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Supplemental Data

Mutations in *ABHD12* Cause the Neurodegenerative Disease PHARC: An Inborn Error of Endocannabinoid Metabolism

Torunn Fiskerstrand, Dorra H'mida-Ben Brahim, Stefan Johansson, Abderrahim M'zahem, Bjørn Ivar Haukanes, Nathalie Drouot, Julian Zimmermann, Andrew J. Cole, Christian Vedeler, Cecilie Bredrup, Mirna Assoum, Meriem Tazir, Thomas Klockgether, Abdelmadjid Hamri, Vidar M. Steen, Helge Boman, Laurence A. Bindoff, Michel Koenig, and Per M. Knappskog

Figure S1.

Areas of homozygosity/haplotype sharing on chromosome 20 in individuals with PHARC disease from 11 families

Panel showing regions of homozygosity pericentric on chromosome 20 in one individual from each of the 11 families with PHARC disease from four countries. One exception is family 1, where haplotypes for the third cousins 1.2 and 1.3 are shown. In families with more than one affected sib (1, 2, 6, 8, 9 and 10), only one affected is shown, as they shared identical haplotypes beyond the region of interest. Family 1 has been described earlier (Fiskerstrand T, et al. *Neurology*. 2009 Jan 6;72(1):20-7.). The *ABHD12* gene is found 25.2-25.3 Mb from pter (dashed line). The haplotype regions presented are created on the basis of information generated also from other family members (not shown). Unique haplotypes have been given the same colour. All the individuals in the Norwegian families are homozygous for parts of the same ancestral haplotype (red), and this is also found in the Algerian families (ancestral haplotype in yellow). The minimal common region of homozygosity (6.4 Mb) in the Norwegian families is delineated by the affected persons in family 2 (on 20p, 23,553,833 bp from pter) and in family 5 (on 20q, 29,936,849 bp from pter). This represents a genetic interval of 1.4cM sex-averaged (52.3 - 53.7 cM, Rutgers Map Interpolator v.2). The number of generations from present to the most recent common Norwegian ancestor may be estimated to around 70 (Génin E et al. *Ann Hum Genet*. 1998 Sep;62:419-29), thus this indel mutation is more than 1500 years old. In the Algerian families, the minimal common region of homozygosity (6.1 Mb) is delineated by the affected persons in family 8 (24,393,550 to 30,538,033 Mb from pter). In the Emirati family, the minimal linkage region ranged from 23,295,553 to 29,940,293 Mb from pter. One

healthy sister was homozygous for the region ranging from 29,940,293 -37,449,930 Mb from pter (shown in lighter blue colour) , thus limiting the linkage region in this family. All positions are given according to NCBI Build 36.3.

Figure S1

Figure S2.

Three deleterious mutations in the *ABHD12* gene.

The mutations found in the *ABHD12* gene in the Norwegian (exon 3), Algerian (exon 9) and American (exon 12) PHARC patients are shown in the lower panels, with respective wildtype sequences in upper panels. In the Norwegian family (left panels), a homozygous indel mutation in exon 3 in the *ABHD12* gene (c.337_338delGAinsTTT) was identified in all eight patients (GATTT is marked on the wildtype sequence). This frameshift mutation predicts the replacement of an asparagine (D) at codon 113 with phenylalanine (F) leading to a downstream premature stop codon (p.Asp113PhefsX15). The Algerian patients (middle panels) were homozygous for a 7 bp duplication in exon 9 (c.846_852dupTAAGAGC), which directly replaces the histidine (H) residue at codon 285 with a stop codon (p.His285fsX1). A patient of French-Canadian heritage from USA (right panels) was found to be homozygous for a nonsense mutation (c.1054C>T, marked by arrow) in exon 12, leading to a predicted stop codon in position 352 in the protein (p.Arg352X).

