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Determination of regulatory motifs and pathogenicity of intronic variants of *GNPTAB*, *GNPTG*, and *NAGPA* genes in individuals with stuttering

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Abstract

Background: Stuttering is a fluency disorder typically characterized by part-word repetitions, voiced or voiceless sound prolongations, and broken words. Evidence suggests that 1% of the world population stutters. Compelling evidence from past research suggests that stuttering is caused by non-synonymous coding sites. This study evaluates the intronic regions of *GNPTAB*, *GNPTG*, and *NAGPA* genes for possible pathogenicity of intronic variants from unrelated non-syndromic stutterers in a cohort of the south Indian population.

Results: High-throughput sequencing revealed 41 intronic variants. Computational tool Reg-SNP Intron identified three intronic variants rs11110995 A>G, rs11830792 A>G, and rs1001171 T>A of having a plausible pathogenic impact which was identified in 37.9%, 26.5%, and 59.4% of stutterers, respectively. RegulomeDB identified the regulatory motifs and susceptible loci of the intronic variants.

Conclusions: This study imparts the identification, association, and interpretation of pathogenicity and regulatory significance of the intronic variants in the context of the noncoding DNA elements. Future work is warranted to better understand the role of the intronic variants in a larger cohort of stutterers, and a cohort of fluent controls would be valuable.

Keywords: Stuttering, Introns, Allele frequencies, *GNPTAB*, *GNPTG*, *NAGPA*

Background

Stuttering is a fluency disorder resulting in various forms of speech interruptions affecting all language groups which typically arise in children aged ~2 to 5 years when they begin to develop more complex speech and language (Reilly et al. 2013; Didirková et al. 2021; Polikowsky et al. 2022). Stuttering occurs predominantly in males than females with a male-to-female ratio of 5:1 and most of them; particularly the females recover spontaneously

or with the aid of speech therapy (Drayna and Kang 2011; Yairi and Ambrose 2013). It has long been observed that stuttering frequently runs in families and is highly heritable (Fedyna et al. 2011; Barnes and Neutel 2016; Bloodstein et al. 2021). Various Studies have elucidated a solid genetic influence on stuttering risk and identified coding variants in *GNPTAB*, *GNPTG*, and *NAGPA* genes which have been linked to mutations in the lysosomal enzyme-targeting pathway (Riaz et al. 2005; Kang et al. 2010; Raza et al. 2016; Frigerio-Domingues and Drayna 2017; Gunasekaran et al. 2021).

Over the years, research on neurological aspects of stuttering has been carried out to understand the nature and metabolism of the disorder (Alm 2021). Expression

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of stuttering genes (*GNPTAB* and *NAGPA*) in children with persistent stuttering and non-stuttering controls revealed gray matter differences linked to lysosomal deficits (Chow et al. 2020). Lysosomal deficits likely reduce the processing of biomolecules (Alm 2021); energy metabolism was observed in mice carrying the mutant *GNPTAB* gene which had fewer astrocytes in the brain which could be the result of a reduced peak rate of energy supply to the motor system (Barnes and Neutel 2016).

The genes *GNPTAB* [NM_024312.4] (*N*-acetylglucosamine-1-phosphate transferase subunits alpha and beta) located on chromosome 12q23.2 together with *GNPTG* [NM_032520.4] (*N*-acetylglucosamine-1-phosphate transferase subunit gamma) located on chromosome 16p13.3 encodes for a phosphotransferase enzyme, while *NAGPA* [NM_016256.3] (*N*-acetylglucosamine-1-phosphodiester alpha-*N*-acetylglucosaminidase) also located on chromosome 16p13.3 encodes an enzyme responsible for the removal of *N*-acetylglucosamine, thus uncovering the mannose 6 phosphate (M6P) targeting acid hydrolases to lysosomes (Kazemi et al. 2018; Gunasekaran et al. 2021). Some candidate missense variants in stuttering such as *GNPTAB*: rs137853824, rs137853823, rs137853825; *GNPTG*: rs137853827; and *NAGPA*: rs139526942 were previously detected in stutterers (Kang et al. 2010).

