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Heterozygous missense mutations in *SMARCA2* cause Nicolaides-Baraitser syndrome

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Nicolaides-Baraitser syndrome (NBS) is characterized by sparse hair, distinctive facial morphology, distal-limb anomalies and intellectual disability. We sequenced the exomes of ten individuals with NBS and identified heterozygous variants in *SMARCA2* in eight of them. Extended molecular screening identified nonsynonymous *SMARCA2* mutations in 36 of 44 individuals with NBS; these mutations were confirmed to be *de novo* when parental samples were available. *SMARCA2* encodes the core catalytic unit of the SWI/SNF ATP-dependent chromatin remodeling complex that is involved in the regulation of gene transcription. The mutations cluster within sequences that encode ultra-conserved motifs in the catalytic ATPase region of the protein. These alterations likely do not impair SWI/SNF complex assembly but may be associated with disrupted ATPase activity. The identification of *SMARCA2* mutations in humans provides insight into the function of the Snf2 helicase family.

Nicolaides-Baraitser syndrome (NBS; MIM 601358) was first described in 1993 (ref. 1) but only recently has been well delineated[2]. Its main characteristics include sparse hair, typical facial morphology, short stature, microcephaly, brachydactyly, interphalangeal joint swellings, epilepsy and intellectual disability with marked language impairment (**Fig. 1**). This syndrome occurs in individuals from various ancestry groups without substantial differences in the frequency of its occurrence between the sexes. No familial cases are known, with the exception of one pair of concordant monozygotic twins, and no parental consanguinity has been reported[2], suggesting that NBS is caused by dominant *de novo* mutations. We collected DNA and clinical data from 22 of the 27 individuals previously described to have NBS and from 22 additional affected individuals (cases). Subjects were classified into two categories comprising 37 and 7 individuals that had high and low certainty of NBS diagnosis, respectively (**Fig. 1**, **Table 1** and **Supplementary Table 1**). Neither karyotype analysis in 36 cases nor microarray analysis in 28 cases revealed any notable abnormalities.

Initially, we sequenced the exomes of four individuals with a clinical diagnosis of NBS (NBS01–NBS04). Following targeted exome enrichment, we obtained 5.1–6.7 Gb of sequence data per individual by massively parallel sequencing. The mean exome coverage was 40-fold, with 80% of the exome covered at least 10 times. We focused on nonsynonymous variants, splice acceptor or donor site mutations and coding insertions and/or deletions (indels). Given the probable dominant, *de novo* nature of the disorder, we filtered out previously described SNPs using the dbSNP132 and 1000 Genomes Project databases and over 311 unpublished exomes. We selected genes in which at least three of the four affected individuals carried single previously undescribed nonsynonymous variants at different genomic positions, strengthening the likelihood that these variants were causative. Five genes were affected by distinct missense variants in three unrelated cases. To gather additional evidence, we performed exome sequencing in six additional individuals with NBS (NBS05–NBS10). *SMARCA2* (encoding SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 2) was the only gene with previously undescribed nonsynonymous mutations present in eight of the ten affected individuals. The potential impact of the encoded amino-acid substitutions on protein structure and function was predicted to be damaging in all eight cases (**Table 2**). Sanger sequencing validated the heterozygous state of all detected variants and confirmed *de novo* origin in four individuals for whom parental samples were available.

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Figure 1 Photographs of four individuals with NBS in whom *SMARCA2* mutations were identified. (**a**,**b**) NBS08 (female), the first reported individual with NBS[1], at 12 years (**a**) and 32 years (**b**). (**c**,**d**) NBS33 (male) at 9 years and 9 months (**c**) and 10 years and 11 months (**d**). (**e**–**g**) NBS21 (male) at 1 year (**e**), 1 year and 9 months (**f**) and 5 years and 3 months (**g**). (**h**–**l**) NBS13 (female) at birth (**h**), 1 year (**i**), 4 years (**j**), 14 years (**k**) and 21 years (**l**). Note the clinical variability and changing phenotype with age, with progressive facial coarsening. NBS13 shows many but not all classical features: note that sparseness of hair was present to a mild degree at a young age and was later no longer visible. We obtained written informed consent to publish clinical photographs from these individuals.

