

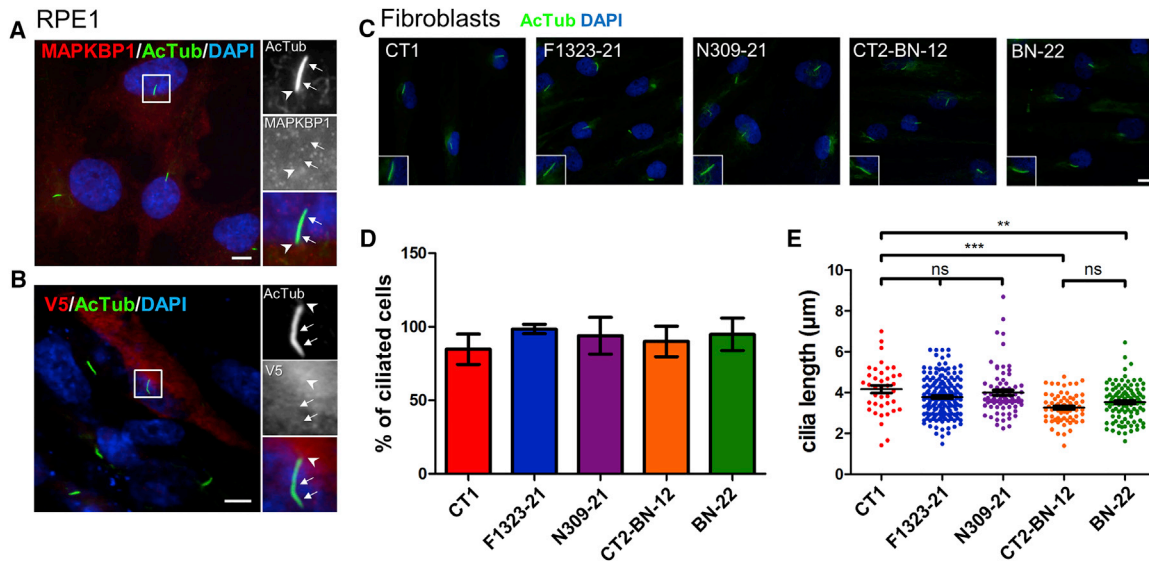
(C) PAS trichrome staining of a kidney biopsy from individual NPH2139-21 showing tubular atrophy (arrowheads in C1), interstitial fibrosis (F) and focal interstitial infiltration (Inf, C2), and sclerotic glomeruli (G, C2) surrounded by a thickened capsular basement membrane (arrows, C1 and C2). Scale bar represents 100  $\mu$ m.

(D1) Histological analysis of individual A3977-22 shows massive interstitial fibrosis and atrophic tubules. Scale bar represents 100  $\mu$ m.

(D2 and D3) Upon renal ultrasound, kidneys present small with narrowed parenchyma (white arrow), increased echogenicity, and single cysts on both sides (white asterisk). D2 right kidney, D3 left kidney.

NPH in the affected individuals was not associated with other classically found extra-renal manifestations of NPH-RCs, except retinitis pigmentosa (RP [MIM: 613810]) found in two out of three siblings of the BN family, suggesting Senior-Loken syndrome (SLS).<sup>1</sup> However, only individ-

ual BN-23 presented with both RP and NPH whereas his siblings presented with either NPH (BN-22) or RP (BN-21). Interestingly, while *MAPKBP1* mutations segregated only with NPH but not with RP, WES of individual BN-23 revealed the co-occurrence of a homozygous mutation in



**Figure 3. MAPKBP1 Is Not Involved in Ciliogenesis**

(A and B) Serum-starved (24 hr) nontransfected RPE1 cells (A) or RPE1 cells transiently transfected (FuGENE [Promega]) with V5-tagged MAPKBP1 encoding plasmid (B) were fixed (paraformaldehyde; PFA) and stained for acetylated  $\alpha$ -tubulin (AcTub, green; cilia; mouse monoclonal antibody [6-11-B-1, Sigma], dil 1/10,000) and for endogenous MAPKBP1 (A) (red; rabbit polyclonal [HPA030832, Sigma], dil 1/300) or overexpressed MAPKBP1 (B) (red, V5 rabbit polyclonal antibody [Sigma V8137], dil 1/10,000). Insets on the right show higher magnifications of representative cilia indicated by a white square in the corresponding images. Arrows indicate cilia, arrowheads cilium base region.

