

ARTICLE

Mucopolysaccharidosis types II and III and non-syndromic stuttering are associated with different variants in the same genes

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Homozygous mutations in *GNPTAB* and *GNPTG* are classically associated with mucopolysaccharidosis II (ML II) alpha/beta and mucopolysaccharidosis III (ML III) alpha/beta/gamma, which are rare lysosomal storage disorders characterized by multiple pathologies. Recently, variants in *GNPTAB*, *GNPTG*, and the functionally related *NAGPA* gene have been associated with non-syndromic persistent stuttering. In a worldwide sample of 1013 unrelated individuals with non-syndromic persistent stuttering we found 164 individuals who carried a rare non-synonymous coding variant in one of these three genes. We compared the frequency of these variants with those in population-matched controls and genomic databases, and their location with those reported in mucopolysaccharidosis. Stuttering subjects displayed an excess of non-synonymous coding variants compared to controls and individuals in the 1000 Genomes and Exome Sequencing Project databases. We identified a total of 81 different variants in our stuttering cases. Virtually all of these were missense substitutions, only one of which has been previously reported in mucopolysaccharidosis, a disease frequently associated with complete loss-of-function mutations. We hypothesize that rare non-synonymous coding variants in *GNPTAB*, *GNPTG*, and *NAGPA* may account for as much as 16% of persistent stuttering cases, and that variants in *GNPTAB* and *GNPTG* are at different sites and may in general, cause less severe effects on protein function than those in ML II alpha/beta and ML III alpha/beta/gamma.

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INTRODUCTION

Stuttering is a common disorder characterized by involuntary disruptions in the flow of speech that has been shown to have strong genetic contributions.^{1–5} The disorder has been associated with variants in *GNPTAB* (encoding *N*-acetylglucosamine-1-phosphotransferase alpha/beta subunits precursor), *GNPTG* (encoding *N*-acetylglucosamine-1-phosphotransferase gamma subunit), and *NAGPA* (encoding *N*-acetylglucosamine-1-phosphodiester alpha-*N*-acetylglucosaminidase).⁶ In the initial report, 6.3% of unrelated subjects with persistent stuttering carried a variant in one of these three genes.⁶ Using a larger sample we have re-examined the frequency of rare non-synonymous coding variants in these genes in this disorder. Variants in *GNPTAB* and *GNPTG* are classically associated with mucopolysaccharidosis II (ML II) alpha/beta (OMIM #252500), mucopolysaccharidosis III (ML III) alpha/beta (OMIM #252600), and mucopolysaccharidosis III (ML III) gamma (OMIM #252605), which are rare, severe lysosomal storage disorders caused by homozygous mutations in these genes. We also compared the rare coding variants observed in *GNPTAB* and *GNPTG* in stuttering subjects with those reported in mucopolysaccharidosis patients.

MATERIALS AND METHODS

Unrelated individuals with persistent developmental stuttering and no other neurological conditions were enrolled in the United States and England (HCRI +NIH and NAF series, 634 total subjects), Pakistan (PKST series, *N*=124), Cameroon (STCR series, *N*=96), and Brazil (BRCS series, *N*=159), and population/gender-matched controls from the United States (NDPT series, *N*=276), Pakistan (RPP series, *N*=96), Cameroon (RC series, *N*=94), and Brazil (BRCO series, *N*=211) with written informed consent under NIH protocol #97-DC-0057, as previously described.⁶ The DNAs from North American subjects determined to be normal following extensive neurological examination were obtained from spouse controls in a large population-based study of Parkinson's Disease (National Institute of Neurological Disorders and Stroke (NINDS) panels NDPT006, -020, -023, -079, -082 and -093) from the Coriell Cell Repository. Extensive clinical data on these subjects and access to these DNAs is available at <http://ccr.coriell.org/Sections/Collections/NINDS/DNApanels.aspx?PgId=195&coll=ND>. All affected subjects reported a family history of stuttering. Sequences from large population samples were obtained from the 1000 Genomes Project database (<http://www.1000genomes.org>) and the NHLBI Exome Sequencing Project (ESP, comprising ~6400 exomes) (<http://evs.gs.washington.edu/EVS/>). Variants associated with ML II alpha/beta