SMARCA2 is located on chromosome 9p24.3 and consists of 34 exons. All eight mutations were located in the region of the gene encoding the ATPase domain (exons 15–25; 490 amino acids; **Fig. 2**), which is 100% conserved in chimpanzee and mouse and 94.7% conserved in zebrafish compared to the human protein (**Supplementary Fig. 1**). We performed Sanger sequencing of exons 15 to 25 in 34 additional affected individuals and identified 27 nonsynonymous mutations and one splice-site mutation 2 bp downstream of exon 24 (**Fig. 2b** and **Table 2**). None of these mutations was present in over 1,300 exomes of unaffected individuals (**Supplementary Table 2**). Taking these results together, we identified nonsynonymous *SMARCA2* mutations in a total of 36 of the 44 individuals studied. In each of the 15 individuals for whom DNA from both parents was available, the *SMARCA2* variant was confirmed to be *de novo*. The splice-site variant could only be assessed as absent in a single parent. The 36 exonic mutations were clustered in exons 15 (n = 4), 18 (n = 11), 19 (n = 3), 24 (n = 4) and 25 (n = 14). We sequenced all remaining exons in mutation-negative cases, but the variants we detected in these individuals were also observed in the control exome group (**Supplementary Table 2**) and were therefore considered not to be causal. We could not distinguish any specific phenotypic differences between individuals with the different mutations, nor could we identify substantive specific differences between the mutation-positive and mutation-negative cases. Nevertheless, the overall subjective certainty of the clinical diagnosis was shown to be important, as mutations were found in 34 of 37 individuals with certain diagnosis and in only 2 of 7 individuals with uncertain diagnosis (**Table 1**).

SMARCA2 (previously known as BRM) is classified in the Snf2 family of helicase-related proteins that are characterized by the presence of the conserved Snf2 family ATPase domain[3] (**Fig. 2**). These nuclear ATPases are the core enzymatic subunits of chromatin remodeling protein complexes. SMARCA2 is the catalytic subunit of the main human BRM-associated factors (BAF) complex, with BAF belonging to the SWI/SNF family of ATPase-dependent chromatin remodelers that regulate gene expression, differentiation and development[4]. SWI/SNF proteins mediate gene expression by repositioning or removing nucleosomes, thereby making DNA more accessible to transcription factors and key cellular proteins, facilitating both the induction and repression of genes[5]. In mammals, SWI/SNF family complexes are very diverse in subunit composition and, hence, are present in multiple forms. BAF is highly conserved in eukaryotes and is composed of multiple subunits, including either SMARCA2 or SMARCA4 (previously known as BRG1) as the central ATPase subunit. This subunit diversity suggests that different complexes may have tissue- and stage-specific roles during development and cell differentiation. The unique composition of BAF correlates grossly with the specific gene expression required for maintaining cell state. Although the exact mechanistic basis for this maintenance is unknown, exchange of SMARCA4 for the SMARCA2 subunit helps to drive the transition from pluripotency to multipotency[4,6]. Somatic loss of both *SMARCA2* and *SMARCA4* has been reported in 15–25% of malignant cell lines and solid tumors, suggesting that they act as tumor suppressor genes[5,7].

