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Supplemental Data

De Novo and Inherited Mutations in *COL4A2*, Encoding the Type IV Collagen α2 Chain Cause Porencephaly

Yuriko Yoneda, Kazuhiro Haginoya, Hiroshi Arai, Shigeo Yamaoka, Yoshinori Tsurusaki, Hiroshi Doi, Noriko Miyake, Kenji Yokochi, Hitoshi Osaka, Mitsuhiro Kato, Naomichi Matsumoto, and Hirotomo Saitsu

Table S1. Primers and PCR Conditions

Exon	Forward primer (5'>3')	Reverse primer (5'>3')	Product size (bp)	PCR conditions
Ex2	ATGGGCTGCCTCCCTCATCCT	GAGAGTTACACCGAAGGGTCCATGC	202	KOD-FX 2step
Ex3	GCATGGACCCTTCGGTGTAACTCTC	CCACTCAAACGTCCCAACCACTCTC	198	KOD-FX 2step
Ex4	TTGGAAGGATTCTCAACAGATG	AGCGAGGCATGACTGTATGA	230	HotStar
Ex5&6	TCGTGGAAATTGAACCTTTG	CCTAGGATGCACGCAATGTT	344	HotStar
Ex7	GCCGGGAACATGGCTTATGAGAATA	GTTATGCTTCCGTTCTGGCCACAGT	332	KOD-FX 2step
Ex8	CTGCACCGAATGTTAATGGA	GATTATGCCGCCATTCTAGG	269	HotStar
Ex9&10	GGGCTGATCTGTTTGATATGC	CCAGAGTGGGCACCTGTGT	343	HotStar
Ex11	CAGAAACCTCCATGCATCCT	CAAACAAACCCACAAACACCT	230	HotStar
Ex12	TTGCCGATAAATAGGCCTTG	TTTCCTGGCTGAGAAATGCT	201	HotStar
Ex13	TTTCCTTTCGATTTAAAGACAACTGC	TGGAATGTGGTTGAATACAATTGAAGA	233	KOD-FX 3step
Ex14	CATGTCATGAACCCTGATTGA	ATGAGAGACTGGCGGTGTG	231	HotStar
Ex15	AGTCCTGGAGCAGAGGATGA	AAACCAAACCAAACCGACAA	186	HotStar
Ex16	CGTAGTCAAGCCCTCTGGAA	TGAGATGCCAAGGCCTATTT	197	HotStar
Ex17	TTTGGAGTTATACATCAGAGACAAAAA	GTGGGCGAGACACCATAAGT	192	HotStar
Ex18	CTCGGGTTTCTTCTTTGGAA	GCTCTGTGTCCCTAACAGGAG	223	HotStar
Ex19	CTCATCAGGCCGCATACAG	GACCTGAGTGCAGGTGCTTT	288	HotStar
Ex20	TCTGGACACGAACACAAAGG	CGGGCTTCATCTGAACATTA	277	HotStar
Ex21	CCTGCATCTGTGGTTGTCTC	GGGGATGGATTTCACCTTCT	199	HotStar
Ex22	GCTAAGAGGAATGCGGAACA	GGAGGCCTCAGAGTGTCTTG	260	HotStar
Ex23	GCCAGCTGTGTGAGATGAAA	GTCCCCGCTCACCTAGAAAG	270	HotStar
Ex24	TCCAGAACAATCACAACCAAAGGTGA	GGGTGTTTGGAGAACCTGAAGGATG	286	KOD-FX 2step
Ex25	GGAAGTCGAGGCGATCTTTA	CAAAGGAAAGCGTGGAATGT	325	HotStar
Ex26	CCCAGACGAGCCAGTAACTC	TTATCCCACGCATACTGCAA	215	HotStar
Ex27	TAGGATTGCTTGGGCTCATC	TTTGTGCTGAGATGCTGGAC	235	HotStar
Ex28	TTATCCTCGTGGAGCCTGAT	CTCCCAAGGACAAATGCAAA	300	HotStar
Ex29	CCATGCTAACTTGTGGTTTGG	CACTGTGCATCTGGGATGG	314	HotStar
Ex30	AGTGTGTGGAGGGAGATGCT	GTGAGGACCCCACTCGTTTA	279	HotStar
Ex31	TGTTTGTCCACCCTGTTTGA	CCAGCAGAGCTGTCTCAGGT	291	HotStar
Ex32	CGAAATGTTACGGAGACGTG	TGCCACCAAGAAAGGGTAAG	297	HotStar

