Supplementary Online Content

Kumar KR, Lohmann K, Masuho I, et al. Mutations in *GNAL*: a novel cause of craniocervical dystonia. *JAMA Neurol*. Published online February 17, 2014. doi:10.1001/jamaneurol.2013.4677.

eTable 1. Primers used in *GNAL* study

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eTable 1. Primers used in *GNAL* study

Exon	Forward Primer	Reverse Primer	Annealing temperature
NM_001142339_ex1*	GGAGCCGTCGCAGGAGCCG	TTAAACCCATTGACGTGCA	58
ex2	GGATTGCTCAGACCCGGCT	ATTGTTTAGAGAAAGCACT	57
ex3-4	ATGTCTTGGGGGTTGATACTG	GCATTTAGAGAGCTGGATTTCG	55
ex5	CTATGCAGACTTCCACAAA	TGCACTCAAGAAACTTCAG	55
ex6	TGCTGTACTTGGGTGATGTC	AAGGCACAAGGTTTGATGAC	55
ex7	CATGCTCGGCCAATGTTGG	AGTAGGCAGTAAAATCAAC	55
ex8	TGCTGTCTGCTTGATCTGTC	ACTCCTGACTTCAGGTTATCCG	60
ex9	CAGGCTGTTCTGTGACTGA	CAGGCTAGTCTCCAACTGCT	55
ex10	GGAATGAGGATACTGGTGTACTG	GGGATCCGATTACAAATAGGAC	55
ex11	GCTGGGAATATACAGCAGTCTAAC	CCTTGGGTGAGAAATCTAACC	55
ex12	CATTCCTGCCTCTAAGTGC	ACTGTGTTTTCTCCCCATCTAC	55
cDNA_ex4(F), ex12(R)	TAGCCCCTATCACTGACT TTG	CCTCACAAGAGCTCATACTGC	60
Ex9_qPCR	GCTTTCCTTTTTCTCCCCA	AATTTGCTATTTTTGTCAC	55
β globin_qPCR	ACACAACTGTGTTCACTAGC	CAACTTCATCCACGTTCACC	55

*A GC rich kit (GC Rich PCR system, Roche Diagnostics GmbH, Mannheim, Germany) was used for sequencing of this exon.

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eTable 2. Results of software analyses.*

Patient	Genomic change	Protein change	Polyphen-2[4]	Mutation Taster[5]	SIFT[6]
L4486	c.637G>A	p.Gly213Ser	Probably damaging with a score of 0.999 (sensitivity 0.14; specificity 0.99)	Disease causing	Damaging
L1589	c.931G>A	p.Ala311Thr	Benign with a score of 0.299 (sensitivity 0.91; specificity 0.89)	Disease causing	Damaging
DYT_family7	c.1057G>A	p.Ala353Thr	Probably damaging with a score of 1.000 (sensitivity 0.00; specificity 1.00)	Disease causing	Damaging

*We used the Ensembl transcript ID ENST00000423027 and protein ID ENSP00000408489 for the software analyses.

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eMethods. Bioluminescence energy transfer assay and quantitative duplex polymerase chain reaction assay

Bioluminescence energy transfer assay

Genetic Constructs

masGRK3-Nluc construct contained amino acids G495-L688 of bovine GRK3 (NP_776925), preceded by a myristic acid attachment peptide (mas; MGSSKSKTSNS). The stop codon of GRK3 was replaced with a GGGS linker, which was followed by the NanoLuc.[1]

Analysis of Gαβγ trimer formation and D1-Golf coupling by fast kinetic BRET assay

$G\alpha_{olf}$ function in living cells was analyzed by monitoring the kinetics of its association and dissociation with Gβ1γ2 subunits following activation of D1R by agonist. The assay measures agonist-dependent changes in bioluminescence resonance energy transfer (BRET) between Gβ1γ2 tagged with Venus and its effector GRK3 tagged with Nluc and was conducted as previously described [2]. Briefly, N-terminal 3xHA-tagged dopamine D_1 receptor, $G\alpha_{olf}$, Venus156-239-Gβ1, Venus1-155-Gγ2, masGRK3ct-Nluc, and Flag-tagged Ric-8B constructs were transfected into HEK293T/17 cells at a 1:6:1:1:1:2 ratio with 5 µg total DNA delivered per 4 x 10^6 cells. 16 h post transfection cells were stimulated with 10 µM dopamine followed by treatment with 100 µM haloperidol that has reported 63 nM Kd for D1 receptor [3]. Stimulation of the D1R by dopamine results in the dissociation of $G\alpha_{olf}$ from the heterotrimer. Released Gβγ subunits tagged with Venus become available for the interaction with Nluc-tagged GRK3ct reporter producing the BRET signal which is determined by the change in the emission ratio at wavelengths 535 nm and 475 nm. Mean BRET ratios were calculated from 4 wells per condition. One-way ANOVA followed by the Holm-Sidak method was performed to determine statistically significant differences relative to wild-type control. Similar results were obtained from two independent experiments.

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Quantitative duplex polymerase chain reaction assay

To screen for whole gene deletions/duplications, a quantitative polymerase chain reaction (qPCR) assay was performed on a LightCycler 480 (Roche Diagnostics, Mannheim, Germany). We amplified exon 9 of *GNAL* and a reference gene, *β globin*, using a LightCycler Fast start DNA Master plus SYBR Green I kit (Roche Diagnostics GmbH, Mannheim, Germany, primers listed below). A relative ratio (concentration of *GNAL* exon 9: β globin) between 0.8 and 1.2 was considered normal; a ratio between 0.4 and 0.6 would have suggested a heterozygous deletion; and a ratio of 1.3 and 1.7 would have indicated a heterozygous duplication.

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