DYSTONIA

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Mechanisms of *GNAL* linked dystonia

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Mutations in the *GNAL* gene, encoding $G\alpha_{olf}$, are causative for an adult-onset, isolated dystonia that may provide unique insights into the etiology of adult-onset idiopathic dystonia. $G\alpha_{olf}$ is an alpha subunit of heterotrimeric G protein that replaces $G\alpha_s$ in the striatum and has unique expression patterns outside of the striatum. $G\alpha_{olf}$ additionally has defined molecular functions in GPCR signaling. These defined molecular pathways and expression pathways point to defined circuit deficits underlying the causes of this adult-onset dystonia that may provide additional insights into broader idiopathic dystonia. Here, we will review the available evidence for normal $G\alpha_{olf}$ function, and how this is corrupted by *GNAL* mutations to cause dystonia. Thes include the molecular signaling and expression profiles of $G\alpha_{olf}$ and the other G proteins, $\beta_2\gamma_7$, complexedwith it., Additionally, we will discuss the circuits that $G\alpha_{olf}$ influences, and how *GNAL* mutations may reorganize these circuits to cause dystonia.

KEYWORDS

***GNAL*, dystonia, *GNAL* dystonia, mutations in *GNAL*, models of dystonia**

Introduction

In 2013, the first mutations in *GNAL* were reported through whole exome sequencing in 2 families with dystonia which revealed a nonsense mutation in one family and a missense mutation in the other [1]. Further screening in 39 other families with dystonia revealed another 6 autosomal dominant mutations in *GNAL* including nonsense, missense, frameshift, and deletions [1]. The frequency of mutations in these families was 19%, and suggested that mutations in *GNAL* may be a common cause of familial dystonia. Subsequent studies have revealed at least 30 more pathogenic variants in *GNAL* as well as rare autosomal recessive mutations that are linked to dystonia [1–8]. Additionally, recent evidence has also suggested that sporadic *GNAL* mutations are linked to dystonia, and that >50% of all *GNAL* dystonia cases are sporadic [8–10]. These genetic studies have suggested that *GNAL* linked dystonia is rarer than originally believed with a frequency as low as 0.5% of familial dystonia attributed to mutations in *GNAL* [3].

Clinically, patients with mutations in *GNAL* are largely indistinguishable from idiopathic dystonia [1–5, 10, 11]. Age of onset in patients is typically in adulthood with age range at diagnosis between 7 and 68 years (average ages 31–42 years) [1, 8, 11]. Also, *GNAL* dystonia patients present with an isolated dystonia that often starts focally, sometimes becomes segmental, and rarely becomes generalized [8]. The exception to this

is the rare autosomal recessive mutations in *GNAL* which also present with intellectual disabilities [6]. This high overlap in clinical characteristics with idiopathic disease, as well as defined gene function of *GNAL* discussed below, may make this form of genetic dystonia an interesting model system to understand synapses, circuits, and cells that are disturbed in dystonia, and how these alterations can be manipulated for novel therapeutic targets. Here, we review the known mechanisms of *GNAL* mutations, the functions of its protein product $G\alpha_{olf}$, and discuss how these mutations may lead to dystonia.