(C–E) Serum-starved fibroblasts from controls (CT1 [unrelated healthy], CT2-BN-12 [mother of BN-22, heterozygous for the c.592C>T (p.Arg198\*) mutation]) or from the affected individuals (BN-22, F1323-21, NPH309-21) were stained for acetylated-tubulin (AcTub, green) to identify cilia (C). Insets on the right show higher magnifications of representative cilia. Ciliogenesis (% of ciliated cells [D]) and length of cilia (E) were analyzed and quantified by ImageJ from three independent experiments ( $n > 50$  cells for each experiment). ns, \* $p < 0.01$ , and \*\*\* $p < 0.0001$  were calculated via Dunn's multiple comparison test after the analysis of variance ANOVA test. Note that cilia length in cells from individual BN-22 is not significantly different from that of cilia from cells of his mother (CT2-BN-12). Cilia in cells from individuals F1323-21 and NPH309-21 were not significantly different than in control cells (CT1) but longer than in cells from individuals of the BN family. Scale bars represent 5  $\mu$ m.

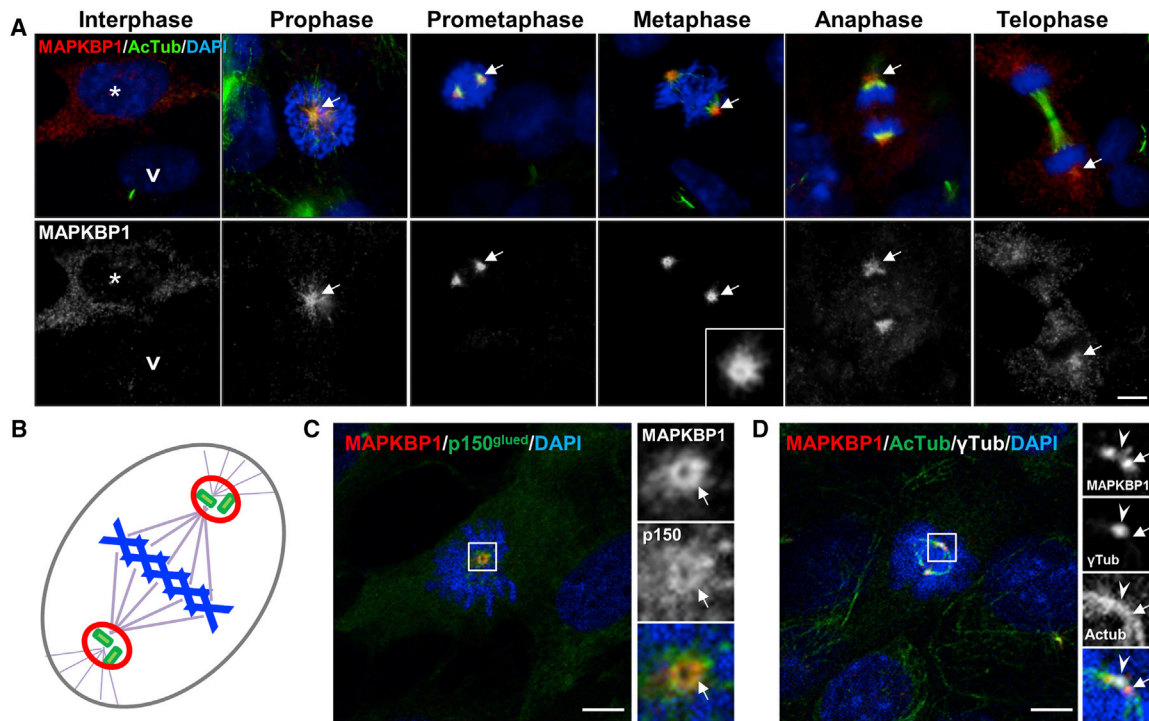
*PDE6A* (c.2053G>A [p.Val685Met]), previously associated with RP<sup>13</sup> (MIM: 180071), that segregated with RP in affected siblings but was not found in individual BN-22 (Figure S5). Altogether, these results show that RP and NPH in the BN siblings are caused by biallelic mutations in two different genes and that mutations in *MAPKBP1* are associated with NPH without RP as observed in individual BN-22. Additional manifestations were observed in several affected individuals from different families including facial dysmorphism (F1323-21 and NPH309-23) and/or scoliosis (NPH309-21, -22, -23). Finally, congenital heart disease and meningomyelocele were observed in a BN family member (BN-23). It remains speculative at this point whether defects of *MAPKBP1* are truly related to these conditions since none of them were observed in animal models (see below).