We evaluated the correlation of *SMARCA2* expression with some of the major NBS features (intellectual disability, microcephaly and hypotrichosis) by performing immunohistochemical studies of Smarca2 expression in the developing cerebral cortex and hair follicles of mouse embryos, using two independent antibodies to Smarca2 on paraffin-embedded sections. At embryonic day (E) 14.5 and E15.5, Smarca2 levels were high in the post-mitotic neurons of the cortical plate (**Supplementary Fig. 2a**–**c**) and were generally low in the progenitor regions (ventricular and subventricular zones). Consistently, high levels of *Smarca2* gene expression have been reported in the cortical plate (GenePaint) at E14.5 (**Supplementary Fig. 2d**). There was a

Table 1 Summary of clinical findings in individuals with NBS examined in this study

Clinical features	Cases with nonsynonymous *SMARCA2* mutation (n = 36)	Cases without nonsynonymous *SMARCA2* mutation (n = 8)
Clinical diagnosis of NBS, reliable	34/36	3/8
Clinical diagnosis of NBS, possible	2/36	5/8
Prenatal growth retardation	10/34	2/7
Postnatal growth retardation	19/36	4/8
Intellectual disability (mild-moderate-severe/total)	3-9-24/36	0-1-7/8
Seizures	22/35	2/8
Microcephaly	19/35	3/8
Sparse hair	35/36	7/8
Increased skin wrinkling	18/36	2/8
Thick, anteverted alae nasi	32/36	6/8
Broad philtrum	31/36	8/8
Long philtrum	29/36	6/8
Large mouth	34/36	7/8
Thin upper vermillion	27/36	5/8
Thick lower vermillion	32/36	8/8
Prominent interphalangeal joints	28/35	4/8
Prominent distal phalanges	21/35	5/8
Short metacarpals and/or metatarsals	16/32	3/6

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decrease in expression around E16.5 (**Supplementary Fig. 2e**,**f**), after which we found neurons that were intensely stained for Smarca2 in an area corresponding to layer 5 (**Supplementary Fig. 2g**–**i**). In the adult cortex and hippocampus, Smarca2-positive cells resided in the cortex and hippocampus (**Supplementary Fig. 2j**). Cells within the epithelium of developing early hair follicles produced Smarca2 (**Supplementary Fig. 2k**,**l**), suggesting a possible function in hair follicle development. These data show that Smarca2 is expressed at sites consistent with the phenotypes observed in individuals with NBS[2].

Considering that deletions encompassing human *SMARCA2* do not cause NBS[8], that mice lacking functional Smarca2 do not present major developmental abnormalities[6,9] and that none of the variants we identified were truncating, we predict that the mutations identified in NBS act in a dominant-negative or gain-of-function manner. All nonsynonymous *SMARCA2* mutations altered ultra-conserved amino acids (**Fig. 2b** and **Table 2**), including seven helicase-related sequence motifs that define the SNF2 domain: motifs I, II, III and VI and the SNF2 domain D motif (**Fig. 2**). Motifs I, II and III have been associated with nucleotide binding and motif D with DNA interaction and ATP hydrolysis[3]. In addition, seven mutations were localized to motifs that were not previously known to be highly conserved. The SNF2 domain is identical in both SMARCA4 and SMARCA2