Molecular signaling and expression of $G\alpha_{olf}$

GNAL encodes $G\alpha_{olf}$ which is an alpha subunit of a heterotrimeric G protein [3, 12]. $G\alpha_{olf}$ is part of the $G\alpha_S$ family of alpha subunits [13]. Upon neurotransmitter binding to the G protein coupled receptor, $G\alpha_{olf}$ switches from a GDP bound state to GTP bound state, dissociates from the receptor, and the G βγ subunits. Active $G\alpha_{olf}$ then binds to adenylate cyclase type 5, activates this enzyme, and leads to the production of the second messenger cAMP which then acts on its many downstream targets including protein kinase A, exchange proteins activated by cAMP (EPAC proteins), and cyclic nucleotide gated ion channels (See Figure 1) [14]. In order to inactivate $G\alpha_{olf}$, GTP must be hydrolyzed to GDP which can achieved by the slow intrinsic GTPase activity of $G\alpha_{olf}$ and can be accelerated by regulator of G signaling (RGS) proteins [13]. Normal signaling of $G\alpha_{olf}$ is critical to the function of the striatum as $G\alpha_{olf}$ is the determining factor in cAMP second messenger production in the striatum [15–18]. $G\alpha_{olf}$ has an expression pattern that points to a unique role for the above signaling in normal basal ganglia function. $G\alpha_{olf}$ replaces $G\alpha_S$ in the predominant neuron types in the striatum, spiny projection neurons. Additionally, $G\alpha_{olf}$ is co-expressed with $G\alpha_s$ in cholinergic interneurons, and possibly other interneuron classes, in the striatum [8, 19, 20]. This indicates that $G\alpha_{olf}$ is the major signal transducing alpha subunit for pro-excitatory neurotransmitters and their G protein coupled receptors in the striatum with $G\alpha_{olf}$ coupling to the D1 dopamine receptor in direct pathway spiny projection neurons and the adenosine 2A receptor in indirect pathway spiny projection neurons [15]. While the signaling role for $G\alpha_{olf}$ in the striatum is better defined, the role for $G\alpha_{olf}$ outside of the striatum is not well understood. $G\alpha_{olf}$ is expressed in multiple brain nuclei. IHC studies indicate $G\alpha_{olf}$ expression in Purkinje Cells of the cerebellum and dopaminergic cells of the substantia nigra pars compacta [8]. RNAseq and proteomics studies have supported even further widespread expression including in multiple cortical regions, thalamus, and amygdala [21–23]. However, receptors to which $G\alpha_{olf}$ couples in these regions are not understood, and in many cases the cell type or types that express $G\alpha_{olf}$ in these regions are not well understood. Understanding the complete expression profile of $G\alpha_{olf}$ with cell type specificity will aid in understanding the mechanisms and networks that are corrupted by mutations in *GNAL* to lead to dystonia.

Mechanisms of mutations in $G\alpha_{olf}$

In the original paper describing the first mutations in *GNAL*, bioluminescence resonance energy transfer experiments gave the first evidence that disease associated mutations were loss of function mutations [1]. These experiments tagged the βγ subunits associated with $G\alpha_{olf}$ with the venus acceptor in this system and a downstream effector of βγ, GIRK, with the luciferase donor. Upon stimulation of the D1 dopamine receptor, mutations showed decreased association of βγ with GIRK, and suggested that mutations in *GNAL* represent loss of function mutations as they disrupt normal dopamine signaling through the D1 dopamine receptor [1]. However, the original description of the effects of *GNAL* mutations did not examine $G\alpha_{olf}$ dependent functions such as cAMP production.

More recently, however, the effects of *GNAL* mutations on $G\alpha_{olf}$ function have been examined in exquisite detail [12]. As discussed above, $G\alpha_{olf}$ is critical not only for the activation of adenylate cyclase and the production of cAMP, but also in formation of the heterotrimeric G protein complex, sequestering βγ from being active, and also termination of the signal through hydrolysis of GTP [12, 13]. These studies rigorously examined the effects of the known mutations at the time of the study on the full spectrum of molecular events that $G\alpha_{olf}$ is associated with. These studies revealed that, with respect to normal, well-regulated dopamine signaling, known mutations in *GNAL* are loss of function, but that the effects of the mutations on $G\alpha_{olf}$ function are significantly more nuanced than previously appreciated.

Each of the mutations had distinct effects on stability of $G\alpha_{olf}$, formation of the trimer, G protein activation, signal termination, cAMP production, and basal signaling which led to each mutation having a distinct functional phenotype [12]. Interestingly, some of the mutations led to increased basal or dopamine induced cAMP production which would more traditionally appear as gain of function mutations. However, despite this increased basal or induced cAMP production, these mutations still represent a loss of function with respect to normal response to dopamine release [12]. This challenges the simple assignment of gain or loss of function to *GNAL* linked dystonia, and highlights the complicated molecular mechanisms behind mutations in *GNAL*. Furthermore, when these differential mutation effects on $G\alpha_{olf}$ are compared to dystonia severity or symptom clusters, the mutation effects do not significantly associate with any phenotype [12]. This suggests that although diverse in their mechanism, mutations in *GNAL* lead to an

isolated dystonia phenotype through diverging from a narrow range of normal $G\alpha_{olf}$ activity.