MAP-kinase binding protein 1 (*MAPKBP1*, also known as *JNKBP1*) is the paralog of *WDR62*,<sup>14</sup> the product of a gene mutated in primary microcephaly (*MCPH2* [MIM: 604317]),<sup>15,16</sup> which localizes at the mitotic spindle poles (MSPs) and has been involved in the orientation of the mitotic spindle.<sup>17</sup> *MAPKBP1* and *WDR62* share the same structural organization with their N-terminal half characterized by the presence of WD40 repeats (12 for *MAPKBP1*, see Figure 1B). In addition, *MAPKBP1*,<sup>18</sup> as

well as *WDR62*,<sup>19</sup> binds JNK family members through their C-terminal domain (aa 1,063–1,331 for *MAPKBP1*). It was shown to act as a scaffolding protein for JNK as well as for other signaling pathways.<sup>18,20,21</sup> Finally, *MAPKBP1* and *WDR62* also share a highly conserved C-terminal coiled-coil containing region that is required for *WDR62* homodimerization and some evidence indicates that it might also be involved in *MAPKBP1*-*WDR62* heterodimerization.<sup>22</sup> However, despite their similarity, no functional relationship has been characterized between the two proteins.

Expression of endogenous *MAPKBP1* was tested in vitro and in vivo, using a commercial rabbit polyclonal antibody against *MAPKBP1* that we validated by western blot and immunofluorescence experiments (Figures S6A–S6C). Analysis of the expression of *MAPKBP1* in fibroblasts from both control subjects and affected individuals using the validated antibody led to the expected results (Figure S6D) with a slightly shorter protein for BN-22 (p.Arg1465\*) and no detectable protein for NPH309-21 (p.Gln943\*, loss of the epitope) and F1323-21 (p.Arg440\*, see Figure S3).

We then investigated the subcellular distribution of *MAPKBP1* first in ciliated RPE1 cells. Endogenous (Figure 3A) and V5-tagged *MAPKBP1* (Figure 3B) showed



**Figure 4. MAPKBP1 Is Recruited to Mitotic Spindle Poles during Early Stages of Mitosis**

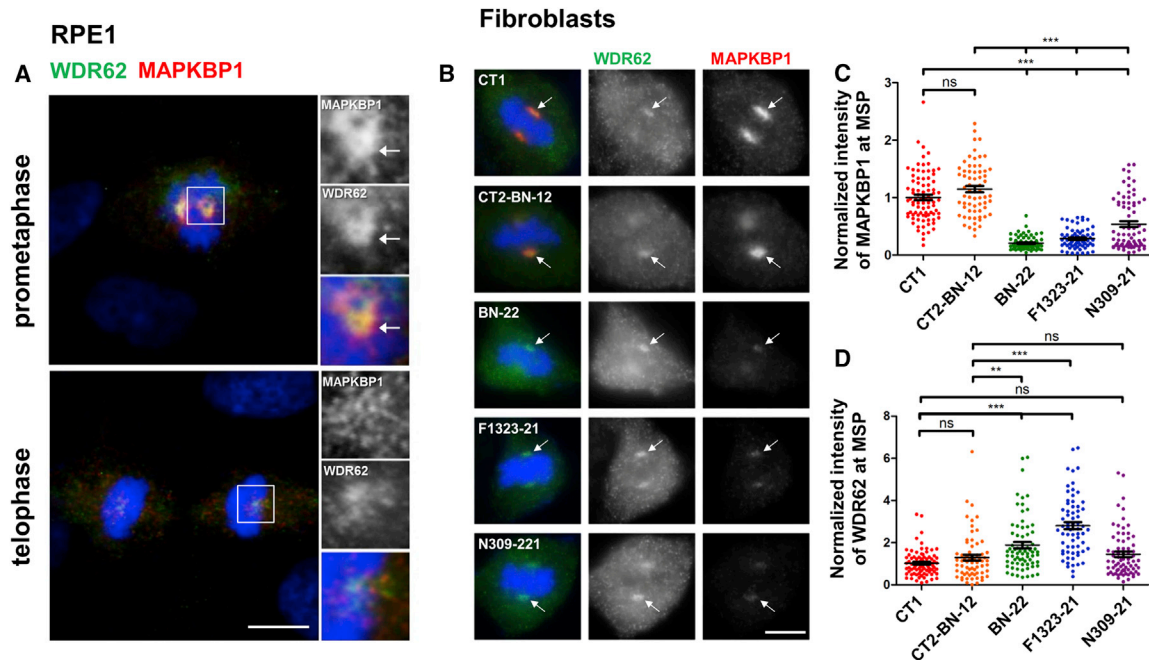
(A) Cycling RPE1 cells were fixed (PFA) and stained for MAPKBP1 (red) and for DNA with DAPI (blue). Arrows stress accumulation of MAPKBP1 staining at spindle poles in mitotic cells. The white arrowhead and star indicate representative ciliated and non-ciliated cells, respectively. Corresponding MAPKBP1 staining are shown in black and white in the lower panels.