Table 2 Annotation of *SMARCA2* mutations identified in individuals with NBS

					Confirmed *de novo*[b]				Predictions[d]			
Subject	Group	Exome sequenced	Mutation[a]	Exon	Mother	Father	Amino-acid change	Chr. 9 position[c]	S	L	M	P
NBS01	1	+	c.3637C>T	25	–	–	p.Arg1213Trp	2116002	D	D	D	D
NBS02	1	+	c.3604G>T	25	+	+	p.Gly1202Cys	2115969	D	D	D	P
NBS03	2	+										
NBS04	1	+	c.3476G>A	25	+	+	p.Arg1159Gln	2115841	D	D	D	P
NBS05	1	+										
NBS06	1	+	c.3473A>T	25	+	+	p.Asp1158Val	2115838	D	D	D	D
NBS07	1	+	c.3475C>G	25	+	+	p.Arg1159Gly	2115840	D	D	D	P
NBS08	1	+	c.2642G>T	18	+	–	p.Gly881Val	2086944	D	D	D	–
NBS09	1	+	c.3485G>A	25	+	+	p.Arg1162His	2115850	D	D	D	P
NBS10	1	+	c.3476G>T	25	–	–	p.Arg1159Leu	2115841	D	D	D	P
NBS11	2	–										
NBS12	1	–	c.2648C>T	18	+	+	p.Pro883Leu	2086950	D	D	D	–
NBS13	1	–	c.3476G>A	25	+	+	p.Arg1159Gln	2115841	D	D	D	P
NBS14	1	–	c.3602C>T	25	+	+	p.Ala1201Val	2115967	D	D	D	B
NBS15	1	–	c.2815C>T	19	+	+	p.His939Tyr	2088545	D	D	D	D
NBS16	2	–	c.2267C>T	15	–	–	p.Thr756Ile	2081914	D	D	D	P
NBS17	2	–	c.3456+2T>G	Intron[f]	+	–		2110419				
NBS18	1	–	c.3313C>T	24	+	+	p.Arg1105Cys	2110274	D	D	D	D
NBS19	1	–	c.2556A>C	18	+	–	p.Glu852Asp	2086858	D	D	D	P
NBS20	1	–	c.2641G>C	18	–	–	p.Gly881Arg	2086943	D	D	D	–
NBS21	1	–	c.3404T>C	24	+	–	p.Leu1135Pro	2110365	D	D	D	P
NBS22	1	–	c.3562G>C	25	+	–	p.Ala1188Pro	2115927	D	D	D	B
NBS23	1	–	c.3314G>C	24	–	–	p.Arg1105Pro	2110275	D	D	D	P
NBS24[e]	1	–	c.2255G>C	15	–	–	p.Gly752Ala	2081902	D	D	D	P
NBS25[e]	1	–	c.2255G>C	15	–	–	p.Gly752Ala	2081902	D	D	D	P
NBS26	1	–	c.2648C>T	18	–	–	p.Pro883Leu	2086950	D	D	D	–
NBS27	1	–	c.2554G>A	18	–	+	p.Glu852Lys	2086856	D	D	D	P
NBS28	1	–	c.2560C>A c.2562C>A	18	–	–	p.His854Leu	2086862	D	D	D	D
NBS29	1	–	c.2648C>T	18	–	–	p.Pro883Leu	2086950	D	D	D	–
NBS30	1	–	c.2837T>C	19	–	–	p.Leu946Ser	2088567	D	D	D	B
NBS31	1	no	c.3614A>G	25	–	–	p.Asp1205Gly	2115979	D	D	D	P
NBS32	1	no										
NBS33	1	no	c.2561A>G	18	+	+	p.His854Arg	2086863	D	D	D	D
NBS34	1	no	c.3602C>T	25	–	–	p.Ala1201Val	2115967	D	D	D	B
NBS35	1	no	c.2264A>G	15	+	+	p.Lys755Arg	2081911	D	D	D	P
NBS36	2	no										
NBS37	1	no	c.3436A>C	24	–	–	p.Ser1146Arg	2110397	D	D	D	P
NBS38	1	no			–	–						
NBS39	1	no	c.2838A>T	19	–	–	p.Leu946Phe	2088568	D	D	D	B
NBS40	2	no										
NBS41	2	no	c.3485G>A	25	+	–	p.Arg1162His	2115850	D	D	D	P
NBS42	1	no	c.3602C>T	25	+	+	p.Ala1201Val	2115967	D	D	D	B
NBS43	1	no	c.2551G>C	18	+	+	p.Asp851His	2086853	D	D	D	P
NBS44	1	no	c.2563C>G	18	+	+	p.Arg855Gly	2086865	D	D	D	P

[a]Mutation numbering is based on NM_003070. [b]For parental samples: +, confirmed not to be present in parental samples; –, no parental sample available. [c]Chromosomal positions based on build hg19 (Feb. 2009, UCSC Genome Browser). [d]Functional predictions retrieved from dbNSFP[24] (S, Sift; L, LRT; M, MutationTaster; P, Polyphen2; D, probably damaging; P, possibly damaging; B, benign; –, no prediction). [e]Monozygotic twins. [f]Mutation in potential splice donor (GT) of intron 24.

