Clinical/Scientific Notes

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HOMOZYGOUS *GNAL* MUTATION ASSOCIATED WITH FAMILIAL CHILDHOOD-ONSET GENERALIZED DYSTONIA

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Heterozygous loss-of-function mutations in the *GNAL* gene encoding the α subunit of the heterotrimeric G protein G_{olf} ($G\alpha_{olf}$) are known to cause isolated dystonia.[1,2] $G\alpha_{olf}$ is enriched in the striatum where it couples D1 dopamine (D1R) and A2A adenosine (A2AR) receptors to the activation of adenylyl cyclase type 5 (AC5). Mutations in *ADCY5*, the gene encoding AC5, are also known to lead to chorea and dystonia.[3,4] Previous functional studies of mutated $G\alpha_{olf}$ variants have revealed deficiencies in activation after D1R stimulation.[1,5]

Patients with heterozygous *GNAL* mutations typically exhibit an adult-onset focal cervical, laryngeal, and/or segmental dystonia.[2] Such cases are typically either familial autosomal dominant or sporadic, resulting from de novo mutations. We describe a multiplex consanguineous Turkish family in which 2 affected children exhibited childhood-onset generalized dystonia. Affected patients were found to harbor a homozygous missense mutation in *GNAL*, representing biallelic mutations rather than the heterozygous *GNAL* mutations typically encountered.

We enrolled the patients in our ethics and institutional review board–approved research study after obtaining written informed consent. The index patient was born at term without complications. The mother took no medications during her pregnancy. Growth and early milestones were attained on time. The girl's parents first became concerned at age 1 year when she began to show evidence of exaggerated muscle tone. As the girl grew older, generalized dystonia affecting the head/neck, trunk, and limbs emerged. She experienced academic difficulties upon starting school, and was diagnosed with mild intellectual disability. At age 15 years, she exhibited generalized dystonia and impaired volitional movement. She experienced action-induced spasms and exacerbations of her baseline dystonia. Laboratory workup was unrevealing and her neuroimaging was unremarkable.

The girl's younger sister was also born at term without complications or prenatal exposures. She too met early motor and language milestones within the first year, but was noted to have hypertonia at age 1 year. She also lagged behind her peers in school and was diagnosed with mild intellectual disability. At age 11 years, she showed generalized dystonia that interfered with purposeful movements, with distressing dystonic spasms. Laboratory evaluations were nondiagnostic, and MRI of the brain was normal for age.

Given the family structure, we suspected an autosomal recessive disorder with identity by descent. Because autosomal recessive dystonia mutations have only recently been described,[6] we began our studies suspecting a novel gene. We applied an approach of tandem homozygosity mapping and whole exome sequencing.

We identified 5 blocks of homozygosity ≥3 Mb shared by the affected sisters but absent from unaffected siblings. Whole exome sequencing was performed using the Complete Genomics platform (appendix e-1 at Neurology.org/ng), and revealed a novel homozygous c.1216C>T (p.R329W) missense variant in *GNAL* (NM_182978) within a prominent block of homozygosity on chromosome 18 (hg19; chr18:7,490,028-15,143,714). This variant was predicted to be deleterious across several algorithms (SIFT, PolyPhen-2, MutationTaster, and PhyloP) and affects a residue that is highly conserved across species. This variant was not observed in the Exome Variant Server and ExAc Browser databases, and segregated in a homozygous form with affected status in the family (figure, A). Both parents and several siblings were heterozygous but did not display any overt dystonia.

We suspected this missense change to be the probable cause of the patients' phenotype. To test this idea, we evaluated the ability of mutated $G\alpha_{olf}$ to be activated by the D1R upon reconstitution in HEK293T cells using our previously published bioluminescence resonance energy transfer (BRET) strategy[1] (figure, B). In mutants, the basal BRET ratio is elevated, indicating a marked deficit in Gβγ binding and/or protein stability (figure, C). We further observed a markedly diminished activation of mutated $G\alpha_{olf}$ in response to D1R stimulation with dopamine (figure, D), indicating an additional partial loss of function.

Taken together, these findings suggest that our cases represent bona fide autosomal recessive *GNAL*-associated disease. The mutation seen in our patients seems to lead primarily to impaired $G\alpha_{olf}$ functional