Genome-wide association studies (GWAS) by high-throughput sequencing have identified several loci linked with the trait and identified additional candidate genes. Exonic mutations in the *SLC6A3* gene (rs2617604, rs28364997, rs28364998) and *DRD2* gene (rs6275, rs6277) were detected among the Hans Chinese patients with speech disfluency (Lan et al. 2009); AP4E1 gene (rs760021635, rs556450190) variants among a large African family (Raza et al. 2013, 2015); *CYP17A1* gene (rs743572) variant among the Kurdish (Mohammadi et al. 2017); and *CYRIA* gene (rs12613255) variant in patients of European ancestry (Shaw et al. 2021). Also, high-throughput sequencing has transformed to detect an abundance of variants of noncoding segments (introns) through several GWAS (Reuter et al. 2015; Elliott and Larsson 2021). Sequence elements within the nuclear introns may modulate significant functions in gene expression, mRNA export, splicing, alternative splicing and transcription coupling (Berk 2016; Panaro et al. 2022). Studies on intronic variants in stuttering are limited, and only a few studies have reported the presence of fewer intronic alleles. Therefore, the current study was performed to reveal the intronic single-nucleotide variants (iSNVs) of three candidate genes (*GNPTAB*, *GNPTG*, and *NAGPA*) to conceal the possible pathogenicity of intronic variants in the south Indian cohort who stutter.

Methods

Recruitment and stuttering examination

The study included 100 participants (94 male and 6 female) > 18 years of age, who enrolled for speech impairment assessment at the All India Institute of Speech and Hearing (AIISH). The study participants had a detailed speech pathology examination. Individuals without any associated communication, cognition, psychological, and neurological problems except for developmental stuttering were selected. Among the 100 participants, 67/100 (67%) had a family history of stuttering and the remaining 33/100 (33%) participants had no family history. The distribution of severity ranged from very mild 46/100 (46%), moderate 36/100 (36%) to very severe 18/100 (18%) stuttering with an average onset age of 2–5 years. The stuttering Severity Instrument (Riley and Bakker 2009) was used to document the severity of overt stuttering.

Sample and DNA isolation

About 5 ml of peripheral venous blood was collected from the study participants ($n=100$) by standard phlebotomy. DNA isolation was done using PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific) as per the manufacturer's protocol.

Massively parallel sequencing and analysis

Among the 100 samples, only 79 samples (75 males: 4 females; mean age \pm SD = 26 \pm 6.49 years) were selected based on the DNA quantitation. Custom-targeted libraries were constructed by Ion AmpliSeq Library Kit Plus (Life Technologies) and PCR enrichment was done using Ion AmpliSeq Exome RDY panel (Life Technologies) according to the manufacturer's protocols. Sequencing was processed on the Ion Proton™ next-generation sequencing systems (Life Technologies) following the manufacturer's guidelines. All sequencing data passed specific minimal quality control requirements, and the sequence read alignment and variant calling were performed with the reference genome (hg19) using TMAP Alignment (Thermo Fisher Scientific). Variants were detected using the Ion Reporter (Thermo Fisher Scientific).

Allele frequency estimation, functional annotation, and pathogenicity prediction of iSNVs

Intronic variants were filtered based on the allele frequencies. The allele frequencies of the variants were compared with the gnomAD database (Karczewski et al. 2020) (<https://gnomad.broadinstitute.org/>) that served as a control. RegulomeDB (<http://regulomedb.org>) is a database integrating information from the Encyclopedia of DNA Elements (ENCODE) that was used to annotate single-nucleotide variants (Boyle et al. 2012). Reg-SNP

Intron (<https://regsnps-intron.cccb.iupui.edu/>), a computational framework, was used to predict the pathogenic impact of intronic single-nucleotide variants (Lin et al. 2019).