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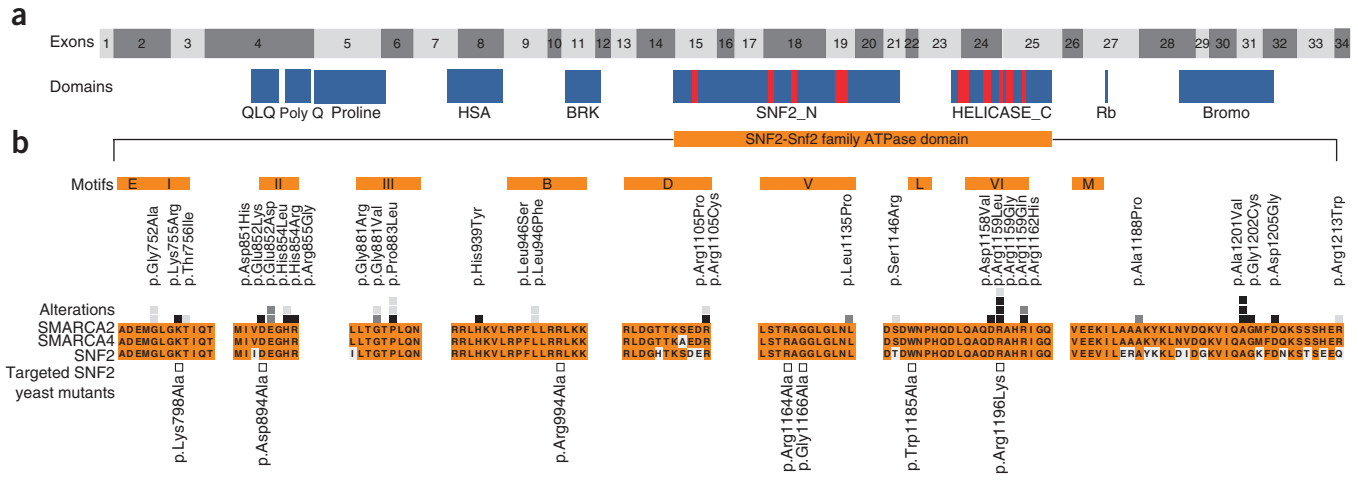

Figure 2 Exons, protein domains, conserved motifs and mutation spectrum for *SMARCA2*. (**a**) *SMARCA2* is composed of 34 exons that encode 9 conserved domains (blue)[7]. QLQ, glutamine-leucine-glutamine domain; proline, proline-rich domain; HSA, small helicase/SANT-associated domain; BRK, brahma and kismet domain; SNF2_N and HELICASE_C, DNA-dependent ATPase domains. The Snf2 family is characterized by seven helicase-related sequence motifs defining the ATPase domain. (**b**) Amino-acid alignment for the mutated conserved motifs in the catalytic ATPase domain for human SMARCA2 and SMARCA4 and yeast Snf2, showing the conserved structural motifs in Snf2 subfamilies[3]. The amino-acid substitutions in individuals with NBS are indicated with rectangles above the alignment (black, confirmed *de novo*; dark gray, mutation is absent in one available parent; light gray, no parental DNA available). Targeted alterations in yeast used for functional assays[11] are indicated by white rectangles below the alignment.

