The American Journal of Human Genetics Volume *88*

Supplemental Data

Loss of BRCC3 Deubiquitinating Enzyme Leads to Abnormal Angiogenesis and Is Associated with Syndromic Moyamoya

Snaigune Miskinyte, Matthew G. Butler, Dominique Hervé, Catherine Sarret, Marc Nicolino, Jacob D. Petralia, Francoise Bergametti, Minh Arnould, Van N. Pham, Aniket V. Gore, Konstantinos Spengos, Steven Gazal, France Woimant, Gary K. Steinberg, Brant M. Weinstein, and Elisabeth Tournier-Lasserve

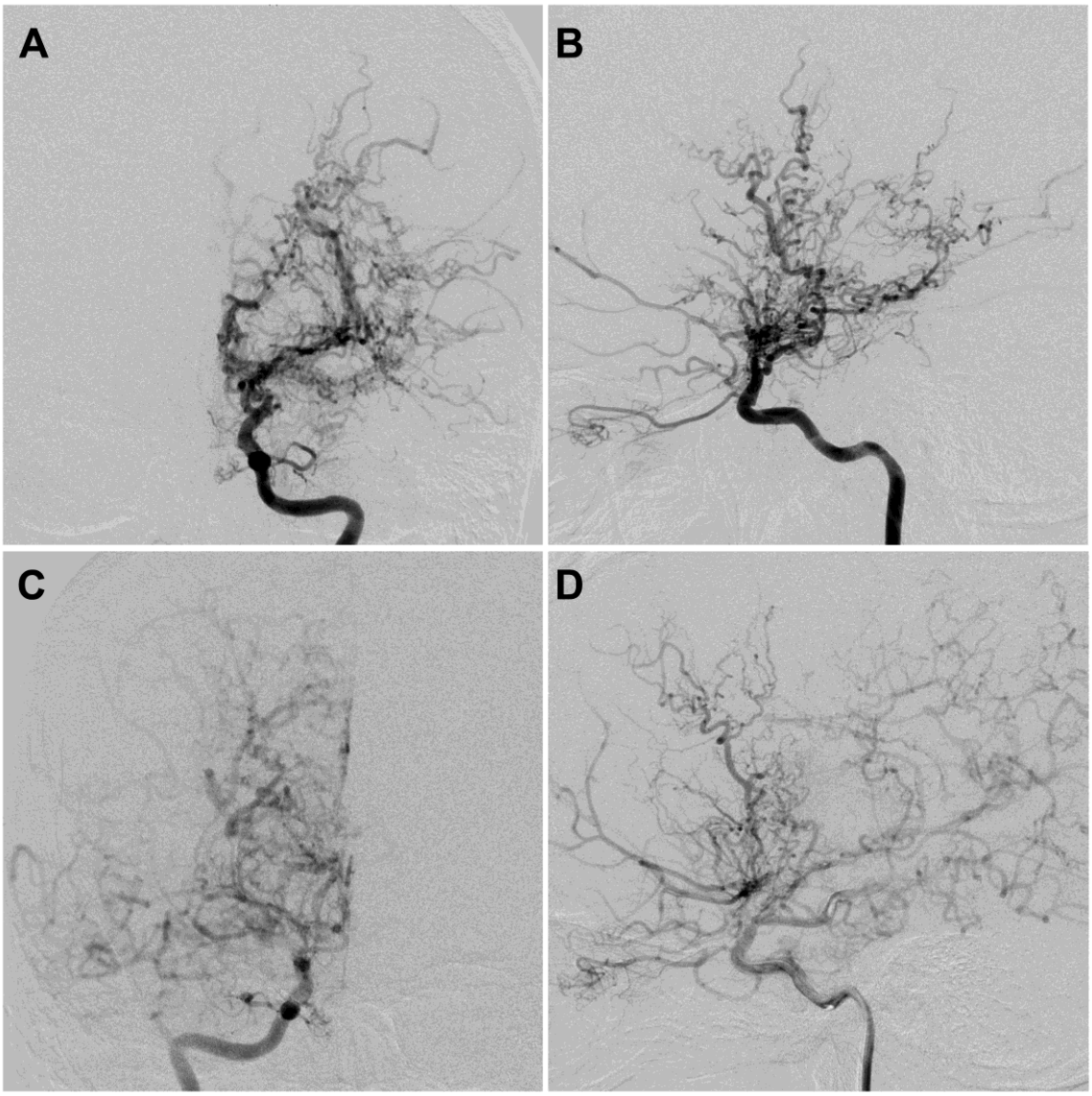


Figure S1: Moyamoya angiopathy in F3 III-7, aged 18 yo. AP/lateral left internal carotid artery (ICA) injections (A/B) and AP/ lateral right ICA injections (C/D) showing bilateral supraclinoid ICA occlusions and extensive Moyamoya collaterals.