Figure S2

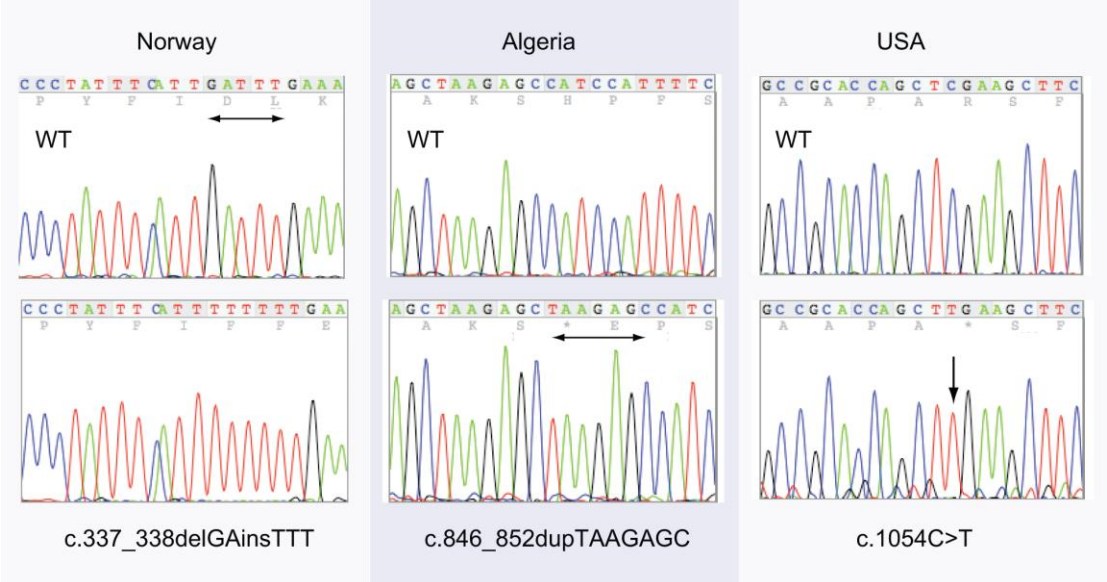
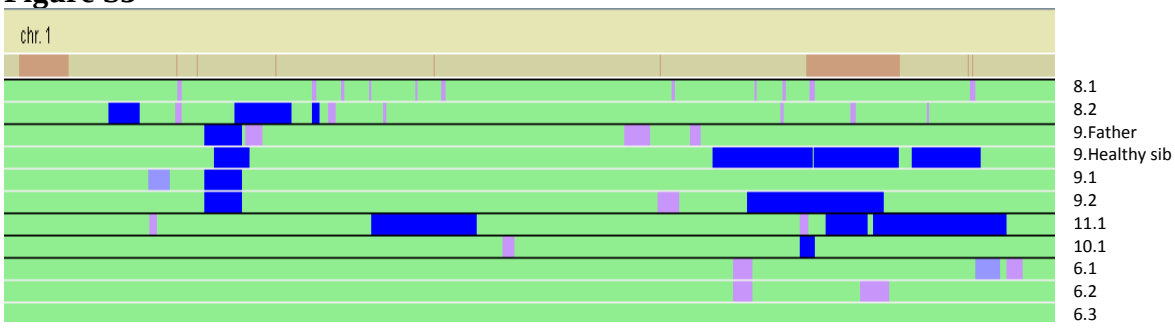


Figure S3.

Genotyping Results of the Genome-wide Scan in Families 6 and 8-11.

The graphic interface (HomoSNP software) reveals shared regions of homozygosity in the consanguineous families. Each panel represents the result of one chromosome indicated on the left of the top bar. Chromosome pter to qter orientation is represented from left to right. The green bars show individual results of the following patients (from the top): 8.1, 8.2, father in family 9, healthy brother in family 9, 9.1, 9.2, 11.1, 10.1, 6.1, 6.2, 6.3 , as indicated on the right in some panels. A white line separates two bars from individuals of the same family. A black line separates two bars from individuals of different families. The regions with more than 30 consecutive homozygous SNPs for family 8 (Genechip 50K), and with more than 25 consecutive homozygous SNPs for all other families (Genechip 10K), are in dark blue. Regions with 20 to 24 consecutive homozygous SNPs are represented in purple (Genechip 10K). All affected individuals share a region of homozygosity by descent on chromosome 20cen.

