The American Journal of Human Genetics, Volume *93*

Supplemental Data

De Novo Mutations in *GNAO1*, Encoding a $G\alpha_o$ Subunit of Heterotrimeric G Proteins, Cause Epileptic Encephalopathy

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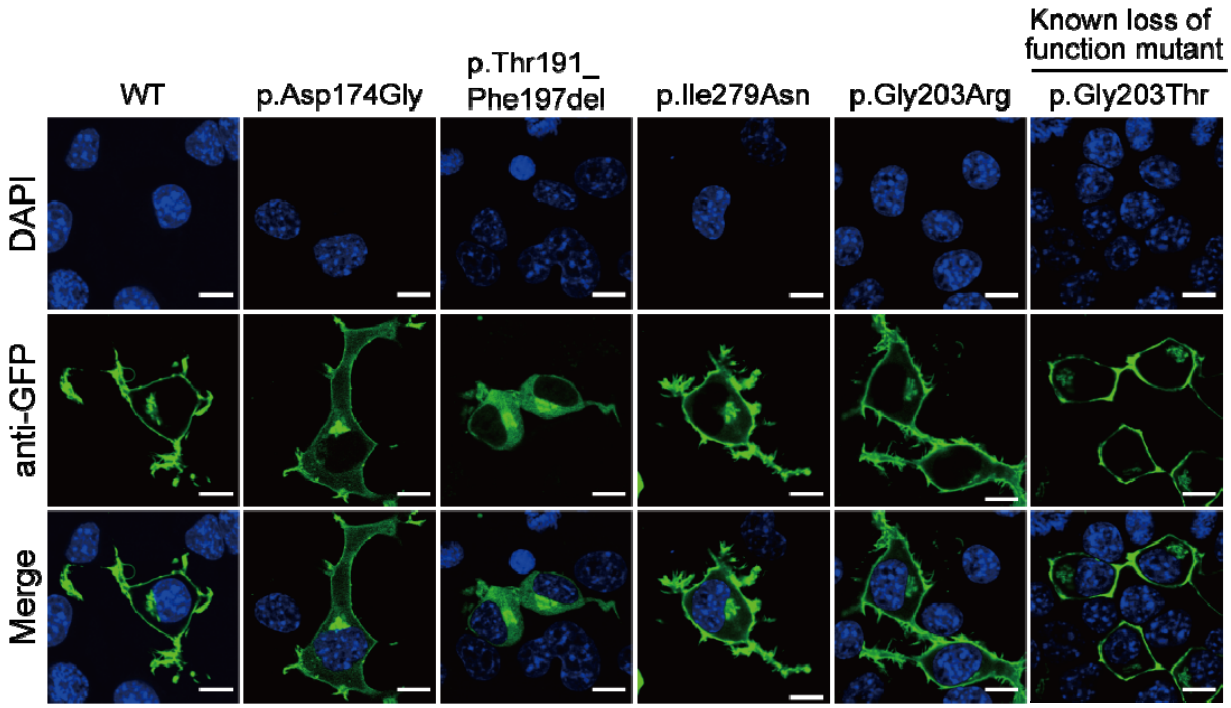

Figure S1. Expression of AcGFP1-Tagged $G\alpha_{o1}$ in N2A Cells

Human *GNAO1* cDNA was inserted into pAcGFP1-N2 vector (Clontech, Mountain View, CA, U.S.A.) to generate C terminal AcGFP1-tagged $G\alpha_{o1}$ expression constructs. Expression of AcGFP1-tagged wild-type $G\alpha_{o1}$ (WT), p.Gly203Arg, and p.Gly203Thr mutants were observed at the cell periphery. In contrast, p.Thr191_Phe197del was expressed in the cytosolic compartment. The p.Asp174Gly and p.Ile279Asn mutants were localized to the cell periphery with weak signal in the cytosol. The nucleus was stained with DAPI. The scale bars represent 10 μm.