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Table 1 Rare non-synonymous variations in GNPTAB (NG_021243.1) in stuttering cases and control populations

cDNA position	Rare variants in GNPTAB		Total		Population-specific stuttering cases						Population-specific controls				
	AA change	SNP ID	Cases	Controls	NIH (W)	NIH (Af)	NAF	PKST	STCR	BRCS	RPP	RC	BRCO	NDPT	
			ESP6400	N = 677	N = 434	N = 56	N = 144	N = 124	N = 96	N = 159	N = 96	N = 94	N = 211	N = 276	
c.70 T>G	p.(Phe24Val)	rs141329633	4	2	2	—	—	—	—	—	—	—	—	—	
c.137G>A	p.(Arg46Gln)	rs117566084	10	11	5	4	—	—	—	2	—	—	—	3	
c.232_234del	p.(Val78del)	rs281864952	1	1	1	—	—	—	—	—	—	—	—	—	
c.337 A>G	p.(Lys113Glu)	rs140656599	2	3	1	—	—	—	—	2	—	—	—	—	
c.500 T>A	p.(Ile167Asn)	rs143907628	4	—	—	—	—	—	—	—	—	—	1	—	
c.560G>C	p.(Ser187Thr)	Unknown	—	1	1	—	—	—	—	—	—	—	—	—	
c.580G>A	p.(Ala194Thr)	Unknown	—	1	—	—	—	—	—	—	—	—	—	1	
c.886 A>G	p.(Thr296Ala)	Unknown	—	1	—	—	—	—	—	—	—	—	1	—	
c.961 A>G	p.(Ser321Gly)	rs137853824	1	1	—	—	—	1	—	—	—	—	—	—	
c.1253 A>G	p.(Asp418Gln)	Unknown	—	1	—	—	—	—	—	1	—	—	—	—	
c.1283 A>G	p.(Lys428Arg)	rs145281185	2	1	—	—	—	—	—	—	—	—	—	—	
c.1363G>T	p.(Ala455Ser)	rs137853822	—	2	2	—	—	—	—	—	—	—	—	—	
c.1429 T>G	p.(Tyr477Asp)	rs145586576	4	—	—	—	—	—	—	—	—	—	—	—	
c.1433 T>C	p.(Ile478Thr)	rs149718548	5	1	—	—	—	—	—	—	—	—	—	—	
c.1669 A>C	p.(Ile557Leu)	rs142025274	1	1	1	—	—	—	—	—	—	—	—	—	
c.1803 A>G	p.(Ile601Met)	Unknown	—	1	—	—	—	—	—	—	—	—	—	1	
c.1875C>G	p.(Phe625Leu)	rs137853823	—	1	—	—	—	1	—	—	—	—	—	—	
c.1931_1932inv	p.(Thr644Met)	Unknown	—	43	3	2	8	—	23	7	4	6	—	—	
c.1943G>A	p.(Gly648Asp)	Unknown	—	2	—	—	—	—	—	2	—	—	—	—	
c.1948G>A	p.(Glu650Lys)	Unknown	—	1	1	—	—	—	—	—	—	—	—	—	
c.1985C>T	p.(Ala662Val)	rs142172397	—	1	—	—	—	—	1	—	—	—	—	—	
c.2081 A>C	p.(Lys694Thr)	Unknown	—	1	—	—	—	—	—	—	—	—	—	—	
c.2341G>A	p.(Val781Met)	rs183435240	1	1	—	—	—	—	—	1	—	—	—	—	
c.2582G>A	p.(Arg861Lys)	rs139411012	—	2	—	—	—	—	1	—	—	—	—	—	
c.2900 A>G	p.(Gln967Arg)	rs192687061	3	1	—	—	—	—	1	—	—	—	—	—	
c.3571C>T	p.(Arg1191Cys)	Unknown	—	1	—	—	—	—	1	—	—	—	—	—	
c.3572G>A	p.(Arg1191His)	rs376398528	—	1	—	—	—	—	—	—	—	—	—	1	
c.3598G>A	p.(Glu1200Lys)	rs137853825	1	8	2	—	—	—	—	—	—	—	—	—	
c.3707 A>G	p.(Lys1236Arg)	Unknown	—	1	1	—	—	—	—	—	—	—	—	—	
Total			15	125	87	22	4	12	9	27	15	2	5	9	6