Figure S3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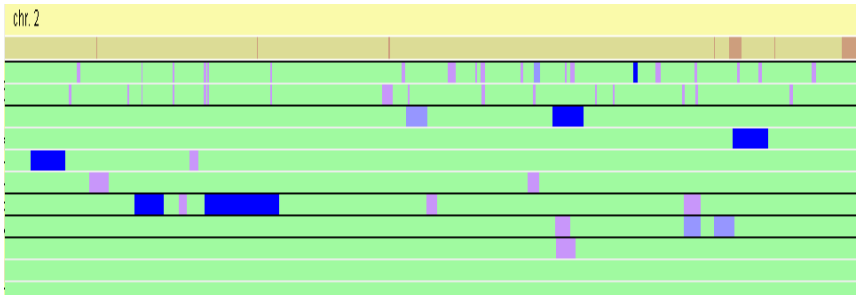
8.2
9.Father
9.Healthy sib
9.1
9.2
11.1
10.1
6.1
6.2
6.3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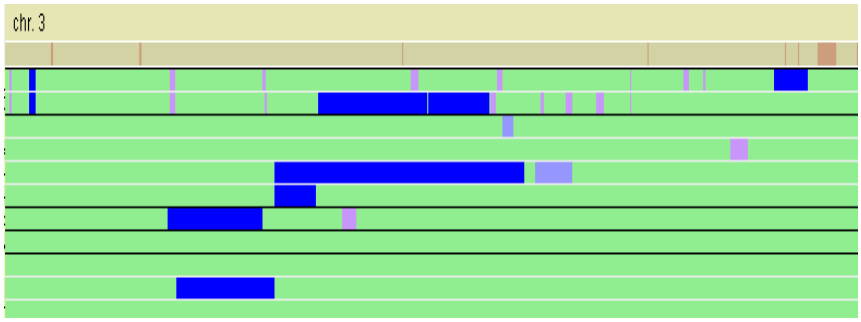
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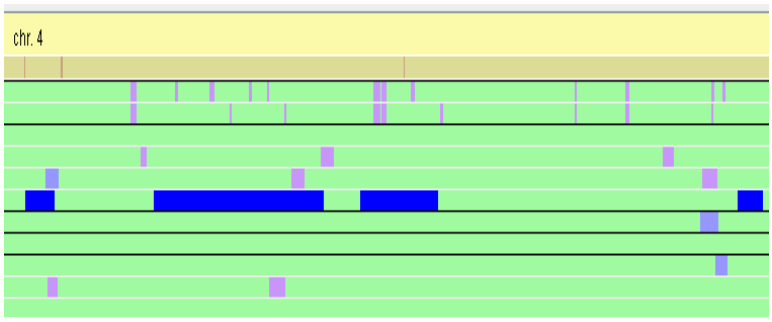
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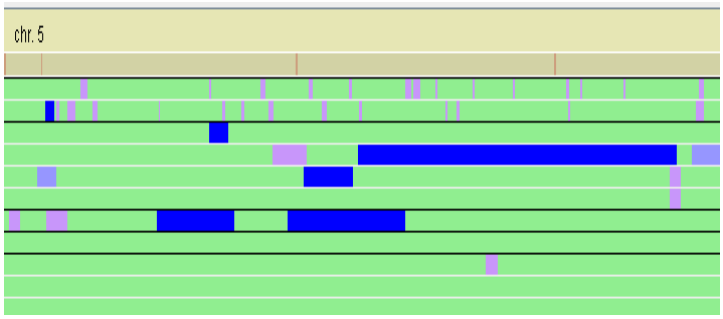
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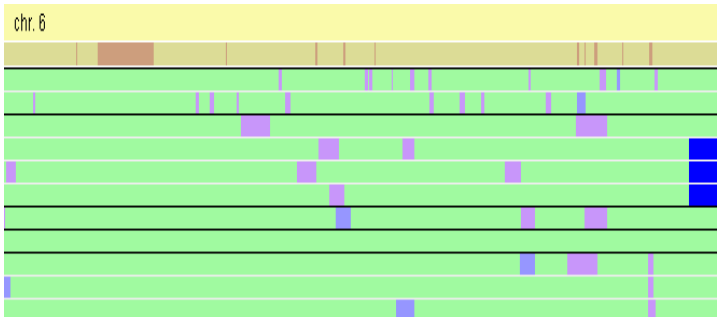
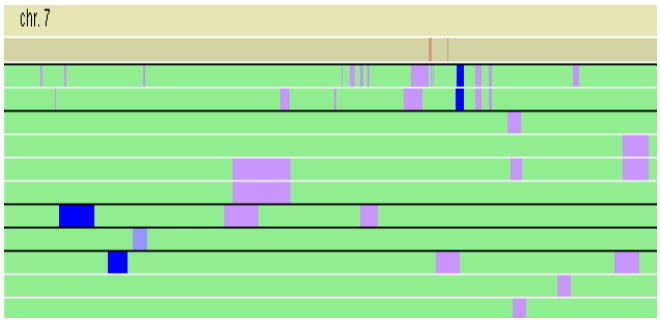
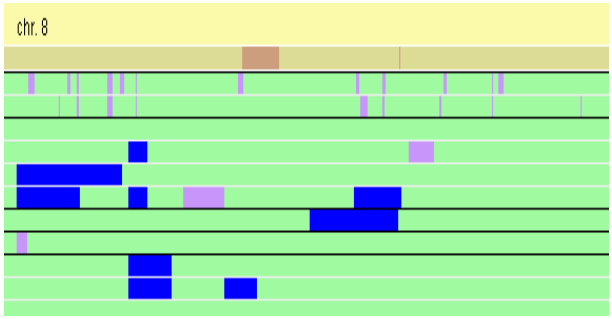
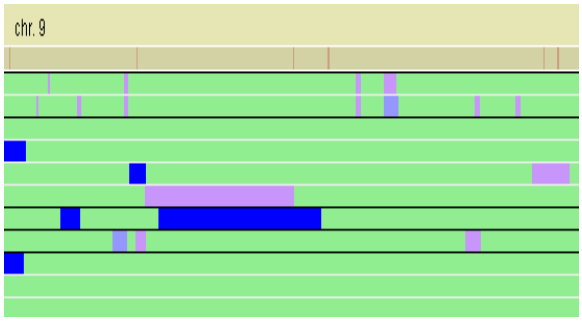
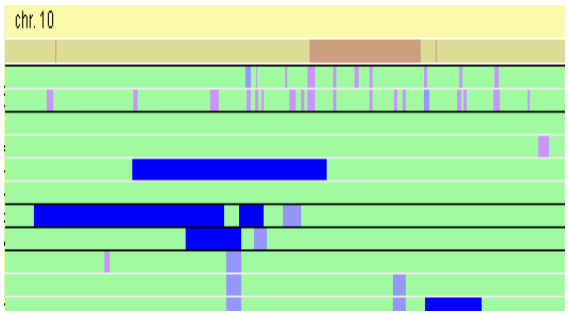