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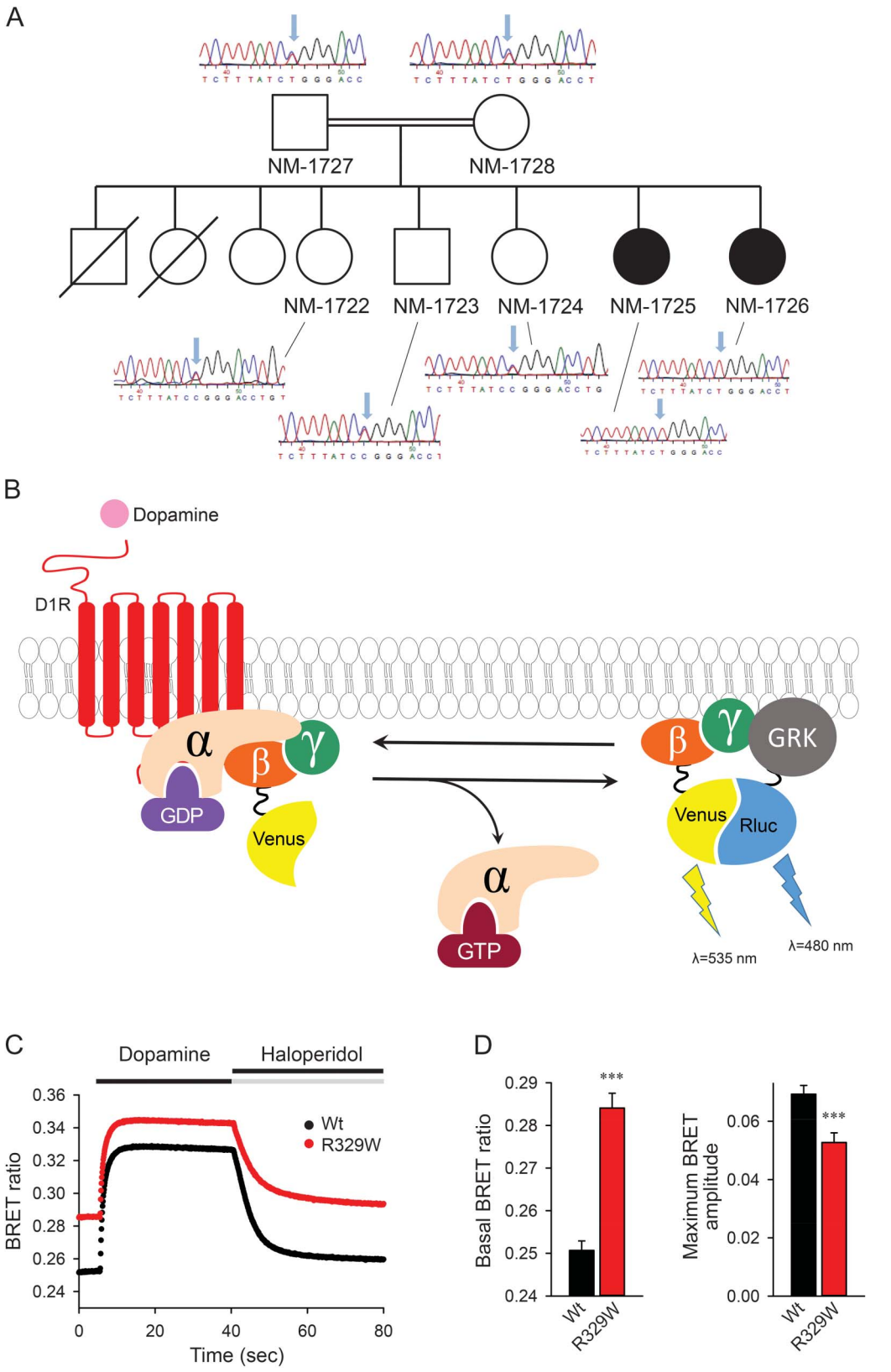
Figure ***GNAL* mutation and functional characterization**



(A) Family pedigrees. Filled symbols correspond to affected individuals. Empty symbols represent healthy individuals. Electropherograms show the missense mutation that leads to the (p.R293W) variant of *GNAL*. (B). Schematic of $G\alpha_{olf}$ functional coupling to D1R and bioluminescence resonance energy transfer (BRET) assay. Stimulation of the D1R by dopamine results in the dissociation of $G\alpha_{olf}$ from the heterotrimer. Released $G\beta\gamma$ subunits tagged with Venus become available for interaction with Nluc-tagged GRK reporter producing the BRET signal, which is determined by the change in the emission ratio at

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Figure legend, continued:
wavelengths 535 and 480 nm. (C) Time course. BRET signal changes upon stimulation of cells with dopamine and subsequent deactivation by haloperidol. (D) BRET ratios. Basal ratios calculated before the application of dopamine reflect the extent of the $G\alpha_{olf}$-$G\beta\gamma$ heterotrimer formation, and changes in the BRET ratio from basal signal to maximal response reflect the amplitude of the response. Results represent the mean of quadruplicate wells from a typical experiment. Similar results were seen in 2 independent experiments. Error bars represent the SEM. An unpaired *t* test was performed to determine statistically significant differences. Asterisks indicate statistical significance from wild-type control: $***p < 0.001$.

coupling to dopamine D1 receptors, differing from previously described mutations in *GNAL* that lead to a strict loss-of-function phenotype.[1,5] It is likely that, although the coupling of $G\alpha_{olf}$ with dopamine D1 receptors is impaired, enough normal protein is present in heterozygotes to retain the ability to bind the receptor efficiently and maintain adequate biological activity. Thus, only biallelic (p.R329W) mutations impair $G\alpha_{olf}$ functional coupling enough to cause the observed phenotype. Our findings indicate that mutations in *GNAL* may manifest through either autosomal dominant or recessive modes of inheritance, with the present patients exhibiting generalized dystonia with onset in childhood.

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1. Fuchs T, Saunders-Pullman R, Masuho I, et al. Mutations in GNAL cause primary torsion dystonia. Nat Genet 2013; 45:88–92.
2. Vemula SR, Puschmann A, Xiao J, et al. Role of Gα(olf) in familial and sporadic adult-onset primary dystonia. Hum Mol Genet 2013;22:2510–2519.
3. Chen YZ, Matsushita MM, Robertson P, et al. Autosomal dominant familial dyskinesis and facial myokymia: single exome sequencing identifies a mutation in adenylyl cyclase 5. Arch Neurol 2012;69:630–635.
4. Carapito R, Paul N, Untrau M, et al. A de novo ADCY5 mutation causes early-onset autosomal dominant chorea and dystonia. Mov Disord 2015;30: 423–427.
5. Kumar KR, Lohmann K, Masuho I, et al. A. Mutation in GNAL: a novel cause of craniocervical dystonia. JAMA Neurol 2014;71:490–494.
6. Zech M, Lam DD, Francescatto L, et al. Recessive mutations in the α3 (VI) collagen gene COL6A3 cause early-onset isolated dystonia. Am J Hum Genet 2015; 96:883–893.

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