Abbreviations: AA, amino acid; Af, African; BRCO, Brazilian control; BRCS, Brazilian stuttering cases; ESP, Exome Sequencing Project; NAF, North American families (European descent); NDPT, neurologically normal; NIH, National Institutes of Health; PKST, Pakistani stuttering; RC, random Cameroonian control; RPP, random Pakistani population; STCR, stuttering Cameroonian random; W, white (European descent); —, 0.

Table 2 Rare non-synonymous variations in GNPTG (NG_016985.1) in stuttering cases and control populations

cDNA position	Rare variants in GNPTG		Total		Population-specific stuttering cases						Population-specific controls				
	AA change	SNP ID	Genome databases	Cases	Controls	NIH (W)	NIH (Af)	NAF	PKST	STCR	BRCS	RPP	RC	BRCO	NDPT
			1000g	ESP6400	N = 677	N = 434	N = 56	N = 144	N = 124	N = 96	N = 159	N = 96	N = 94	N = 211	N = 276
c.7G>A	p.(Ala3Thr)	Unknown	—	—	—	—	—	1	—	1	—	—	—	—	—
c.8C>T	p.(Ala3Val)	Unknown	—	2	—	1	—	—	—	1	—	—	—	—	—
c.15_23dup	p.(Ala6_Leu8dup)	Unknown	—	1	—	1	—	—	—	—	—	—	—	—	—
c.19C>T	p.(Arg7Trp)	Unknown	—	—	1	—	—	—	—	—	1	—	—	—	—
c.28T>G	p.(Leu10Thr)	Unknown	—	3	—	—	—	—	—	3	—	—	—	—	—
c.74C>A	p.(Ala25Glu)	rs137853826	—	2	—	2	—	—	—	—	—	—	—	—	—
c.74C>G	p.(Ala25Gly)	rs137853826	—	1	—	1	—	—	—	—	—	—	—	—	—
c.93G>C	p.(Glu31Asp)	rs8052503	—	11	—	—	1	—	—	—	—	—	—	—	—
c.161 A>T	p.(Asp54Val)	rs368478807	—	6	—	—	1	—	—	—	—	—	—	—	—
c.230C>A	p.(Ser77Tyr)	Unknown	—	—	2	—	—	—	—	—	—	2	—	—	—
c.316G>A	p.(Gly106Ser)	rs137852885	—	—	1	—	—	—	—	—	—	—	—	1	—
c.388C>T	p.(Arg130Cys)	Unknown	—	—	—	1	—	—	—	—	—	—	—	—	—
c.401G>A	p.(Arg134Gln)	Unknown	—	—	1	—	—	—	—	—	—	—	—	1	—
c.450 T>G	p.(His150Gln)	rs147109185	8	51	—	—	—	—	—	1	—	—	—	—	—
c.472G>A	p.(Val158Ile)	rs138018487	1	9	1	2	—	2	—	—	—	—	—	—	1
c.479C>T	p.(Ala160Val)	rs374470431	—	1	1	—	1	—	—	—	1	—	—	—	—
c.499dup	p.(Leu167Profs*32)	Unknown	—	8	—	—	—	—	—	—	—	—	—	—	—
c.502G>A	p.(Val168Ile)	rs190614894	—	—	1	—	—	—	—	—	1	—	—	—	—
c.557G>A	p.(Arg186Gln)	rs139997459	1	1	—	—	—	1	—	—	—	—	—	—	—
c.574G>C	p.(Glu192Gln)	Unknown	—	1	—	—	—	—	—	1	—	—	—	—	—
c.688C>G	p.(Leu230Val)	rs137853827	—	—	—	1	—	—	—	—	—	—	—	—	—
c.814 A>G	p.(Arg272Gly)	rs8062558	6	26	1	—	1	1	—	2	1	—	—	—	1
c.878 A>C	p.(Glu293Ala)	rs372062796	—	2	—	—	—	2	—	—	—	—	—	—	—
c.887G>A	p.(Arg296Gln)	Unknown	—	—	—	2	—	—	—	—	—	—	—	—	—
c.904C>T	p.(Arg302Cys)	Unknown	—	—	1	—	—	—	—	—	—	—	—	1	—
c.910 A>G	p.(Ser304Gly)	rs7187001	10	40	2	—	1	—	—	6	—	2	—	—	—
Total			26	155	12	12	5	8	0	15	5	3	4	3	2