Signaling partners of $G\alpha_{olf}$ also are associated with dystonia

Giving further validity to the critical nature of normal levels of $G\alpha_{olf}$ signaling are further human genetic studies that point to proteins both upstream and downstream of $G\alpha_{olf}$ as causative for dystonia. Mutations in five genes (*GCH1, TH, PTS, SPR,* and *QDPR*) that lead to disruption of the synthesis of dopamine are causative for dystonia [24–28]. Also upstream of $G\alpha_{olf}$, mutations in the D1 dopamine receptor (*DRD1*) are linked to infantile parkinsonism-dystonia and tardive-like dystonia [29, 30]. Downstream of $G\alpha_{olf}$, there are dystonia associated mutations in *ADCY5*, encoding adenylate cyclase 5, the enzyme responsible for the second messenger cAMP [31]. Further downstream enzymes, including *PDE2A* and *PDE10A* which are phosphodiesterase's responsible for metabolizing cAMP to AMP are causative for a neurological syndrome that includes dystonia as a symptom [32, 33]. Also, mutations in *DARPP-32*, a signal transducing protein of the cAMP effector protein kinase A are dystonia linked [34]. These mutations upstream and downstream of $G\alpha_{olf}$ show that disrupting normal dopamine signaling can cause dystonia. However, there is one key difference between *GNAL* mutations and mutations for the other proteins in this pathway, Other than rare bi-allelic autosomal recessive mutations in *GNAL* [6], these autosomal dominant mutations in *GNAL* lead to an isolated dystonia which is usually adult onset. However, these other mutations in the $G\alpha_{olf}$ pathway often produce a neurological disorder that has dystonia as a symptom of a larger clinical syndrome, rather than in isolation, with juvenile age of onset. Why *GNAL* mutations are unique in this regard are not known, but point to a unique opportunity for utilizing *GNAL* to understand the pathophysiology of dystonia.

$G\alpha_{olf}$ dysfunction is associated with other movement disorders

While not genetically linked to other movement disorders, alterations in expression and activity of $G\alpha_{olf}$ are associated with Parkinson's Disease and treatment induced dyskinesia [35]. This is most notable in levodopa induced dyskinesia, which is an adverse effect of the gold-standard of treatment for Parkinson's disease. In levodopa induced dyskinesia, expression levels of $G\alpha_{olf}$ are associated with development and severity of dyskinesia in experimental models of this disorder, and not upstream receptors or neurotransmitters [35–38]. This points to an important role of $G\alpha_{olf}$ activity in the regulation of normal movement, and also a unique role of aberrant $G\alpha_{olf}$ activity in producing abnormal movement. However, how $G\alpha_{olf}$ activity can differentially alter striatal output and basal ganglia activity to produce different disease states is not known.

Possible βγ contributions to *GNAL* dystonia

Gα subunits in the GDP bound state sequester Gβγ, and prevent the Gβγ subunits from interacting with their effector proteins [13]. In the striatum, $G\alpha_{olf}$ couples to $\beta_2\gamma_7$ to form a form the functional heterotrimeric G protein [17, 18]. While the levels of expression of individual components of the heterotrimeric G protein are linked, decreased expression or removal of a single component the heterotrimeric G protein does not completely eliminate the other G protein components. For example, genetic removal of $G\alpha_{olf}$ or γ_7 reduces the expression of the other, but does not eliminate it [12, 39]. Additionally, even in *GNAL* mutations that increase turnover of $G\alpha_{olf}$ or reduces stability of the trimer, there is still observable expression of $\beta_2\gamma_7$ [12]. This has led to all mutations in *GNAL* having observable unsequestered $\beta_2\gamma_7$ [12]. The consequences of this unsequestered and freely active $\beta_2\gamma_7$ are unknown. However, there are several possibilities. βγ subunits modulate the activity of several effector proteins including activation of phospholipase C, activation of GIRK channels, and inhibition of N, P, and Q type calcium channels [40–43]. Interestingly, one of the primary effectors of Gβγ, GIRK, are not expressed in striatal spiny projection neurons [44]. Unregulated modulation of these effectors by unsequestered $\beta_2\gamma_7$ could have profound effects on neuronal physiology and alter striatal activity. However, how unsequestered $\beta_2\gamma_7$, and possibly other βγ subunits in cells expressed outside the striatum, contribute to the development, maintenance, or expression of dystonia remains untested.