and is highly conserved in yeast SNF2. In addition, a chimera of yeast SNF2 and human SMARCA4 with the DNA-dependent helicase domain of SNF2 replaced by the corresponding human sequence restored normal mitotic growth and capacity for transcriptional coactivation in *snf2*-deficient yeast cells, proving functional conservation of this domain[10]. Mutations of yeast *snf2* affecting conserved motifs resulted in dominant-negative activity in a functional assay[11], and two of these mutations are identical to *SMARCA2* mutations in individuals with NBS, while four map to the same motifs (**Fig. 2**). This clustering of mutations provides genetic evidence that abolishing the ATP hydrolyzing engine, which provides energy directed toward the repositioning of histones on DNA, causes functional inactivation[12,13]. Crystal structures of helicases have shown that all seven conserved motifs within the SNF2 domain lie in close proximity to the nucleotide-binding site and are thus probably involved in nucleotide binding and ATP hydrolysis[14,15]. Structural and mutagenesis studies have shown that each of the conserved motifs has a role in the transformation of chemical energy from ATP hydrolysis to mechanical motion. These data, together with the mutations identified in individuals with NBS, support a model in which dysfunctional but structurally undamaged SMARCA2 generates a BAF complex that is intact with respect to its composition and interacts properly with chromatin but is nonetheless functionally inactive, resulting in dominant-negative effects.

The identification of clustered mutations in a large number of individuals with NBS substantiates the notion that NBS is a distinct clinical entity. In addition, the collection of 44 individuals with NBS in a short time span since the syndrome's delineation shows that NBS is more common than was originally anticipated. Mutations in *SMARCA2* add it to a growing list of chromatin remodeling genes, including *SMARCAL1* (causing Schimke immunoosseous dysplasia (SIOD); MIM 242900)[16] and *CHD7* (causing CHARGE syndrome; MIM 214800)[4,5,17], that cause developmental abnormalities variably associated with intellectual disability. The CHD7 protein was recently shown to interact with SMARCA2 in human neural crest-like cells[18]. We anticipate that mutations in genes encoding other helicase family members and BAF complex proteins will be identified as the cause of developmental disorders.

URLs. GenePaint, http://www.genepaint.org/, Annotate-it!, http://www.annotate-it.org/; Picard, http://picard.sourceforge.net/; GoNL, http://www.dutchgenomeproject.com/; BGI, http://en.genomics.cn/navigation/index.action; Big Grid, http://www.biggrid.nl/.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Accession codes. Exome sequencing data are available upon request to J.R.V. Exome variant data are available at the Annotate-it! website (see URLs).

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

J.R.V., K.D., R.H. and S.B.S. designed the experiments. J.K.J.V.H., A.S., Y.M., A.H.C.v.K. and B.D.C.v.S. performed bioinformatic analyses. S.B.S. and R.H. collected the study subjects. B.A.N. performed the sequencing. E.S. and D. Hu performed immunostaining. B.A.N., N.A. and P.v.D. performed functional studies.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Subjects. Subjects were recruited through genetics centers in Europe, Canada and the United States. Informed consent was obtained from all subjects, and the study was approved by the appropriate Institutional Review Boards involved in this investigation. The diagnosis of NBS was based on the typical morphology of face and limbs, radiographic data, growth pattern and cognitive development as published[2] (**Supplementary Table 1**). Affected individuals were classified into high- and lower-certainty groups by consensus among three clinical geneticists (R.H., K.D. and S.B.S.). The parental status was tested with the Profiler plus kit from Applied Biosciences.

Targeted enrichment and exome sequencing. Genomic DNA was sheared by sonication, platform-specific adaptors were ligated, and the resulting fragments were size selected. The libraries from subjects NBS01–NBS04 were captured using the SureSelect All Exon Target Enrichment System (Agilent, Human All Exon Kit, 38Mb) according to the manufacturer's protocols. The enriched libraries underwent paired-end (2 × 76 bp) sequencing on a Genome Analyzer IIx (Illumina). NBS05 and NBS06 libraries were enriched with the SeqCap EZ Human Exome Library v2.0 (Roche, NimbleGen), and 2 × 76-bp paired-end reads were generated on a HiSeq2000 (Illumina). The NimbleGen exome capture array (version 1) was used to produce the enriched libraries for NBS07–NBS10 that were sequenced on the Applied Biosystems SOLiD system.