(B) Scheme indicating mitotic spindle poles (MSP, red) surrounding centrosomes (green) at each pole of the mitotic spindle (pink) during metaphase (chromosomes in blue).

(C and D) Cycling RPE1 cells were fixed (PFA) and stained for MAPKBP1 (red) and either with p150 glued (MSP, green [C]; mouse monoclonal antibody [610473, BD Biosciences], dil 1/100) or acetylated  $\alpha$ -tubulin (AcTub, spindle, green [D]) and  $\gamma$ -tubulin ( $\gamma$ -tub, centrosomes, white [D]); goat polyclonal antibody [sc-7396, Santa Cruz], dil 1/2,000) together with DAPI (blue). Insets on the right show higher magnifications of representative MSP indicated by a white square in the corresponding images. Arrows and arrowheads point to MAPKBP1 staining at the MSP and to  $\gamma$ -tub, respectively. Scale bars represent 5  $\mu$ m.

a similar diffuse or punctate cytoplasmic distribution and did not show any specific association with either the axoneme (acetylated  $\alpha$ -tubulin, arrows) nor the basal body region (arrowhead) of primary cilia, an expected localization for a *NPHP* gene product. Similar results were obtained in ciliated human primary fibroblasts and IMCD3 kidney cells (Figure S7A) as well as in human adult kidney tubular cells *in vivo*, where MAPKBP1 showed a similar faint cytoplasmic staining not associated with cilia (Figure S8). The absence of MAPKBP1 from cilia and basal body was in agreement with proteomic and recent proximity-dependent biotinylation studies in which MAPKBP1 was not detected either at cilia or at centrosomes/basal bodies.<sup>23–27</sup> Therefore, as expected, mutations in *MAPKBP1* did not show any impact on ciliogenesis since fibroblasts obtained from affected individuals (NPH309-21, F1323-21, BN-22) formed cilia in similar proportion and with similar length as in cells from control individuals (Figures 3C–3E). Cilia with normal shape were also observed at the apical membrane of tubular epithelial cells from individual BN-23 (Figures 2A, panel 4, and S8). In addition, animal models that were generated, including zebrafish morphants (Figure S9) and mutant (Figures S10 and S11)

as well as knock-out (Figure S12) mice, did not show any of the classical ciliopathy-associated phenotypes including body axis curvature, situs inversus, or pronephric cysts in the zebrafish, or situs inversus, polydactyly, or renal tubular cysts in the mouse. In agreement, in the zebrafish larvae, *mapkbp1* was not expressed in the pronephros nor in the Kupffer vesicle and was mostly expressed in somites and in the brain (Figure S13), similarly as reported in mice.<sup>18</sup> Altogether, these data show that MAPKBP1 is not directly nor indirectly involved in ciliary functions. Examination of 18-month-old fish, in which *mapkbp1* is expressed at low level in the kidney (Figure S10B), revealed a slight proportion of *mapkbp1* mutant with dilated tubules associated with fibrosis (1/8; Figure S11). This result, although anecdotal, may reflect a potential role for MAPKBP1 in kidney homeostasis in a long term. The lack of obvious kidney phenotype in mice models even in adults (up to 10 months; Figure S12) was already reported in other *Nphp* knockout mice, including *Nphp1*, *Nphp4*, and *Nphp5*,<sup>28–30</sup> respectively mutated in juvenile isolated NPH<sup>1</sup> and SLS-associated NPH.<sup>1,31,32</sup> In the case of *MAPKBP1*, the lack of kidney phenotype in vertebrate models is possibly linked to either residual functional