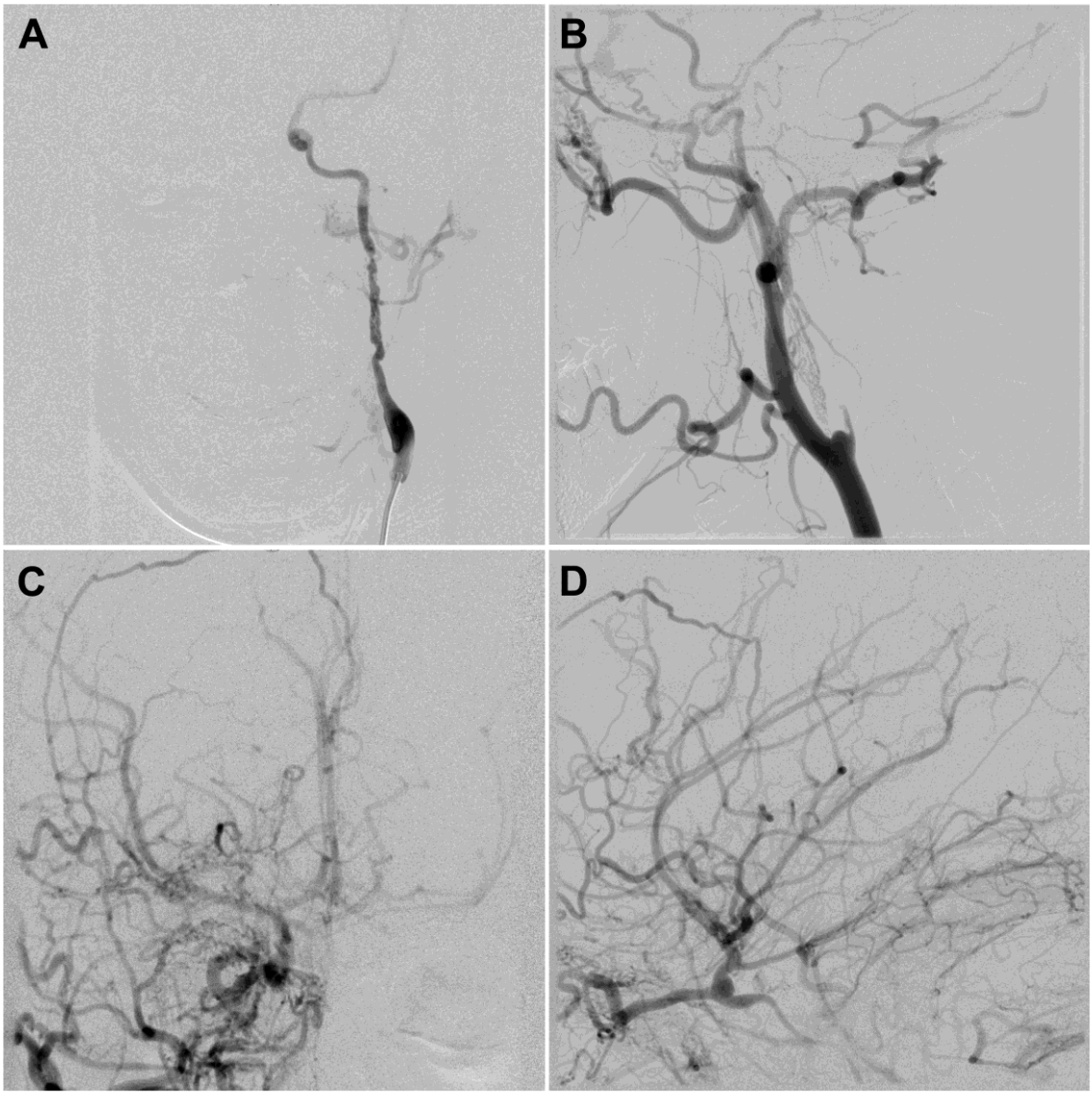


Figure S2: Cerebral angiography in F3 III-2, aged 21 yo. AP left internal carotid artery (ICA) injection (A) showing multiple areas of irregularity and narrowing suggesting prior occlusion with partial recannalization and occlusion of the left A1 anterior cerebral artery. Right ICA injection demonstrates occlusion of the cervical ICA (lateral, B) with reconstitution of the right cavernous ICA through multiple collaterals including moyamoya vessels (AP, C; lateral, D).