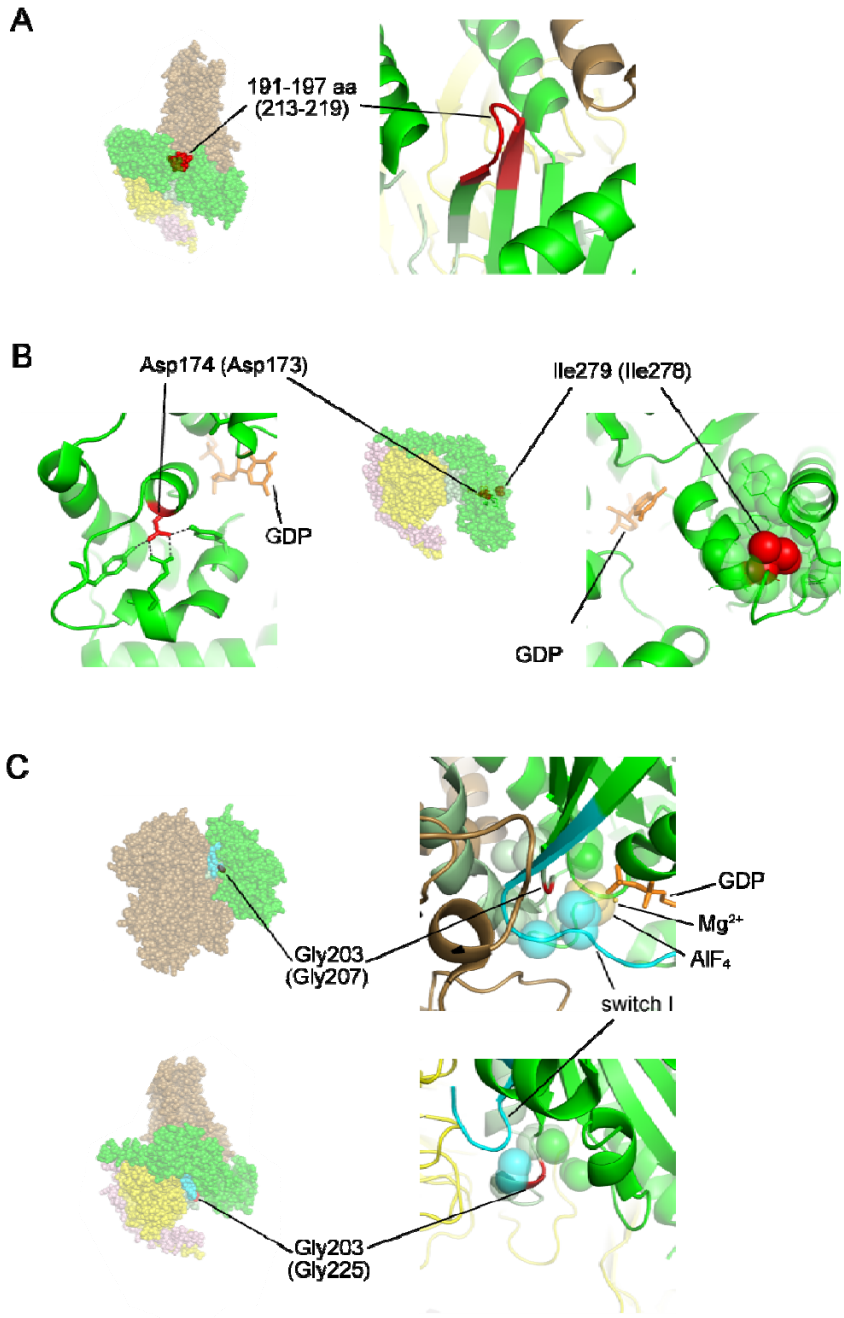

Figure S2. Structural Consideration of the Gα Mutations in Some Gα-Containing Complexes

Overall and close-up views of the positions in the Gα subunit-containing complexes corresponding to the mutation sites found in human *GNAO1*; p.191_197del in the $G\alpha_s\beta\gamma$-β2AR complex (PDB code 3SN6) (A), p.Asp174Gly and p.Ile279Asn in the GDP-bound $G\alpha_i\beta\gamma$ heterotrimer (PDB code 1GG2) (B), and p.Gly203Arg in the transition-state analogue of GTP (GDP+AlF_4^-)-bound $G\alpha_q$-PLCβ complex (PDB code 3OHM) (top) and the $G\alpha_s\beta\gamma$-β2AR complex (bottom) (C). Overall views are shown in the space-filling representation as in Figure 4. In the close-up views, α helices, β strands

and loops are depicted as ribbons, arrows and threads, respectively. Colors are as in Figure 4 except for nucleotides in orange and the mutated residues in red. The amino acid numbering shown is for human *GNAO1* with bovine $G\alpha_s$ (A and C), rat $G\alpha_i1$ (B), and mouse $G\alpha_q$ (C) in parentheses. In B, side chains of some residues involved in a hydrophobic core are shown in the space-filling representation, and hydrogen bonds are shown as black dotted lines. In C, the switch I region is colored cyan, and heavy atoms within 5 Å distance from the atom corresponding to the α carbon of Gly203 in the human $G\alpha_{o1}$ are shown in the space-filling representation.