**Figure 5. Localization of MAPKBP1 at Mitotic Spindle Poles Is Disturbed in Fibroblasts from Affected Individuals**

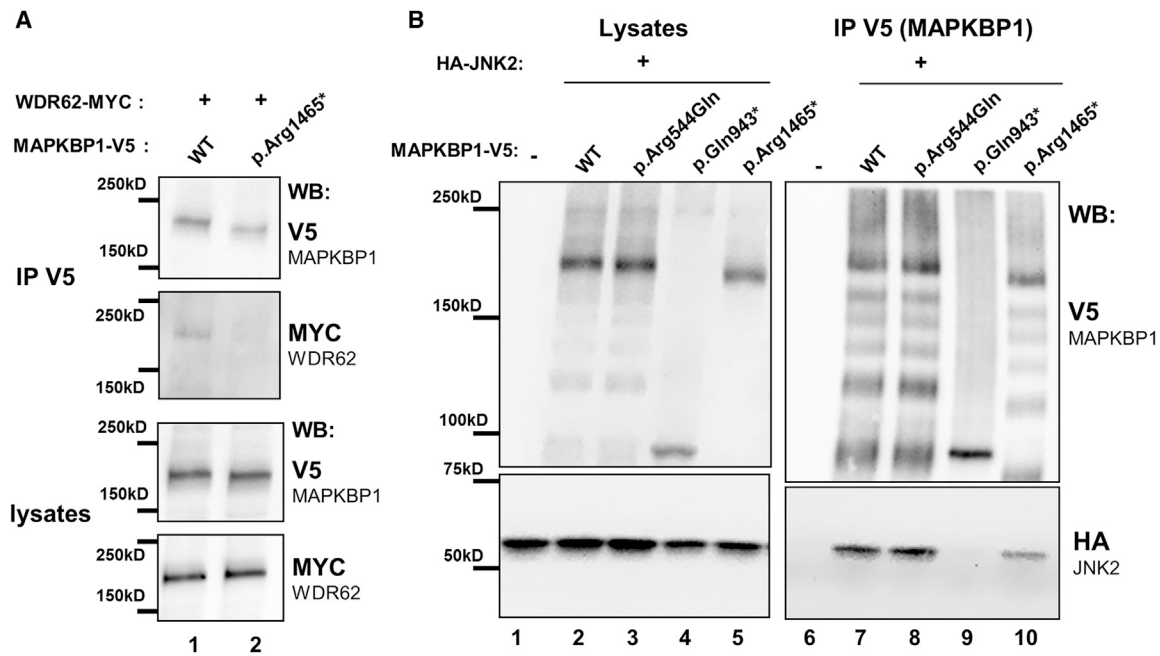
(A) Cycling RPE1 cells were fixed (PFA) and stained for endogenous MAPKBP1 (red) and WDR62 (green; mouse monoclonal [Clone 3G8, Sigma], dil 1/1,000) together with DAPI (blue). Representative pictures of cells in prometaphase and telophase are shown. Insets on the right show higher magnifications of representative MSP indicated by a white square in the corresponding images.

(B–D) Cycling fibroblasts from control subjects (CT1 and CT2-BN-12) or from affected individuals (BN-22, F1323-21, NPH309-21) were stained for both MAPKBP1 (red) and WDR62 (green). Representative pictures of cells in metaphase are shown in (B). Arrows stress MSPs. Intensity of MAPKBP1 (C) and WDR62 (D) stainings at the mitotic spindle poles was quantified with ImageJ (3 independent experiments,  $n > 65$  cells). ns,  $**p < 0.001$  and  $***p < 0.0001$  were calculated via Dunn's multiple comparison test after the analysis of variance ANOVA test. Scale bars represent 5  $\mu\text{m}$ .

protein (zebrafish mutant) or compensatory effects by its paralog WDR62 or species- and tissue-specific differences in the function of MAPKBP1.

As MAPKBP1 was absent from cilia in quiescent cells where it showed non-specific cytoplasmic localization (Figures 3 and S7A), we analyzed its distribution in cycling cells. Interestingly, while MAPKBP1 staining was very faint in ciliated quiescent cells, it globally increased in the cytoplasm of non-ciliated cycling cells (Figure 4A, asterisk) and showed a strong accumulation around the poles of the mitotic spindle from prophase to anaphase in mitotic cells (Figure 4A, arrows). Interestingly, this distribution during mitosis was similar to the one reported for WDR62 that is recruited to MSP during early phases of mitosis.<sup>15,17</sup> The localization of MAPKBP1 at MSP was further confirmed by the fact that MAPKBP1 staining showed a typical ring-shaped organization (Figure 4A, metaphase, arrow) that colocalized with the MSP marker p150 glued (Figure 4C, arrows) and it surrounded centrioles as observed by confocal transverse sections (Figure 4D, arrows). MAPKBP1 also colocalized with WDR62 at MSP during early phases of mitosis (Figure 5A), as expected. We therefore investigated the impact of the mutations on the localization of MAPKBP1 and WDR62 during mitosis. As shown in Figure 5B, WDR62 was observed at MSP in mitotic fibroblasts from both control subjects and