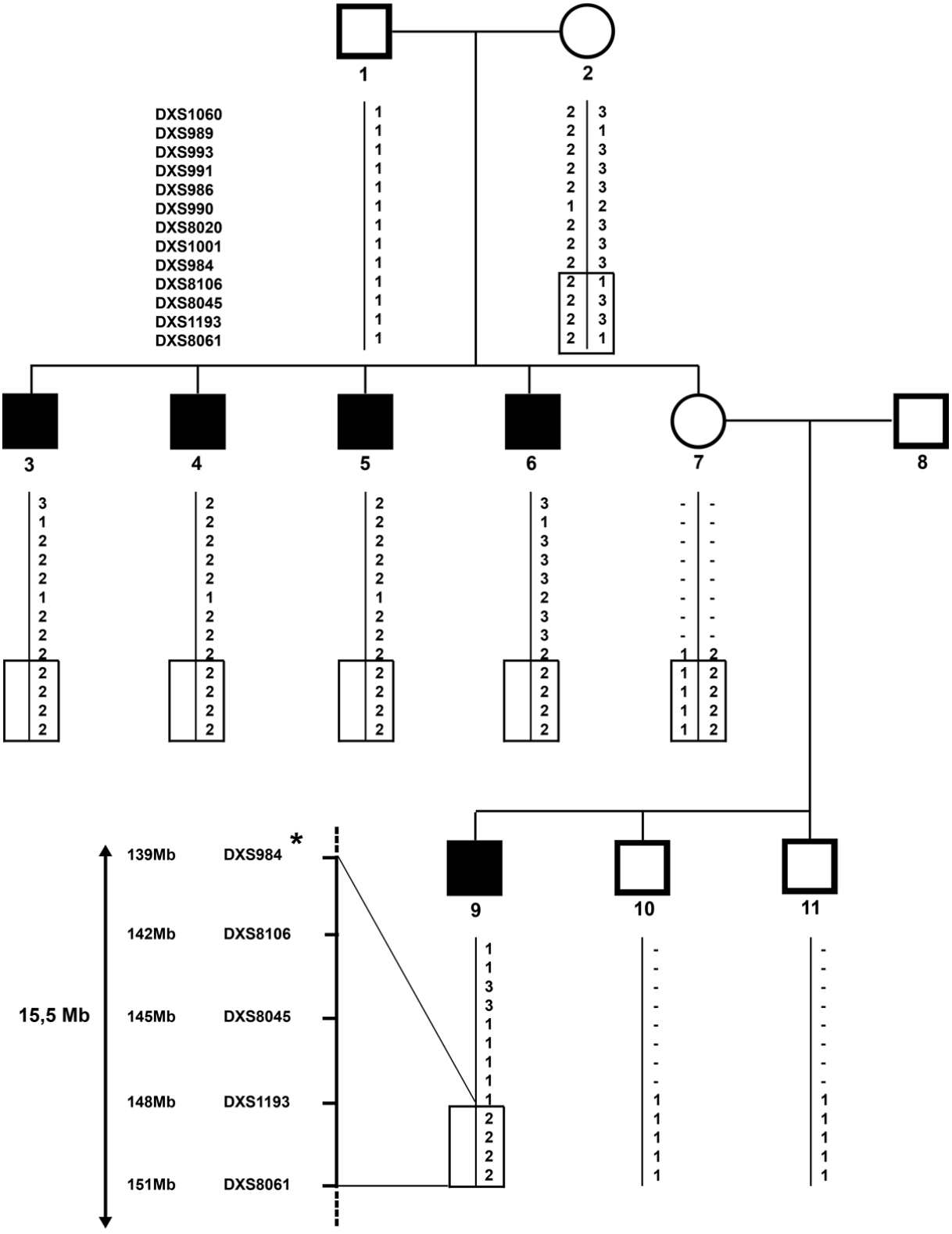


Figure S3: X chromosome haplotype analysis in family F1. Informative microsatellite markers and the haplotype cosegregating with the disease are shown.