Statistical analysis

Descriptive statistics, i.e., mean, standard deviation (SD), and probability values of the allele frequencies, were analyzed using Statistical Package for Social Sciences (SPSS v21 IBM Corp New York).

Results

In this study, massively parallel sequencing of the three genes *GNPTAB*, *GNPTG*, and *NAGPA* identified 41 iSNVs in 79 samples (75 males and 4 females; mean age \pm SD = 26 \pm 6.49 years). Among the indexed patients, mild stuttering (46%) was more prevalent followed by moderate (36%) and severe (18%) and all the study participants were of south Indian descent. Allele frequencies of the 41 iSNVs were compared with the allele frequencies of the South Asian record and total allele frequency record using the gnomAD database; the allele frequency was highly significant and consistent with both south Asian ($p=0.001$) and total allele frequency ($p=0.001$) from gnomAD database (Table 1).

Functional annotation of intronic SNVs identified in this study

RegulomeDB was used to identify the potential regulatory/functional iSNVs. Overall 41 iSNVs were identified in this study, out of which 38 revealed RegulomeDB scores of 1- 6 and 3 with a score of 7 (Table 1 and Additional file 1: Table S1) Further, 6 iSNVs showed comparatively more evidence for the regulatory element with a score of 1, which included 5 iSNVs (rs11111002, rs4764814, rs4764813, rs1001171, and rs1001170) with a score of 1f and 1 iSNV (rs11110995) with a score of 1d. Expression quantitative trait loci (eQTLs) were observed in *GNPTAB* and *NAGPA* gene variants which describes a fraction of the genetic variance of a gene expression phenotype (Nica and Dermitzakis 2013). It is noticeable that the lesser the RegulomeDB score, it is more likely that it would be the variant that lies within a potential functional region (Liao et al. 2016). Detailed information about the regulatory iSNVs and functional annotation of other variants observed in the study, viz. likely/less likely affecting binding, and minimal binding are shown in Additional file 1: Table S1.

Pathogenicity of iSNVs

The pathogenic impact of intronic SNVs was analyzed using RegSNPs-intron which measures the impact of splicing on an intronic variant with structural features

corresponding to potential alternatively spliced exons. The assay identified three iSNVs: *GNPTAB*: c.3603 - 1359A>G (rs11110995) in 30/79 (37.9%) of the cases and c.324 - 457A>G (rs11830792) in 21/79 (26.5%) cases and *NAGPA*: c.543 - 404T>A (rs1001171) in 47/79 (59.4%) cases with the prediction score of having a potentially deleterious effect (Table 2), and the remaining 38 iSNVs were benign (Additional file 1: Table S2).

Discussion

Stuttering is a disorder of speech interruptions or disfluency which is highly heritable and has a strong genetic influence. This study describes the potential regulatory and pathogenic effect of intronic SNVs which has been discussed. Apart from the coding exonic variants, the noncoding intron plays a vital part in gene regulation (Rose 2019). The assortment of proteins is enhanced by alternative splicing where introns play important roles in producing multiple variant proteins from a single gene in a eukaryotic cell (Wang et al. 2015; Yang et al. 2021). Conservations in flanking introns of conserved alternative exons regulate alternative splicing (Pan et al. 2008; Vaz-Drago et al. 2017; Yang et al. 2021). In this study, we investigated the intronic variants of *GNPTAB*, *GNPTG*, and *NAGPA* genes and predicted the pathogenic impact of intronic SNVs using the RegSNPs-intron tool. This study identified three possibly pathogenic intronic variants rs11110995, rs11830792, and rs1001171. Previous studies have reported an intronic variant g.10985G>A in the *GNPTG* gene among the Iranian stutterers (Kazemi et al. 2018), and another intronic variant c.192+618G>A (rs7837758) in the *ZMAT4* gene in stutterers of African ancestry was also reported (Shaw et al. 2021).