affected individuals (NPH309-21, F1323-21, BN-22), while MAPKBP1 staining at MSP was severely decreased in affected individuals compared to control subjects (arrows). Quantification of WDR62 and MAPKBP1 staining intensities at MSP confirmed these observations (Figures 5C and 5D). Similar results were obtained upon transient transfections of corresponding V5-tagged MAPKBP1 encoding constructs in cycling HeLa cells (Figure S14), showing that all variants of MAPKBP1, except the p.Arg544Gln, impair its recruitment to MSP. Interestingly, in fibroblasts from two out of three affected individuals (F1323-21 and BN-22, but not NPH309-21), the level of WDR62 staining at MSP was significantly increased compared to control subjects (Figure 5D) suggesting that WDR62 might compensate for the lack of MAPKBP1. In addition, the last conserved coiled-coil domain of MAPKBP1 (aa 1,488–1,514), which is lost in the p.Arg1465\* protein (BN-22), was involved in WDR62-MAPKBP1 heterodimerization.<sup>22</sup> As shown in Figures 6A and S15, WDR62 was efficiently co-immunoprecipitated by WT MAPKBP1 but not by the p.Arg1465\* variant, confirming the interaction between the two proteins, the role of the last coiled-coil domain in MAPKBP1-WDR62 heterodimerization, and the negative impact of p.Arg1465\* on this interaction. As expected, the interaction with WDR62 was also lost for the p.Gln943\* shorter protein



**Figure 6. Mutations in *MAPKBP1* Affect JNK2 and/or WDR62 Binding**

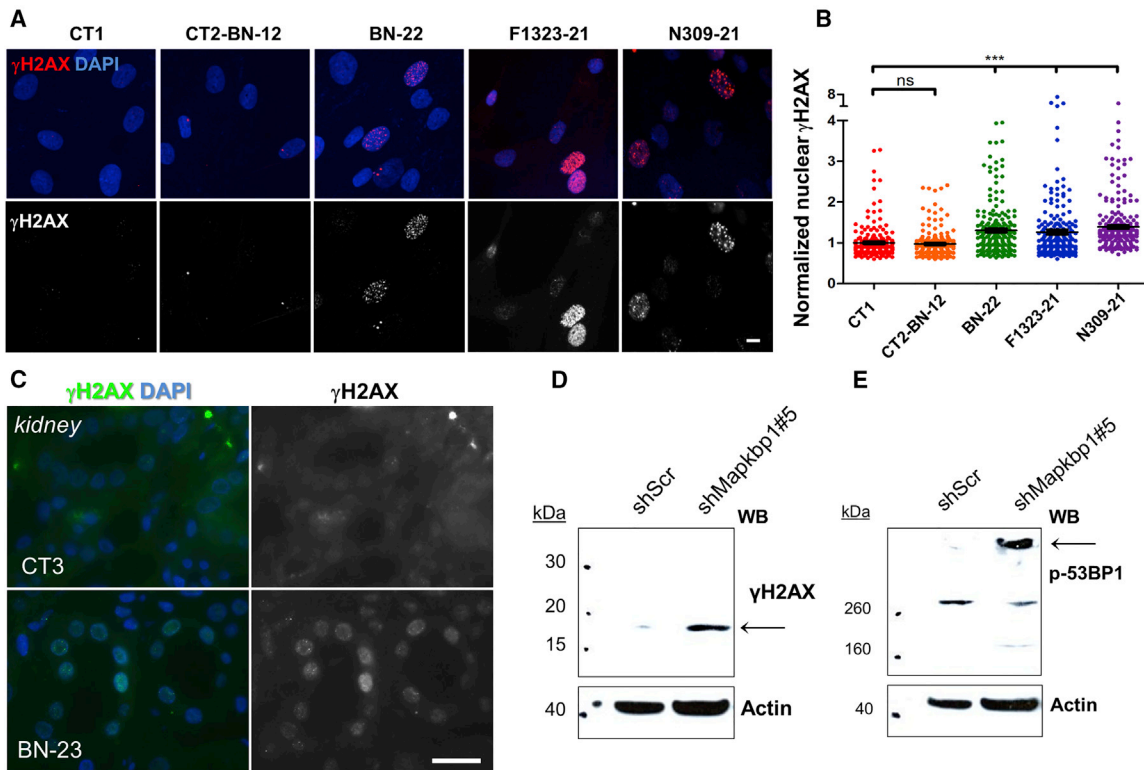
HEK293 cells were transiently transfected (lipofectamine [ThermoFischer]) with plasmids encoding V5-tagged WT and mutant forms of *MAPKBP1* and Myc-tagged WDR62 (A) or HA-tagged JNK2 (B), as indicated. Cells were lysed and immunoprecipitated with the anti-V5 antibody (rabbit polyclonal [Sigma, V8137]). Lysates and immunoprecipitates (IP) were analyzed by western blot as indicated (WB; V5: Mouse monoclonal clone SV5-Pk1 [AbD Serotec MCA-1360], dil 1/5,000; HA: mouse monoclonal, clone 16B12 [Biolegend, 901502], dil 1/5,000; MYC: mouse monoclonal, Ab-2 clone 9E10.3 [Fisher Scientific MS-139-P1], dil 1/5,000).