Abbreviations: AA, amino acid; Af, African; BRCS, Brazilian stuttering cases; ESP, Exome Sequencing Project; NAF, North American families (European descent); NDPT, neurologically normal; NIH, National Institutes of Health; PKST, Pakistani stuttering; RC, random Cameroonian control; STCR, stuttering Cameroonian random; W, white (European descent); —, 0.

Table 3 Rare non-synonymous variations in NAGPA (NG_028152.1) in stuttering cases and control populations

cDNA Position	Rare variants in NAGPA		Total		Population-specific stuttering cases							Population-specific controls			
	AA change	SNP ID	Genome databases	Cases	Controls	NIH (W)	NIH (Af)	MAF	PKST	STCR	BRCS	RPP	RC	BRCO	NDPT
			1000g ESP6400	N = 1013	N = 677	N = 434	N = 56	N = 144	N = 124	N = 96	N = 159	N = 96	N = 94	N = 211	N = 276
c.131G>C	p.(Arg44Pro)	rs374266430	—	1	—	1	—	—	—	—	—	—	—	—	—
c.136C>T	p.(Arg46Cys)	Unknown	—	1	—	—	—	—	—	—	—	—	—	—	—
c.220G>C	p.(Gly74Arg)	Unknown	—	—	1	—	—	—	—	—	—	—	—	1	—
c.236G>A	p.(Arg79His)	rs373128375	7	1	—	—	—	1	—	—	—	—	—	—	—
c.252C>G	p.(His84Gln)	Unknown	—	2	—	2	—	—	—	—	—	—	—	—	—
c.257G>A	p.(Arg86Lys)	Unknown	—	1	—	1	—	—	—	—	—	—	—	—	—
c.299C>T	p.(Pro100Leu)	Unknown	—	—	1	—	—	—	—	—	1	—	—	—	—
c.562G>A	p.(Val188Met)	rs138721187	2	1	—	—	—	—	—	—	—	—	—	—	—
c.591G>C	p.(Gln197His)	rs146071322	6	2	—	1	—	—	—	—	—	—	—	—	—
c.641A>G	p.(Asn214Ser)	rs140529374	14	1	1	—	—	—	—	—	—	—	—	1	—
c.667G>A	p.(Glu223Lys)	rs143077001	1	2	3	—	—	1	—	1	—	—	—	—	—
c.676G>C	p.(Glu226Gln)	rs200736428	3	1	—	—	—	1	—	—	—	—	—	—	—
c.785A>G	p.(Gln262Arg)	Unknown	—	—	1	—	—	—	—	—	—	—	—	—	—
c.851A>C	p.(Asn284Thr)	rs139566850	1	—	1	—	—	—	—	—	—	—	—	1	—
c.947G>A	p.(Arg316His)	rs201325687	2	1	—	1	—	—	—	—	—	—	—	—	—
c.982C>T	p.(Arg328Cys)	rs139526942	10	8	1	7	—	1	—	—	—	—	—	1	—
c.1129G>A	p.(Gly377Ser)	Unknown	—	2	—	—	1	—	—	—	—	—	—	—	—
c.1187G>C	p.(Gly396Ala)	Unknown	—	1	—	1	—	—	—	—	—	—	—	—	—
c.1209G>C	p.(Gln403His)	rs141084182	1	1	—	1	—	—	—	—	—	—	—	—	—
c.1243G>C	p.(Asp415His)	rs147782887	1	—	1	—	—	—	—	—	—	—	—	—	1
c.1264A>T	p.(Ser422Cys)	rs367664804	1	—	1	—	—	—	—	—	—	—	—	1	—
c.1319G>C	p.(Gly440Ala)	rs149923128	2	2	3	—	—	—	1	—	1	—	—	1	1
c.1367C>T	p.(Ala456Val)	Unknown	—	1	—	1	—	—	—	—	—	—	—	—	—
c.1407del	p.(Leu469Leufs*26)	Unknown	—	1	—	—	—	—	—	1	—	—	—	—	—
c.1501G>A	p.(Ala501Thr)	rs141568446	27	1	—	—	—	—	—	—	—	—	—	—	—
c.1538_1553del	p.(Phe513Serfs*113)	Unknown	—	1	—	—	—	—	—	—	—	—	—	—	—
Total			4	97	14	17	1	6	1	2	5	2	4	6	2