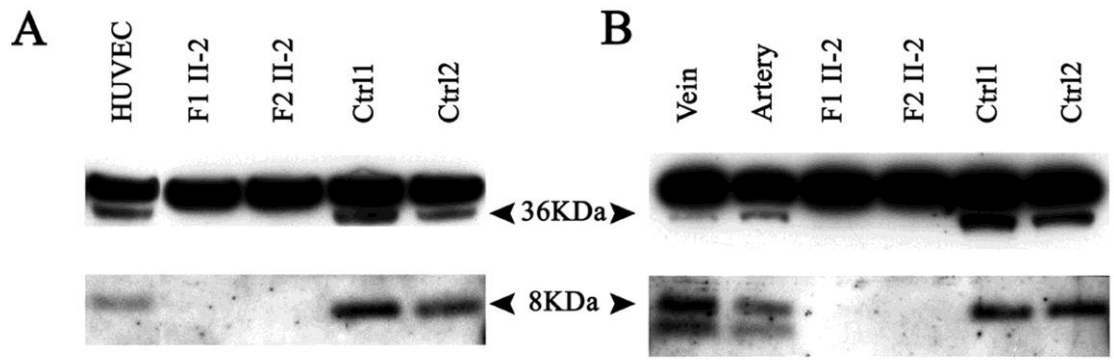
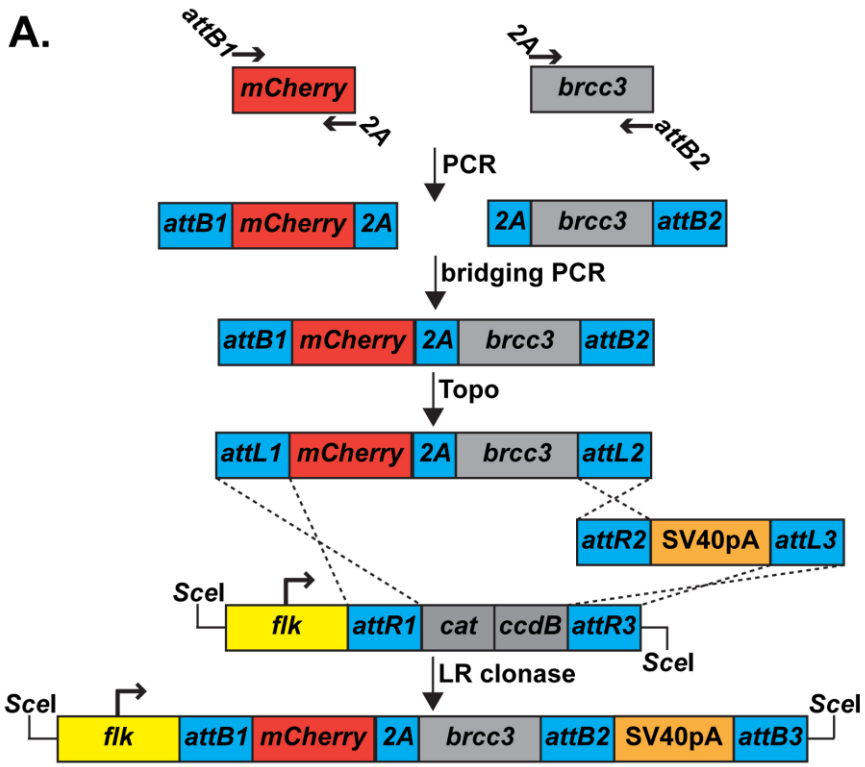


Figure S4 : Western blot analysis of p8^{MTCP1NB} and BRCC3 proteins. **A**: BRCC3 (36 KDa) and p8^{MTCP1NB} (8 KDa) proteins are not detected in EBV cell lines lysates from patients F1 II-2 and F2 II-2 while they are readily detectable in control EBV cell lysates and HUVEC endothelial cells. **B**: BRCC3 and p8^{MTCP1NB} proteins are detected in both human arteries and veins (arteries > veins).