Table S1. Primers and PCR Conditions

Exon	Forward primer (5'>3')	Reverse primer (5'>3')	Product size (bp)	PCR conditions
Ex1	TTTGTTTCCAGCCCAGGAGAGGATA	GATCCTGGGCACATGGTGGTG	386	KOD FX with 2 step PCR 70-64
Ex2	CCCCTGTTCCCTTAAGCTG	GAGGACCCAGAACCAACGTA	286	KOD FX 64-56
Ex3	GACCTGGCCCACAGTCAG	AGGCAGCCCAGCACTATAAA	326	HotStartTaq MM Kit 64-56
Ex4	CTTGGCTGGCAGAGGTCTT	CATCACCAGTCCCTTCCACT	295	HotStartTaq MM Kit 64-56
Ex5	ACAGTGTCCAGGCATTTGGT	CCTTGGCAGAAACACAGACA	347	HotStartTaq MM Kit 64-56
Ex6	CAGCGTGCTCACAGCTTAAT	CTCAGAGGGCTGGCCTATC	280	HotStartTaq MM Kit 64-56
Ex7-variant 1	CCAGTCCCTCTCTGTCAAGC	GAGCAGCCTGTTCTCTGAGC	329	KOD FX 64-56
Ex7-variant 2	AGCCACATTGGTGGACCTT	CATCCACAGAGCACAGGAAG	349	HotStartTaq MM Kit 64-56
Ex8-variant 1	ATCCCACTTCCTGGGACAC	TCTGTGGGTGCTGGAATCAT	350	HotStartTaq MM Kit 64-56
Ex8-variant 2	GTCCATGCCAAGCAGTCC	CAAGTGCAAAGAGTGGTCTGA	348	HotStartTaq MM Kit 64-56

The column "PCR conditions" shows the PCR enzymes used and the range of annealing temperatures. The temperature was lowered by 2°C every 3 cycles to the lowest annealing temperature. Then, the PCR reaction was cycled 30 times.

The details of the PCR conditions are as follows:
Hot Start Taq MM Kit: 95°C for 10 s, 64°C, 60°C, 58°C, 56°C for 30 s, and 72°C for 30 s
KOD-FX: 95°C for 10 s, 64°C, 60°C, 58°C, 56°C for 30 s, and 72°C for 30 s
KOD-FX with 2 step PCR: 98°C for 10 s and 70°C, 68°C, 66°C, 64°C for 30 s

The following PCR amplification enzymes were used: Hot Start Taq MM Kit, Hot Start Taq Master Mix Kit (Qiagen, Tokyo, Japan); KOD FX, KOD FX Neo (Toyobo, Osaka, Japan)

Table S2. Prediction of the Pathogenicity of the *GNAO1* Mutations

Case	Diagnosis	cDNA change	Amino acid change	Inheritance	SIFT	Polyphen2	Mutation Taster	In-house database
1	Ohtahara syndrome	c.836T>A	p.Ile279Asn	*de novo*	0.00	Probably damaging 1.000	Disease causing 0.999	0 / 408
2	Ohtahara syndrome	c.521A>G	p.Asp174GLy	*de novo* mosaic	0.00	Probably damaging 1.000	Disease causing 0.999	0 / 408
3	Ohtahara syndrome	c.572_592del	p.Thr191_Phe197del	*de novo*	N/A	N/A	N/A	0 / 408
4	Epileptic encephalopathy	c.607G>A	p.GLy203Arg	*de novo*	0.00	Probably damaging 1.000	Disease causing 0.999	0 / 408

N/A = not applicable.
SIFT (http://sift.jcvi.org/): scores of less than 0.05% indicate substitutions that are predicted as intolerant.
PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/): scores are evaluated as 0.000 (most probably benign) to 0.999 (most probably damaging).
Mutation Taster (http://www.mutationtaster.org/): rapid evaluation of DNA sequence alterations. The alterations are classified as disease causing or polymorphism.

Table S3. Read Counts in Family of Individual 2 with a Mosaic Mutation in *GNAO1* (c.521A>G)

Methods &samples	Read counts		% of Mutant alleles
	Mutant alleles	Wild type alleles	
Whole exome			
Blood #1	6	29	17.14
Deep sequencing			
Blood #1	2,996	13,430	18.24
Blood #2	2,659	11,784	18.41
Nail #1	4,187	12,174	25.59
Nail #2	2,975	14,948	16.60
Saliva	3,073	14,255	17.73
Father Blood	23*	18,154	0.13
Mother Blood	27*	14,872	0.18

Samples of blood and nail have been independently obtained twice (#1 and #2).
*These small numbers of mutant reads were considered to be PCR or sequencing errors.