Models of *GNAL* linked dystonia

$G\alpha_{olf}$ expression and function were originally described in the olfactory epithelium where it is a key mediator of odorant receptor signaling. A global knockout of *GNAL* was made to study $G\alpha_{olf}$ in this context, and found that homozygous knockout of *GNAL* results in anosmia [19]. Also, due to feeding deficits, the majority of homozygous knockout mice die in early postnatal development. The original report of these mice showed that these homozygous knockout mice were paradoxically hyperactive, but the effects of *GNAL* loss on motor systems was confounded by the failure to thrive of global knockout mice that survived post weaning [19]. Heterozygous mice, however, are viable, have normal olfactory function, and are of comparable weight to wildtype littermate controls. These heterozygous mice do not display alterations in gross locomotor ability, but do have rotarod

deficits that are progressive with age [45]. More recently, a heterozygous *GNAL* rat was developed, but displays similar motor deficits to the mouse with decreased spontaneous locomotion and rotarod deficits [46]. However, both the mouse and the rat *GNAL* model do not display overt dystonia like motor deficits in both visual observation based assays as well as EMG [45, 46].

Key findings from rodent models of dystonia

Despite the general lack of overt dystonic symptoms in the *GNAL* heterozygous mice and rats, these models have driven valuable understanding of how loss of $G\alpha_{olf}$ alters the central nervous system, and have given possible electrophysiological and biochemical endophenotypes of neuronal dysfunction to link to dystonic symptoms. First, the *GNAL* heterozygous mouse can have abnormal movements induced by administration of the non-selective muscarinic acetylcholine receptor agonist oxotremorine either through intraperitoneal injection or microinjection into the dorsal striatum but not the cerebellum [45]. *GNAL* heterozygous mice are more sensitive than littermate controls to administration of oxotremorine, regardless of route of administration, which points to an important role for cholinergic interneurons of the striatum in regulating *GNAL* linked dystonia.

Second, rodent models have pointed to electrophysiological alterations in basal ganglia physiology that may be important to the generation of dystonic motor phenotypes. Interestingly, *GNAL* heterozygosity did not alter intrinsic properties of striatal spiny projection neurons in the rat model of *GNAL* with no changes in resting membrane potential, membrane resistance, or rheobase current [46]. Additionally, no baseline differences in cortico-striatal evoked post-synaptic currents or paired pulse ratio was observed. However, there was significant impairment of cortico-striatal long-term depression that was partially ameliorated through application of D_1 and D_2 dopamine receptor agonists and fully ameliorated through $Adenosine_{2A}$ receptor agonists [46]. These electrophysiological findings have pointed to a potential unique role in altering activity dependent striatal activity, and altering striatal processing of cortical inputs.

Next, rodent models of *GNAL* have pointed to nuclei outside of basal ganglia as being altered by *GNAL* heterozygosity. As discussed above, $G\alpha_{olf}$ is expressed in Purkinje Cells of the cerebellum [20]. Using *in vivo* electrophysiology and optogenetics, recent evidence has shown that in both an asymptomatic state and oxotremorine induced abnormal movement state, cerebellar connectivity to cortical and thalamic nuclei is altered [47]. At baseline, *GNAL* heterozygous mice have decreased cerebello-thalamic plasticity induced by deep cerebellar nuclei stimulation [47]. Also, after abnormal movements were induced through oxotremorine administration, both cortical and thalamic inputs from the cerebellum were altered after deep cerebellar nuclei stimulation [47]. Interestingly, this alteration in cerebellar-thalamic activity may drive striatal dysfunction as well through decreased connectivity to fast spiking interneurons in *GNAL* heterozygous mice [47].