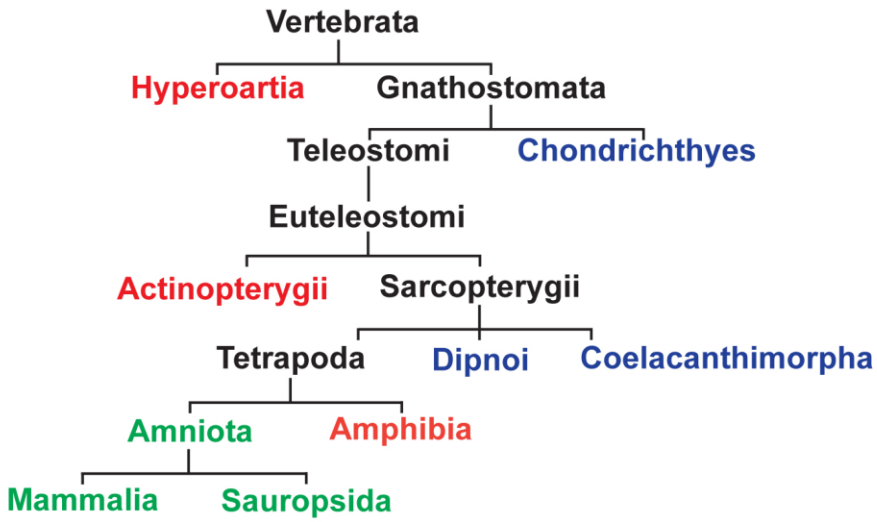


B.

Name	Oligo (5′→3′)
2A-brcc3-F	GGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGA GGAGAACCCTGGACCTATGGCTGTCAACGCGGTGCATTTAGAGTCGGAC
attB2-brcc3-R	GGGGACCACTTTGTACAAGAAAGCTGGGTTTACAATGCCGCCAGCTCTTGCGTCAGC
attB1-mcherry-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTACATGGTGAGCAAGGGCGAGGAG
2A-mcherry-R	AGGTCCAGGGTTCTCCTCCACGTCTCCAGCCTGCTTCAGCAGGC TGAAGTTAGTAGCTCCGCTTCCCTTGTACAGCTCGTCCATGCC

Figure S5 : ***brcc3*** **Endothelial Expression Transgene.** **(A)** The strategy employed to generate an endothelial *brcc3* expression transgene is shown. A red fluorescent *mCherry* cassette (red) was PCR-amplified with primers containing *attB1* and *2A* peptide (blue) sequences[38,39]. The *brcc3* (gray) coding sequence was PCR-amplified with primers containing *2A* peptide and *attB2* sequences. A bridging PCR generated an *attB1-mCherry-2A-attB2* cassette, which was cloned into the pENTR/D-TOPO® vector (Invitrogen™). The resultant vector was recombined with pDEST-I-*SceI-flk* (*unpublished, A.V. Gore*) and p3E-*polyA* to generate a *Sce*I-*flk1-mCherry-2A-brcc3-SV40polyA-Sce*I transgene in a LR clonase reaction. [40] **(B)** Table lists the names and nucleotide sequences for the primers used in the construction of the *flk-mCherry-2A-brcc3* transgene.

A.



B.

Hyperoartia	Actinopterygii	Amphibia	Mammalia	Sauropsida
Petromyzon marinus	*Danio rerio* *Gasterosteus aculeatus* *Oryzias latipes* *Takifug u rubripes* *Tetraodon nigrovirdis*	*Xenopus laevis* *Xenopus tropicalis*	*Ailuropoda melanoleuca* *Bos Taurus* *Callithrix jacchus* *Equus caballus* *Canis familiaris* *Homo sapiens* *Monodelphis domestica* *Mus musculus* *Ornithorhynchus anatinus* *Otolemur garnettii* *Rattus* norvegicus	*Anolis carolinensis* *Gallus gallus* *Taeniopygia guttata*

Figure S6 : Phylogeny of the *MTCP1* locus. (A) A phylogenetic tree showing the presence (green) or absence (red) of *MTCP1* homologs at various stages of vertebrate evolution. *MTCP1* likely arose during tetrapod evolution given its presence in each of the mammalian and sauropsid species surveyed and its absence in amphibians as well as all of the more divergent relatives. (B) Table listing each of the species surveyed for the presence or absence of *MTCP1*.