Among the three possibly pathogenic intronic variants detected, rs11110995A>G in *GNPTAB* gene with a RegulomeDB score of 1d which is an eQTL that likely affects binding and is linked to the expression of a gene target, pathogenicity estimation showed a damaging effect which was detected in 30/79 (37.9%) of the stutterers. The variant rs11830792 A>G in the *GNPTAB* gene with a RegulomeDB score of 6 indicated whether a certain position in the DNA sequence is bound or unbound by the transcription factor, pathogenicity estimation was possibly damaging for the iSNV which was detected in 21/79 (26.5%) of the stutterers. The variant rs1001171T>A detected in the *NAGPA* gene segment with a RegulomeDB score of 1f also indicated to affect binding and was linked to expression of a gene target with pathogenicity estimated to be possibly damaging and was detected in 47/79 (59.4%) of stutterers. No pathogenic iSNVs were detected in the *GNPTG* gene. In summary, these database provided evidence allowing us to examine the nucleotide variations responsible for conservation,

Table 1 Allele frequencies of the 41 variants observed in *GNPTAB*, *GNPTG*, and *NAGPA* genes among the 79 unrelated persistent stutters and their comparison with gnomAD database

Chr	Gene	Variant	rsID	n	%	Allele frequency in this study	Allele frequency South Asian	Allele frequency total	RegulomeDB score
chr12	<i>GNPTAB</i>	c.117+4859_117+4860insATAAC	rs10641124	76	96.20253165	1.853658537			4
		c.3603-1359A>G	rs111110995	30	37.97468354	0.731707317	0.1739	0.1081	1d
		c.3136-257C>T	rs111111002	32	40.50632911	0.780487805	0.1736	0.1102	1f
		c.1408+712T>C	rs4764814	52	65.82278481	1.268292683	0.4128	0.457	1f
		c.2715+427C>G	rs4764813	20	25.3164557	0.487804878	0.2189	0.2622	1f
		c.771+3179C>G	rs919215	79	100	1.926829268	0.6567	0.6209	5
		c.771+3153G>A	rs919214	17	21.51898734	0.414634146	0.1542	0.1859	5
		c.324-340T>A	rs10778150	79	100	1.926829268	0.6443	0.6208	6
		c.203+609T>C	rs4764654	78	98.73417722	1.902439024	0.6439	0.6021	4
		c.117+10307A>G	rs10444509	40	50.63291139	0.975609756	0.2218	0.1805	7
		c.117+4571C>T	rs4764823	69	87.34177215	1.682926829	0.4633	0.2818	4
		c.203+564A>G	rs10860788	28	35.44303797	0.682926829	0.1617	0.02236	7
		c.118-7189A>C	rs10860790	45	56.96202532	1.097560976	0.2446	0.1539	4
		c.3136-295G>A	rs12366292	10	12.65822785	0.243902439	0.05305	0.06437	5
		c.117+4718G>A	rs7960795	12	15.18987342	0.292682927			4
		c.117+10310T>C	rs80178135	28	35.44303797	0.682926829	0.1611	0.02244	7
		chr16	<i>GNPTG</i>	c.324-457A>G	rs11830792	22	27.84810127	0.536585366	0.2281
c.117+10414G>T	rs545549083			2	2.53164557	0.048780488	0.0006211	0.0001971	5
c.772-489T>C	rs17725026			5	6.329113924	0.12195122	0.06084	0.03799	3a
c.1408+686G>A	rs568797492			1	1.265822785	0.024390244	0.0178	0.0006046	4
c.117+10485C>T	rs962073192			1	1.265822785	0.024390244	0	0.000006572	5
c.3603-1345T>C	rs75733424			1	1.265822785	0.024390244	0.002276	0.02443	4
c.178+3230C>T	rs742460			60	75.94936709	1.463414634	0.4372	0.3145	4
c.179-1611G>A	rs2887538			66	83.5443038	1.609756098	0.565	0.6081	5
c.179-4651G>T	rs8060970			32	40.50632911	0.780487805	0.1592	0.1481	4
c.179-4672G>A	rs1008995614			1	1.265822785	0.024390244	0.001241	0.00009197	4
c.178+186C>T	rs11865211			12	15.18987342	0.292682927	0.05314	0.06899	2b
c.179-1582C>T	rs187809520			1	1.265822785	0.024390244	0	0.00001317	5
c.179-4260_179-4259insTTA	rs113850025			2	2.53164557	0.048780488			5
c.117+4887A>G	rs139670026			1	1.265822785	0.024390244	0.001265	0.00236	5
c.178+300G>C	rs572050858			1	1.265822785	0.024390244	0.002698	0.0009201	2b
c.791+300G>A	rs3743841			59	74.6835443	1.43902439	0.4158	0.3431	4
c.791+265G>A	rs3743840			60	75.94936709	1.463414634	0.4125	0.2863	4
c.543-404T>A	rs1001171	48	60.75949367	1.170731707	0.4093	0.3341	1f		
c.543-346A>C	rs1001170	45	56.96202532	1.097560976			1f		
c.920+259G>T	rs62036221	14	17.72151899	0.341463415			5		