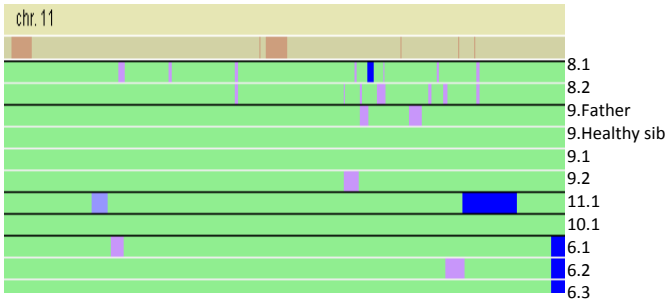
8.1
8.2
9.Father
9.Healthy sib
9.1
9.2
11.1
10.1
6.1
6.2
6.3

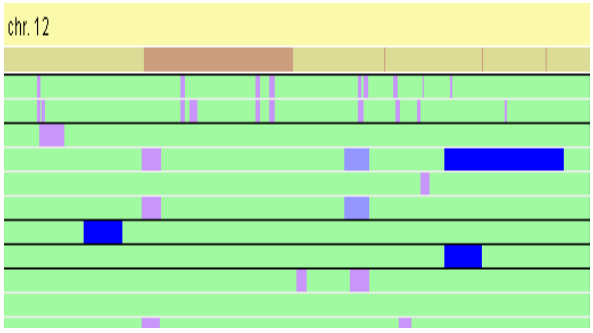









chr. 11
8.1
8.2
9.Father
9.Healthy sib
9.1
9.2
11.1
10.1
6.1
6.2
6.3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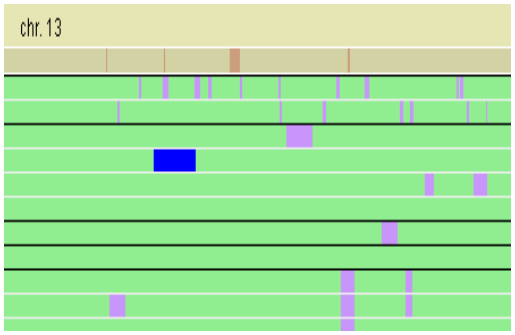
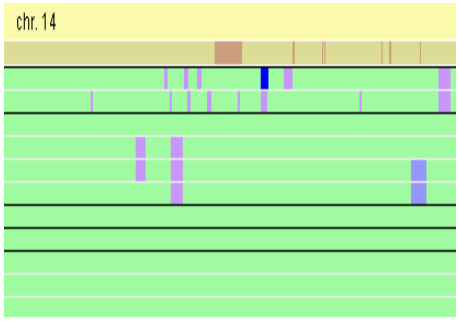