Mapping and variant analyses. The paired-end sequence reads of NBS01–NBS06 were aligned to the human genome (hg19) with the Burrows-Wheeler Aligner (BWA; version 0.5.8a)[19] using default settings, and the read trimming parameter was set to 15. SAMtools (version 0.1.8)[20] was used for converting (SAM/BAM), sorting and indexing alignments. The quality metrics for mapping were calculated with Picard tools (version 1.38). Duplicate reads were marked with Picard tools and excluded from downstream analysis. The GATK framework (version 1.0.4974)[21] was used for performing the local realignment, base call recalibration and SNP calling. Variants with at least Q10 confidence were reported, but, if the confidence was less than Q50, it was reported as LowQual. Indels were called with Dindel (version 1.01)[22] using default parameters. Variants were annotated with ANNOVAR (version 2011)[23] and filtered against known variants by comparison to dbSNP131 and 1000 Genomes SNP data (release November 2010). The sequence reads for NBS07–NBS10 were aligned with BWA (version 0.5.7) to the reference genome (hg18) with default settings. SNPs and indels were called with SAMtools pileup (version 0.1.7) and Varscan (version 2.2), and annotated with ANNOVAR. Variants with an average base quality above 35, SIFT score below 0.05 and variant frequency above 15 were kept for further analysis. Variants that occurred in segmental, duplicated regions or were non-conserved (mce 44), synonymous or known (in dbSNP132 or 1000 Genomes, version July 2010) were excluded from the analysis.

Searches for known variants were performed on 1000 Genomes SNP data (release November 2010), 223 individuals from the Genome of The Netherlands Project (GoNL) and 86 in-house sequenced exomes). In the GoNL Project, individuals (father-mother-child) were sequenced at the Beijing Genomics Institute (BGI) with an average sequence depth of >12×. The GoNL variants for all genes in all individuals were annotated with Annovar[23] on the Dutch grid (Big Grid) using the e-BioInfra framework[24]. The *SMARCA2* variants were extracted from this data set to verify whether the mutations found in NBS were previously unknown. In total, we found ten *SMARCA2* variants in the GoNL data set, of which eight were known from the public databases and two were new (**Supplementary Table 2**). None of the NBS-related mutations were present in the GoNL data set.

Variant confirmation. Primers were designed using Primer3 software. PCR products were purified with ExoSAP-IT (GE Healthcare) and sequenced using BigDye Terminator v3.1 chemistry (Life Technologies) on a 3730 DNA Analyzer (Life Technologies). Sequence traces were aligned to the *SMARCA2* reference sequence using SeqScape software (v2.6) (Life Technologies). Functional predictions for the amino-acid changes according to different models (SIFT, Polyphen2, LRT and MutationTaster) were retrieved from dbNSFP (database for predictions for nonsynonymous SNPs; **Table 2**)[25].

Immunohistochemistry and microscopy. Timed pregnant Swiss females (with E0.5 being the morning of the day of vaginal plug) were dissected and mouse embryos (E14.5) or brains (E15.5–E16.5) were collected and washed in ice-cold PBS, fixed overnight with 4% paraformaldehyde and submitted to progressive alcohol-assisted dehydration and paraffin embedding. Frontal (E15.5 and E16.5) or sagittal (E14.5) 6-µm thick sections were processed for immunohistochemistry using an automated platform (Ventana Discovery, Ventana Medical Systems; details of procedures can be obtained on request) and were dehydrated and mounted with Eukitt (Sigma). Studies were performed using two different primary rabbit antibodies to Smarca2, one from Abcam (ab15597; 1:60 dilution) and one from Sigma (HPA029981; 1:300 dilution). Photographs were taken on a Leica DM5500B microscope.

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