(NPH309-21). It was preserved for the p.Arg544Gln variant (NPH2139-22; Figure S15), a result in agreement also with the fact that this variation does not affect the WDR62 binding site and with the observation that its targeting to MSP was not affected (Figure S14). Altogether, these data indicate that most of the identified variations in *MAPKBP1* affect its recruitment to MSP that is likely to occur through its interaction with WDR62.

The functional impact of the identified mutations was further evaluated by testing their ability to interact with JNK. As shown in Figure 6B, interaction with JNK2 was not affected by the p.Arg544Gln variation (Figure 6B, lane 8; NPH2139-22) as expected by the fact that the N-terminal WD repeat-containing domain is not required for JNK binding.<sup>18,22</sup> Interaction with JNK2 was lost for the p.Gln943\* construct (Figure 6B, lane 9; NPH309-21), in agreement with the loss of the reported JNK binding site in this mutant<sup>18</sup> (see Figures 1B and S6A). Moreover, the ability of the p.Arg1465\* protein (BN-22) to interact with JNK2 was decreased compared to WT (Figure 6B, lane 10). This could be explained by the fact that the p.Arg1465\* variant lacks the last C-terminal coiled-coil that was shown to be involved in dimerization and efficient binding to JNK in the context of WDR62.<sup>22</sup> Despite the effect on JNK binding, *MAPKBP1* mutations did not result in significant change in JNK signaling (phosphorylated JNK) in stable IMCD3 cells knockdown for *Mapkbp1* expression nor in kidney biopsies from affected individual (Figure S16).

Increased DNA damage response (DDR) signaling was recently reported to be associated with NPH-RCs, so we tested whether this was also the case for NPH caused by mutations in *MAPKBP1*. Steady-state DDR signaling was first analyzed in control fibroblasts by immunofluorescence after phosphorylation of H2AX ( $\gamma$ H2AX), a widely used marker of DDR signaling.<sup>33</sup> As a positive control for the use of this assay, nuclear  $\gamma$ H2AX staining was significantly increased in control fibroblasts treated with aphidicolin, an inhibitor of DNA replication known to induce DDR signaling,<sup>34</sup> compared to untreated cells (Figures S17A and S17B). Steady-state DDR signaling was subsequently analyzed in fibroblasts from affected individuals with mutations in *MAPKBP1*. Interestingly, all mutant fibroblasts showed increased levels of nuclear  $\gamma$ H2AX staining compared to healthy controls (Figures 7A and 7B), a phenotype also observed in kidney tubules from affected individual BN-23 (Figure 7C) and in two different murine cell lines (NIH 3T3 and IMCD3) that were knocked-down for *Mapkbp1* expression (Figures 7D, 7E, S17E, and S17F). These data are in agreement with those recently reported in other NPH models (*CEP290/NPHP6*, *NEK8/NPHP9*, *SDCCAG8/NPHP10*, *ZNF423/NPHP14*, and *CEP164/NPHP15*)<sup>5,6,8,9,35</sup> and suggest either direct or indirect function of *MAPKBP1* in DDR signaling. The exact role of *MAPKBP1* in DDR remains to be determined. Interestingly, JNK pathway positively regulates DDR signaling<sup>36</sup> and defects in JNK regulation could then result in the accumulation of non-repaired DNA damage and then in steady-state





**Figure 7. DNA Damage Response Is Increased in MAPKBP1 Mutant Cells**

(A) Fibroblasts from control subjects (CT1; CT2-BN-12) and affected individuals (BN-22, F1323-21, NPH309-21) were fixed and stained for phosphorylated  $\gamma$ H2AX (red; mouse monoclonal, clone JBW301 [MILLIPORE 05-636], dil 1/200) together with DAPI (blue). Scale bar represents 5  $\mu$ m.