chr. 13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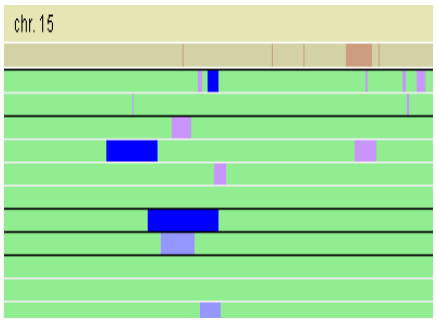
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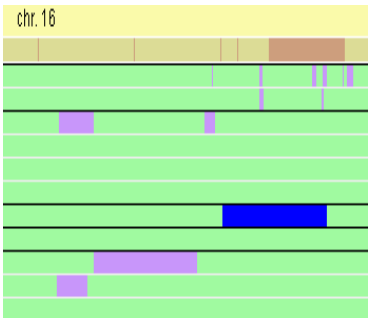
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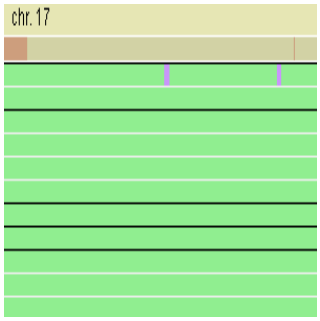
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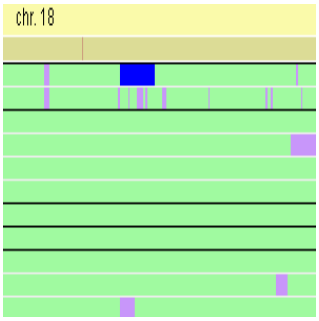
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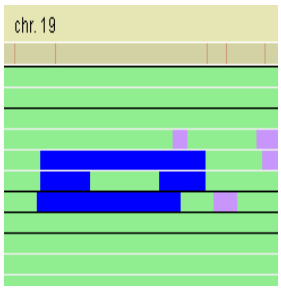
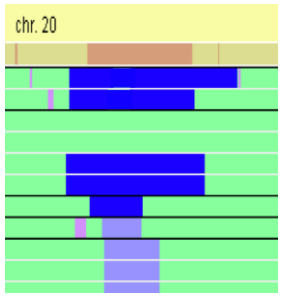
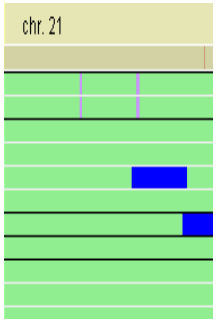




8.1
8.2
9.Father
9.Healthy sib
9.1
9.2
11.1
10.1
6.1
6.2
6.3



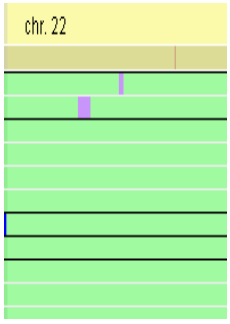


Figure S4.