Primer name	Primer sequence 5' - 3'	Product size (bp)	Position within the gene
ECD21916	F :CAGAAATCAGATCATGTTGAGAGG R : TTCACTGAGCAGAAAAAGAAGC	236	F8 coagulation factor VIII, intron 1
ECD17738	F : GCAGTGGATCAGATGTCAATGT R : TCAGTACCTGGAGGCGAGTT	387	centromeric to exon 1 of *FUNDC2*
ECD21451	F : ATATGGCTTTGCCTTTGCAT R : TGTCAATCCCTTTCTCTGTGG	250	*FUNDC2* intron 1
ECD13623	F :TTTGAGAGGACAAACTAGAGGACT R : TGCTCCACATTAAGTGACCAAT	517	*FUNDC2* encompasses exon 2
ECD07678	F : GGCATATGGCTTCGCATATAG R : TGCAGAACAAGCAGAGTATGAA	676	FUNDC2 encompasses exon 4
ECD07752	F : GCAGTTTTTCAACCCACTCC R : ATCTACCGCGACGAATACCA	674	*MTCP1* exons 2, 3, 4, 5
ECD13141	F : TGGAGCTGAGTGAGTGCAAA R : AGGAGCTTACACCAGGCTCA	526	*MTCP1* intron 1
ECD15347	F : TGTCTCCCCTTAAATGCCAGT R : AAAAAGGAAGTGGGGTTTGG	473	*BRCC3* intron 2
ECD20126	F : CGGTGGATTATTTCCTGGTG R : ACGAGGGCATGAAATCTTGA	297	*BRCC3* intron 3
ECD02005	F : TTCACCCTTGTCCCTTCAAC R : GGAGCTCAGAGGAAAGAACTCA	846	*BRCC3* intron3
ECD05774	F : GACAACTTGCCACTCAACCA R : CATTGTCGCAGTGTTTGACC	728	*BRCC3* encompasses exon 4
ECD22603	F : TTTTCATGCCACTGATGAGG R : TGCCTTTGCAAAAAGTAGGA	215	*BRCC3* intron 5
STS1[a]	F: CCTTGGCACGTTTGACAT R: GCTTGGCTGCTGCTACTTTC	218	*MTCP1* Intron 1
STS2[a]	F: ATCCGCCTTGAGAACAGCTA R: AACTTCGGTTCGAGTGACCT	128	*MTCP1* Exon 1
STS3[a]	F: CGCCGCTCACAGAGTACG R: AGCAGCACCTGGGAAACG	155	*MTCP1* Exon 1
STS4[a]	F :TTTGTCTCAACCACGCTCTG R: CCTAAATCGAGGATGCCTGT	175	*BRCC3* Exon 1/intron 1
STS5[a]	F: GCAAGGCAGGTCAGAAAGAC R: AGGCTCTTCGATACCCTTCA	155	*BRCC3* Intron 1
STS6[a]	F:CTGTAAGGATCACATGGCACA R:GGCACTATATCCCATCACCAA	228	*MTCP1* Intron 1
STS7[a]	F:AGCGCCCTATGAAGATTGTG R:GGCCTGTGTATGAGTCCTGA	194	*MTCP1* Intron 1
STS8[a]	F:CTGCATAACTTGCCAGAACAG R:GGGAGAGGGATTCAGATCAA	166	*MTCP1* Intron 1
Breakpoints sequencing primers			
PrimerF1[b]	TTTGAGAGGACAAACTAGAGGACT		
STSR7[c]	GGCCTGTGTATGAGTCCTGA		

Table S1. Primers used for walking PCR and breakpoints sequencing

[a] Walking PCR primers used in family F2 [b] Breakpoints sequencing primers used in family F1 [c] Breakpoints sequencing primers used in families F2 and F3