Abbreviations: AA, amino acid; Af, African; BRCO, Brazilian control; BRCS, Brazilian stuttering cases; ESP, Exome Sequencing Project; MAF, North American families (European descent); NDPT, neurologically normal; NIH, National Institutes of Health; PKST, Pakistani stuttering; RC, random Cameroonian control; RPP, random Pakistani population; STCR, stuttering Cameroonian random; W, white (European descent); —, 0.

and ML III alpha/beta/gamma were obtained from the ClinVar database (<http://www.ncbi.nlm.nih.gov/clinvar/?term=mucopolipidosis>), and from Cathey *et al.*, Kudo *et al.*, Liu *et al.*, Paik *et al.*, and Tiede *et al.*^{7–11}

Stuttering diagnosis was performed using the Stuttering Severity Index-3 (SSI-3)¹² and as previously described.¹³ All subjects were unrelated by self-report, and no genotypic evidence for relatedness among any subjects was observed.

Coding and adjacent intronic sequences of *GNPTAB* (NM_024312.4, NG_021243.1), *GNPTG* (NM_032520.4, NG_016985.1), and *NAGPA* (NM_016256.3, NG_028152.1) were amplified as previously described.⁶ Sequence variants were referenced from and have been deposited to the Locus Variation Data Base; <http://databases.lovd.nl/shared/genes/GNPTAB> for *GNPTAB*, <http://databases.lovd.nl/shared/genes/GNPTG> for *GNPTG*, and <http://databases.lovd.nl/shared/genes/NAGPA> for *NAGPA*.

RESULTS

Sequencing of the exons and flanking ≥ 50 bp of intronic sequence of the *GNPTAB*, *GNPTG*, and *NAGPA* genes in unrelated individuals with familial persistent developmental stuttering identified many non-synonymous coding variants. Variants that occurred at high frequency in cases and our population-matched controls were not considered further. In our previous studies, all of the stuttering-associated variants found in these three genes were rare coding sequence variants.⁶ We therefore focused on non-synonymous coding sequence variants defined as rare by their presence at a frequency of 0.005 or less in the 1000 Genomes database. These variants are shown in Tables 1 (*GNPTAB*), 2 (*GNPTG*), and 3 (*NAGPA*). In our combined stuttering subject population (North American, Pakistani, Cameroonian, and Brazilian), such variants were observed in *GNPTAB* in 87 of 1013 cases, in *GNPTG* in 45 of 1013 cases, and in *NAGPA* in 32 of 1013 cases, for a total of 164 out of 1013 individuals (16%). All carried a single copy of the variant. In total, these 164 subjects carried 81 different variants in these three genes. In contrast, we observed such variants in a total of 48 individuals out of 677 combined controls. This rate is significantly different from that observed in our combined cases sample ($\chi^2 = 30.63$, $P = 3.13 \times 10^{-8}$).

We also identified an excess of rare non-synonymous coding variants in these three genes in stuttering cases compared with the individuals in the 1000 Genomes database and Exome Sequencing Project database (~6400 individuals), at a rate of 422/7400 in these databases vs 164/1013 in our cases ($\chi^2 = 151.21$, $P = 9.42 \times 10^{-35}$).

The excess of rare variants in cases compared with controls existed within all subpopulations in our sample, including those of European origin (75/578 cases vs 10/276 controls, $\chi^2 = 18.23$, $P = 1.9 \times 10^{-5}$) and those of African ancestry including Brazilians, who have a large component of African ancestry,¹⁴ (79/311 cases vs 31/305 controls, $\chi^2 = 24.4$, $P = 7.93 \times 10^{-7}$).