Ex33	CAGGCCTTCACCTGTGTTCT	GTCTCTGGGGACGGAGAAG	280	HotStar Step down
Ex34	CAGCACGTAGGACAGCAAAA	GCTCACAGAACAAGGGGAGT	321	HotStar
Ex35	ACAGCTAAGCAAACCGCCTA	TCTGAATTGTGGACTCCCTGT	287	HotStar
Ex36	TCCCAGTGGAAAGTCCTGTT	TTGATCTGTTTGGCAAGTCG	205	HotStar
Ex37	GAAGGAGCAGCAGTGTGGTT	AATGTTGACCGCCTTTGTTC	285	HotStar
Ex38	CCAGGACCTCACCACACAG	ACTCTGGGTCTGGGTGACCA	216	HotStar
Ex39	GCTGTCCCACACATGAAATAA	ACACCTCTGCGTGGGACTC	314	HotStar
Ex40	GCTGCCTCTGTTTCTTTGCT	CTCTGGGTGGGTTCTGGTTA	295	HotStar
Ex41	GCACCTCCCATCACTGTCTC	CTACATTAAGCGGGCCATTG	316	HotStar
Ex42	AGAGACTGTCGCCTGAATGGGTGAC	GACGTTAGGGACACGAAAGTCTGTGG	343	KOD-FX 2step
Ex43	CTGGCCACAGTGAGAGGAG	GACCCATGCCAGAGAGGAT	272	HotStar Step down
Ex44	ACTCGGAGCAAGAGAGTGGA	GAACACAAGAGGACGCAATG	293	HotStar
Ex45	CATTGCGTCCTCTTGTGTTC	AGCACTAGGACCTGGGAAGG	248	HotStar
Ex46	GGGCTGCTCTCTCTCTCTTT	AACTTACCAGCCGTGGAGGGTTTG	586	KOD-FX 2step
Ex47-1*	GGCCCTCCAGTAGGTGGCTAAACTC	GGCTGATGTAGGGCTTGATCTCGTC	310	KOD-FX 2step
Ex47-2*	TCCTGTACTGCAACCCTGGTGATGT	CAAAGGCAGCTGTTCTTGCTGTGTC	317	KOD-FX 2step
Ex48	CAGGCTGTGATTCCTAACCCTGTCC	GAATAAGCACCAAAATGGCCCTTCC	341	KOD-FX Step down

*Exon 46 was sequenced in two parts.

PCR was cycled 35 times for each condition as follows:

HotStar, 94°C for 30 sec, 55 or 57°C (for exon 38) for 30 sec, and 72°C for 60 sec;

HotStar Step down, the annealing temperature was lowered by 1°C per cycle from 58°C to 54°C for the initial 5 cycles;

KOD-FX 2step, 98°C for 10 sec and 68°C for 30sec;

KOD-FX 3step, 98°C for 10 sec, 64°C for 30 sec, and 68°C for 30sec;

KOD-FX Step down, the annealing temperature was reduced by 2°C every 5 steps from 72°C to 68°C.

The following PCR amplification enzymes were used: for KOD-FX, KOD-FX DNA polymerase (Toyobo, Osaka, Japan); for HotStar, HotStarTaq (Qiagen).

Table S2. Predictions of the Pathogenicity of the *COL4A2* Mutations

Patient	Mutation	SIFT	PolyPhen	PolyPhen-2	Mutation taster	Align GVGD
1	c.3455G>A p.G1152D	0.00	probably damaging 2.142	probably damaging 1.00	Disease causing	C65
2	c.3110G>A p.G1037E	0.00	probably damaging 2.367	probably damaging 1.00	Disease causing	C65

For the predictions we used SIFT (http://sift.jcvi.org/),
PolyPhen (http://genetics.bwh.harvard.edu/pph/),
PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/),
Mutation Taster (http://neurocore.charite.de/MutationTaster/index.html)
and Align GVGD (http://agvgd.iarc.fr/agvgd_input.php)

SIFT, scores less than 0.05 indicate substitutions are considered to be intolerant;
PolyPhen, scores more than 2.0 are considered to be pathogenic;
PolyPhen-2, scores are evaluated from 0.000 (most probably benign) to 0.999 (most probably damaging);
Align GVGD, class scores are evaluated as Class C0 (less likely) to Class C65 (most likely).