However, an important caveat to all of these key findings is the lack of overt dystonic symptoms in these models which prevents the unambiguous link between electrophysiological and biochemical endophenotypes to dystonic symptoms. Development of rodent models that harbor the patient derived mutations in *GNAL* or a *Gnal* floxed mouse that allows for the conditional removal of *Gnal* may prevent the post-natal lethality of the global *Gnal* knockout mouse, and allow for the development of dystonia like movements in mice. This will be critical for understanding of the neuronal changes induced by mutations in *GNAL* to cause dystonia.

Targeting $G\alpha_{olf}$ for therapeutic benefit

Due to levels and activity of $G\alpha_{olf}$ being associated with other movement disorders, directly targeting $G\alpha_{olf}$ with small molecules may not be the optimal strategy to target *GNAL* linked dystonia [36, 37]. Also, due to the diversity of mechanisms of *GNAL* mutations, directly targeting $G\alpha_{olf}$ may prove impractical as well [12]. Instead, targeting known modifiers of $G\alpha_{olf}$ function may provide greater efficacy. One possibility is the M_4 muscarinic acetylcholine receptor [48, 49]. M_4 directly opposes $G\alpha_{olf}$ and D_1 dopamine receptor activity in direct pathway spiny projection neurons with M_4 activation inhibiting adenylate cyclase and subsequent cAMP production [50]. Removing this inhibition of adenylate cyclase in direct pathway spiny projection neurons through M_4 selective inhibitors may help to restore normal striatal output and basal ganglia activity to reduce dystonic symptoms [48, 49, 51]. Currently used small molecule therapeutics for dystonia may point to this treatment path as being promising. The non-selective muscarinic antagonist trihexyphenidyl remains a mainstay of treatment options for dystonia, but is not well tolerated by patients due to adverse effects [52, 53]. Selective M_4 antagonists may provide efficacy in *GNAL* linked dystonia without the adverse effects of non-selective muscarinic antagonists. The first series of truly M_4 selective antagonists have recently been developed [54], and the extent of their potential efficacy and liabilities has been highlighted in recent reviews [48, 49].

The defined signaling pathway of $G\alpha_{olf}$ also provides several additional possible therapeutic strategies for *GNAL* linked dystonia. Targeting modifiers of $G\alpha_{olf}$ which are downstream of $G\alpha_{olf}$ itself will likely be important, as targeting upstream proteins of $G\alpha_{olf}$ will likely not alter downstream signaling