(B) The intensity of nuclear  $\gamma$ H2AX staining was quantified from three independent experiments ( $n > 200$ ). ns and \*\*\* $p < 0.0001$  were calculated via Dunn's multiple comparison test after the analysis of variance ANOVA test.

(C) Kidney biopsies from a control unrelated individual (CT3) and from the affected individual BN-23 were stained for  $\gamma$ H2AX (green; rabbit polyclonal, dil 1/200). Scale bar represents 20  $\mu$ m.

(D and E) NIH 3T3 cells (fibroblasts) were stably transduced with lentiviral vectors expressing either control scrambled (shScr) or *Mapkbp1* targeting shRNA (shMapkbp1#5; Figures S16 and S17). Immunoblots show the expression of different components of DNA damage response signaling including  $\gamma$ H2AX (Cell Signaling, rabbit polyclonal, dil 1/1,000); (D) and phospho-53BP1 (Cell Signaling, rabbit polyclonal, dil 1/1,000); (E).

increased DDR signaling ( $\gamma$ H2AX). However, we could not detect a major general defect in JNK activation in mutant conditions, suggesting that MAPKBP1 might play a specific function in DDR independent of JNK.

In conclusion, the present study revealed biallelic mutations in *MAPKBP1* in eight affected individuals from five unrelated families presenting with juvenile or late-onset NPH (from 12 to  $>27$  years). We therefore propose *NPHP20* (MIM: 617271) as an alias for *MAPKBP1*. Hypomorphic mutations in ciliary *NPHP* genes have been involved in late-onset NPH including *NPHP3*,<sup>37</sup> *WDR19*,<sup>38</sup> *TTC21B*,<sup>39</sup> and *NPHP5*,<sup>40</sup> whereas truncating or loss-of-function mutations of these genes were associated with early-onset NPH or even more severe cystic kidney disease or syndromic forms of ciliopathies<sup>41–43</sup> in agreement with their key functions at cilia. Affected individuals described here did not share additional manifestations classically observed in ciliopathies, in agreement with the fact that MAPKBP1 is not present at cilia and does not seem to be involved in ciliogenesis. Scoliosis and/or facial dysmorphism were found in several but not all of the affected individuals; however, at this point, we

cannot correlate mutations in *MAPKBP1* with these manifestations that might be linked to other genetic causes in these consanguineous families. Interestingly, MAPKBP1 does not appear to be involved in cilia function in vitro and none of the generated animal models developed ciliopathy-like phenotypes. It rather seems that *MAPKBP1* is likely the first member of a non-ciliary gene family for NPH that may account for non-syndromic forms of NPH for which causative mutated genes remain largely to be identified.

#### Accession Numbers

The phenotype presented in this paper has been named *NPHP20* with the MIM accession number 61721.

#### Supplemental Data

Supplemental Data include 17 figures and 2 tables and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2016.12.011>.

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## Web Resources

Cn3D, <http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml>  
COILS, [http://www.ch.embnet.org/software/COILS\\_form.html](http://www.ch.embnet.org/software/COILS_form.html)  
Ensembl Genome Browser, <http://www.ensembl.org/index.html>  
ESEfinder3.0, <http://krainer01.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home>  
GenBank, <http://www.ncbi.nlm.nih.gov/genbank/>  
Infrafrontier, <https://www.infrafrontier.eu/>  
International Mouse Phenotyping Consortium, <http://www.mousephenotype.org/data/genes/>  
Jalview, <http://www.jalview.org/>  
MutationTaster, <http://www.mutationtaster.org/>  
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, <http://evs.gs.washington.edu/EVS/>  
OMIM, <http://www.omim.org/>  
PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/>  
RefSeq, <http://www.ncbi.nlm.nih.gov/RefSeq>  
UCSC Genome Browser, <http://genome.ucsc.edu>

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