BRCC3 **exons**	**Primer name**	**primer sequence 5' - 3'**	**Product size (bp)**
1	BRCC3ex1Fs BRCC3ex1Rs STSR4	F: GGTCCAGGGAGAGTTGTACG R: CGCACCTTCTGTTAGGCTCT CCTAAATCGAGGATGCCTGT	1235
2	BRCC3F2 [a] BRCC3R2	F:GCCGAAGAGGATGAGGAAAT R:CCCCGGAGTGATAATGGTAA	311
3	BRCC3F3 [a] BRCC3R3	F:TTGTGCATGTGTCCCAGATT R:AGAGCAGCAAGAGGGTGGTA	477
4	BRCC3F4 [a] BRCC3R4	F:TGAGGTTGGGTGTCTTTTATTTG R:GGCATTTAGGAGCAGGAATG	501
5	BRCC3F5 [a] LR-BRCC3R1	F:GCATTGGAAACCATGTGGA R:CCTGGACAGTAATGATATTCAGCA	547
6	LR-BRCC3F2 BRCC3R6 BRCC3ex6 [a]	F:CTATTACCACATTCCATAGCAGAGC R:CTTTCCCCAAGAAATCCACA GCCTGTACAATCTGAATCCTAGG	563
7	BRCC3F7 [a] BRCC3R7	F:GCAGATGTTTGGTTGTGACC R:AAAGGAAATGTCAGGCAGGA	497
8	BRCC3F8 [a] LR-BRCC3R2	F:CCATCTTGGTGTGTGGTTTC R:GCATTCTCACTTTCCTCCTTGATA	328
9 - 10	LR-BRCC3F3 BRCC3R10 BRCC3F10 [a]	F:CTATGGAACTAGGGAATACTGGCTCT R:CCCAGAGTTTACAAGAAATGTTGG AGCTTTGGTGAGAGACAGCA	1096
11	BRCC3F11-2 [a] BRCC3R11	F:CAGGCTTCCCTCTGGCTTAT R:CCTTGCCATTGTACCACTCA	666
MTCP1 **exons**			
1	STSF1 STSR3 stSG604434F [a] LR-BRCC3F1 [a] STSF3 [a]	F:CCTTGGCACGTTTGACAT R:AGCAGCACCTGGGAAACG ATCCGCCTTGAGAACAGCTA GCTGTGAGTTGGAAATAAACAAAAAT CGCCGCTCACAGAGTACG	857
2 – 3 - 4	MTCP1F2* MTCP1Rex	F:GCAGTCAGCAGCACTACCTG R:CACAACTGAATAGAGCCAAAACAA	844
MTCP1NB **exons**			
2	MTCP1NBF MTCP1NBR [a]	F:AAGGGTTTCTTCTGCGAATG R:TGCTAATCACCTGGAGTGAAAA	598
3	MTCPNBF1 [a] LR-MTCP1R	F:TTTCCCTAACGAACCTGTCG R: TGAGTCCTTTCACCTAACATACCTC	682

Table S2. Primers used for *BRCC3, MTCP1/MTCP1NB* amplification and sequencing. [a] Sequencing primers

Probe ID [a]	rs	position	Mother F1 II-2	Affected F1 II-3	Affected F1 II-4
CN_404039		153899986	2	1	1
CN_923663		153903800	2	1	1
CN_923664		153904537	2	1	1
CN_923665		153913201	2	1	1
CN_923667		153921900	1	0	0
CN_923668		153924357	1	0	0
CN_923670		153928244	1	0	0
SNP_A-4304694	rs1448032	153933999	1	0	0
SNP_A-1868149	rs2051161	153939045	1	0	0
CN_404047		153942523	1	0	0
CN_923672		153943426	1	0	0
CN_923673		153947727	1	0	0
CN_923674		153949766	1	0	0
CN_923676		153959531	2	1	1
SNP_A-8311882	rs5945287	153967224	2	1	1
CN_923679		153967285	2	1	1
CN_923681		153971361	2	1	1

Table S3 : Affymetrix SNP 6.0 array data suggesting an Xq28 deletion in family F1: [a] Copy number states of the SNP and copy number probes with their respective positions are shown. Physical probe positions refer to NCBI built 36.3. The GTC v 4.0 software was used to estimate the relative likelihood of each copy number level to be 0, 1, 2, 3 or 4. In the region bracketed by the probes CN-923665 and CN_923676 the copy number level in the obligatory carrier mother (F1 I-2) is estimated at 1 and in her two affected sons (F1 II-3 and F1 II-4) at 0, indicating the loss of genetic material.

Supplemental References

38. Shaner, N.C., Campbell, R.E., Steinbach, P.A., Giepmans, B.N., Palmer, A.E., and Tsien, R.Y. (2004). Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. Nat. Biotechnol. *22*, 1567–1572.

39. Szymczak, A.L., and Vignali, D.A. (2005). Development of 2A peptide-based strategies in the design of multicistronic vectors. Expert Opin. Biol. Ther. *5*, 627–638.

40. Kwan, K.M., Fujimoto, E., Grabher, C., Mangum, B.D., Hardy, M.E., Campbell, D.S., Parant, J.M., Yost, H.J., Kanki, J.P., and Chien, C.B. (2007). The Tol2kit: A multisite gateway-based construction kit for Tol2 transposon transgenesis constructs. Dev. Dyn. *236*, 3088–3099.