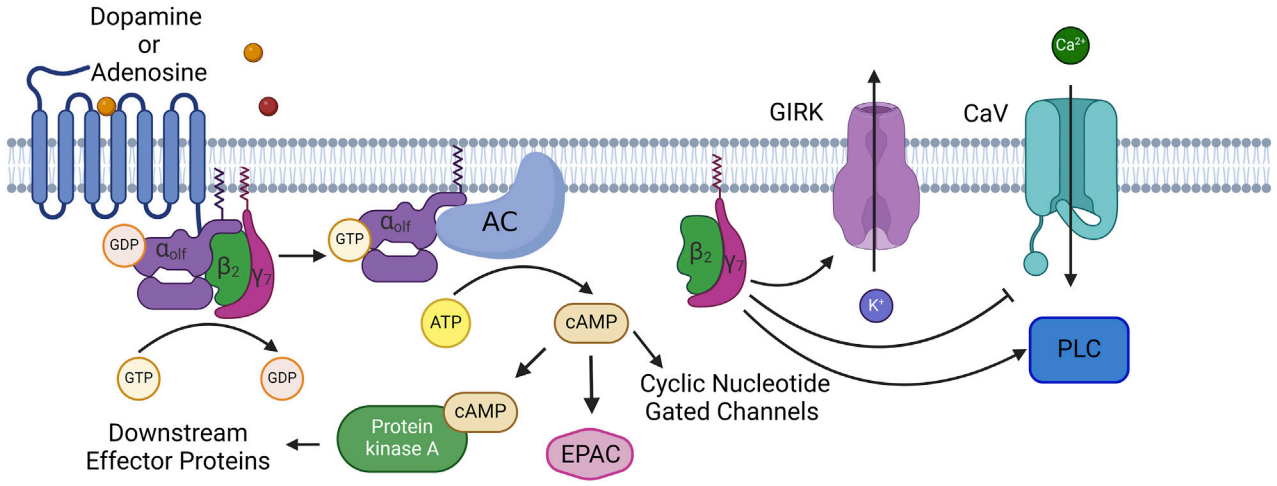

FIGURE 1
Normal Signaling Pathway of $G\alpha_{olf}$: At rest $G\alpha_{olf}$ is complexed with βγ subunits ($\beta_2\gamma_7$ in spiny projection neurons) into an inactive heterotrimer. When neurotransmitter binds to its GPCR, $G\alpha_{olf}$ switches GDP for GTP, causing the heterotrimer to disassociate and its components are now active. $G\alpha_{olf}$ will bind to and activate adenylate cyclase 5, and leads to the production and accumulation of the second messenger cAMP. cAMP has a number of effector proteins including protein kinase A (PKA), exchange proteins activated by cAMP (EPAC), and cyclic nucleotide gated channels. $\beta_2\gamma_7$ also will modulate the activity of several enzymes and channels. These include G-protein gated inward-rectifying potassium channels (GIRK), voltage gated calcium channels, N, P, and Q type calcium channels, as well as phospholipase C. Created with BioRender.com.

sufficiently. Activators of adenylate cyclase to boost cAMP levels itself may provide therapeutic benefit, but keeping the timing of cAMP production in sync with upstream neurotransmitter release will be challenging and may limit therapeutic efficacy [55]. Similarly, increasing cAMP through inhibiting its metabolism through phosphodiesterase may provide therapeutic efficacy, but may not allow for precise control of cAMP production in response to upstream neurotransmitter signaling [56]. Targeting of downstream effectors of cAMP produced by $G\alpha_{olf}$ such as downstream targets of protein kinase A and cyclic nucleotide gated channels also possible [57, 58], but further understanding of how these targets are altered in pre-clinical models of *GNAL* dystonia will be necessary.

Discussion

Mutations in *GNAL* are associated with both sporadic and familial dystonia [8]. These mutations lead to an adult onset dystonia that is usually not co-morbid with other disorders [1, 8]. This and other clinical characteristics of *GNAL* linked dystonia, suggest that this form of genetic dystonia may possibly have significant clinical and pathological overlap with idiopathic dystonia. This may represent a unique opportunity within dystonia research to utilize *GNAL* linked dystonia both in pre-clinical research and clinical research to determine, define and delineate the brain nuclei, cell types, and molecular events that are disturbed to cause disease. Translationally, this may represent unique opportunities for the design of rationale therapeutic strategies for a disease with few efficacious, well tolerated, or non-invasive therapies. Current animal models of *GNAL* linked dystonia have shown both striatal and cerebellar abnormalities, and a possible important role for striatal cholinergic signaling as a consequence of loss of $G\alpha_{olf}$ [45, 47, 59]. However, lack of overt dystonic symptoms in animal models of *GNAL* dystonia have prevented directly tying electrophysiological and biochemical endophenotypes with expression of dystonic motor phenotypes, and resulting in uncertainty over if these pathways are necessary or sufficient to drive dystonic motor phenotypes. Additionally, there are several open questions over the circuits, neuron types, and molecular signaling pathways that are altered to cause dystonia in these model systems. Generation of humanized mice that express the patient specific mutant forms of $G\alpha_{olf}$ or floxed mice that will allow for the conditional removal of $G\alpha_{olf}$ in specific cell types may allow for the development of symptomatic mice to directly address these questions. Further dissection of the mechanisms behind how mutations in *GNAL* lead to dystonia hold promise as a platform not only to mechanistically understand dystonia, but also as a platform to develop and test new anti-dystonic therapies.

Author contributions

MSM designed, conceptualized, and wrote the manuscript.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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