In addition to analyzing the sum of rare variants in cases and controls, we also examined the subset of variants that were observed in cases but not in controls, and compared these with variants observed in controls but not in cases. As shown in Tables 1–3, this subset comprised a total of 66 case subjects carrying variants that occurred in cases and not in controls, and 20 control subjects that carried a variant not seen in cases, a significant difference ($\chi^2 = 10.65$, $P = 1.1 \times 10^{-3}$).

We also surveyed rare variants in adjacent intronic sequences. We defined variants that could affect existing splice sites as those within 10 bp of an exon, and identified a total of four such rare variants in our cases ($N = 1013$) in these three genes. All were present at similar frequencies in our neurologically normal controls, in our population-matched control groups, and in the Exome Sequencing Project and 1000 Genomes databases.

With few exceptions, the coding variants in our stuttering cases were all missense amino-acid substitutions (Tables 1–3). A total of 81 mutations have been previously reported in ML II alpha/beta and ML III alpha/beta/gamma.^{7–11} None of these variants occurred in our stuttering case subjects with one exception, which was the 3 bp in-frame deletion in *GNPTAB* at amino-acid position 78 (Table 1). In particular, several mutations commonly observed in ML II alpha/beta and ML III alpha/beta/gamma, including the c.1399delG and c.3503_3504del frameshift mutations and the c.3335+6 T>C splice site mutation in *GNPTAB* were not observed in our stuttering cases.

DISCUSSION

Our results confirm that rare non-synonymous coding variants in *GNPTAB*, *GNPTG*, and *NAGPA* are significantly more common in stuttering cases compared with controls and to the general populations, and provide an improved estimate of the contribution of mutations in these genes to persistent developmental stuttering. Our present estimate of 16% is substantially higher than the previous estimate of 6.3%. However, we also identified rare variants that met our criteria in control subjects, albeit at a lower rate (7%). If this 7% is considered the background rate of normal variation in these genes, then $16 - 7 = 9\%$ of stuttering cases are attributable to variants in these three genes. However, perhaps as many as 1% of the individuals in the 1000 Genomes and ESP6400 databases are presumed to display stuttering to some degree, and as many as 5% had a history of childhood stuttering.¹⁵ In this case, rare coding variants in such individuals are not normal variation, and we hypothesize that the 16% would be an accurate estimate of the fraction of stuttering cases attributable to such variants.

Previous clinical studies of stuttering individuals who carry a mutation in *GNPTAB*, *GNPTG*, or *NAGPA* failed to reveal any symptoms of ML II alpha/beta and ML III alpha/beta/gamma,⁶ and it has been suggested that the mutations in *GNPTAB* and *GNPTG* that cause stuttering are fundamentally different than those that cause mucopolipidosis. Our current results support this hypothesis. Only one of the 42 different variants in *GNPTAB* and *GNPTG* found in our stuttering subjects has been previously reported in mucopolipidosis. In addition, more than 50 different mutations in *GNPTAB* and more than 30 different mutations in *GNPTG* have been identified in mucopolipidosis patients^{7–11} (www.genecards.org). The majority of these mutations (85%) are frameshifts, stop codons, deletions, or splice site mutations.⁷ Of the variants in *GNPTAB*, *GNPTG*, and *NAGPA* found in our stuttering subjects, 75/81 (92.6%) are missense substitutions, and the common mutations found in ML II and ML III were not observed. The one mucopolipidosis mutation observed in our stuttering cases was not a missense mutation (the in-frame 3 bp deletion encoding the valine at amino-acid position 78 in *GNPTAB*) and was observed in heterozygous state in one stuttering subject. This mutation has been previously reported homozygous in one case of ML III alpha/beta/gamma (www.ncbi.nlm.nih.gov/clinvar/).

Our findings support the view that persistent stuttering is associated with mutations in *GNPTAB* or *GNPTG* that are generally not found in mucopolipidosis and exert a less deleterious effect on protein function, and they provide an improved estimate of the contribution of mutations in these genes to stuttering.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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