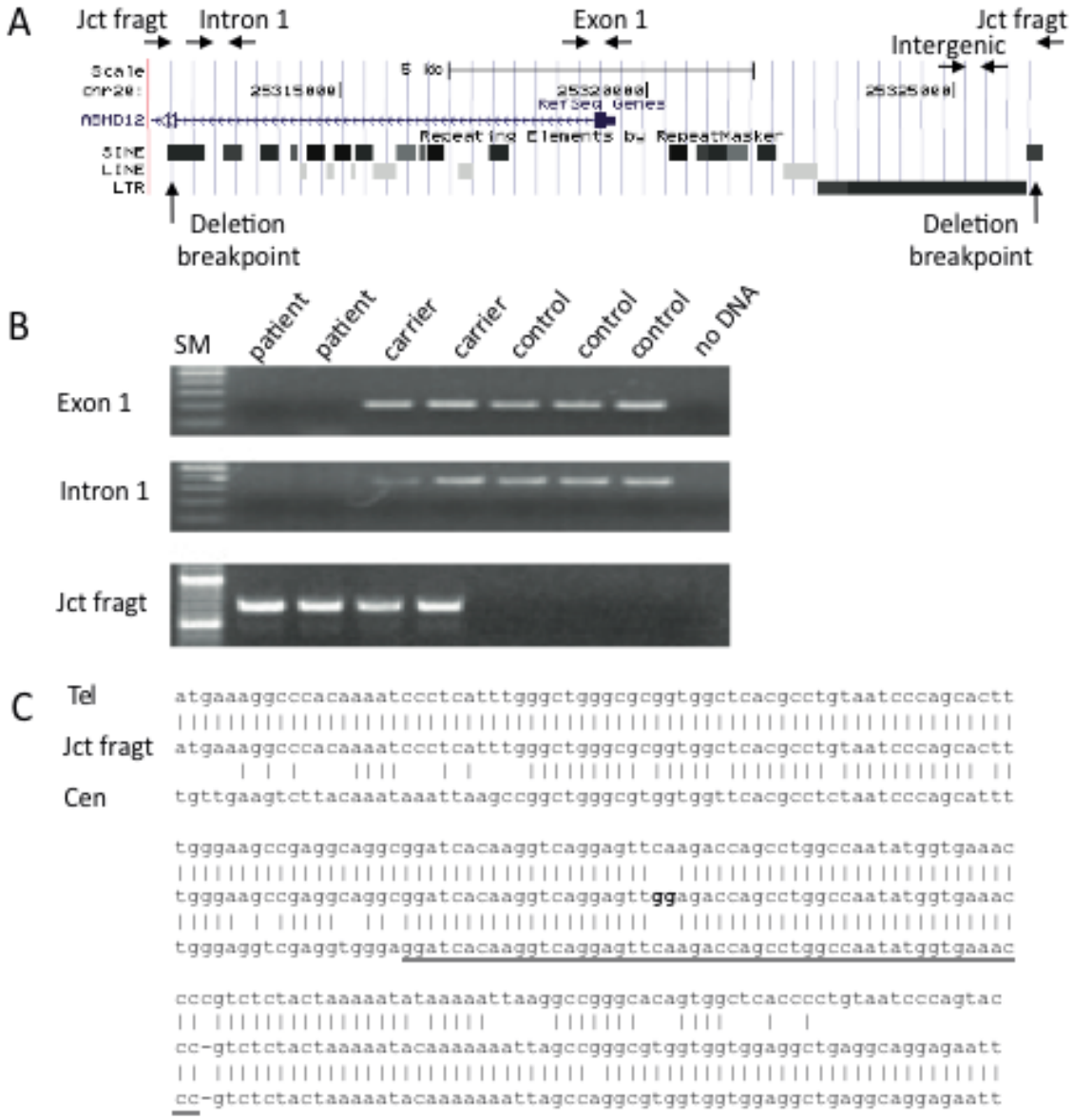
The 14 kb deletion in family 6.

A) Genomic organisation of the region deleted in family 6. The position of the primers used to identifiy and delineate the deletion by PCR are indicated at the top. The position of *ABHD12* exon 1 and of the surrounding repeated elements are shown in the middle (obtained from http://genome.ucsc.edu/). The position of the deletion breakpoints are indicated by vertical arrows at the bottom. They fall within Alu repeated elements (SINE).

B) Identification and delineation of the deletion involving exon 1 of *ABHD12* in family 6. Fragments containing exon 1 and portions of intron 1 of *ABHD12* failed to amplify from genomic DNA of patients 1 and 2, while they amplified from DNA of the healthy brother and sister (carriers) and from unrelated individuals (controls); similar results were obtained with *ABHD12-GINS1* intergenic sequences (not shown). The junction fragment (jct fragt, 700 bp, overlapping the deletion) amplified from genomic DNA of patients 6.1 and 6.2, and of the healthy carriers, but failed to amplify from the controls, as the normal fragment would be 15 kb in size. SM: size marker (size of exon 1 fragment : 200 bp, size of intron 1 fragment : 380 bp).

C) Sequence of the junction fragment overlapping the deletion and alignment with the non-deleted telomeric (tel) and centromeric (cen) sequences. The sequences highly similar between the tel and cen sequences correspond to Alu elements. The sequence that is identical between the telomeric and centromeric Alu elements is underlined. The breakpoints likely occurred at the two mismatching guanines (gg, in bold) which were probably inserted during the non-homologous recombination.

Figure S4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Intron 1
Exon 1
Jct fragt
Intergenic
Scale
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25320000
25325000
RefSeq Genes
ASHD12
Repeating Elements by RepeatMasker
SINE
LINE
LTR
Deletion breakpoint
Deletion breakpoint
B
SM
patient
patient
carrier
carrier
control
control
control
no DNA
Exon 1
Intron 1
Jct fragt
C
Tel
Jct fragt
Cen
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