Table 1 (continued)

Chr	Gene	Variant	rsID	n	%	Allele frequency in this study	Allele frequency South Asian	Allele frequency total	RegulomeDB score
		c:920+266G>A	rs55936034	6	7.594936709	0.146341463	0.03196	0.008494	5
		c:791+356A>C	rs571237436	1	1.265822785	0.024390244	0.008506	0.004635	4
		c:*43C>A	rs1270775930	1	1.265822785	0.024390244			4
		c:920+298G>A	rs12709122	8	10.12658228	0.195121951			5
		c:920+270A>G	rs1307047484	1	1.265822785	0.024390244	0	0.000006576	5

Table 2 Pathogenicity prediction of the variants using Reg-SNP Intron tool

Chr	Position (hg19)	Gene	Reference allele	Alternate allele	rsID	Probability score	Pathogenicity	N (%)
chr12	102144328	<i>GNPTAB</i>	T	C	rs11110995	0.71	Damaging	30 (37.9%)
chr12	102182824	<i>GNPTAB</i>	T	C	rs11830792	0.56	Possibly Damaging	21 (26.5%)
chr16	5082289	<i>NAGPA</i>	A	T	rs1001171	0.56	Possibly Damaging	47 (59.4%)

Off splicing site probability score: 0.0–0.56 (benign); 0.56–0.61 (possibly damaging); 0.61–1.0 (damaging); chr–chromosome

chromatin state, and their effect on regulatory motifs. However, these regulatory variants are only associated with altered gene expression which is not the risk loci on disease pathogenesis and progression, and may not be as disruptive as the coding region variants which may modify the genes.

Conclusions

This study identified three intronic variants of pathogenic impact (rs11110995, rs11830792, and rs1001171) using the RegSNPs-intron tool in stuttering patients that are known to be associated with a certain genetic trait, as well as the regulatory function of the intronic variants were identified using RegulomeDB database which documented a few potential regulatory variants and susceptible loci. Thus, the combination of the two computational approaches may be helpful to understand the regulatory regions and derive a valid hypothesis as to their function. The limitations of this study included the relatively small sample size, and the patients were chosen from a single center, which may limit the generalizability. Therefore, future work confirming the current findings is warranted to better understand the role of the intronic variants in a larger cohort of stutterers and a cohort of fluent controls would be valuable.

Abbreviations

CYP17A1: Cytochrome P450 family 17 subfamily A member 1; CYRIA: CYFIP-related Rac1 interactor A; DDR2: Dopamine receptor D2; DNA: Deoxyribose nucleic acid; ENCODE: Encyclopedia of DNA elements; eQTLs: Expression quantitative trait loci; GnomAD: Genome aggregation database; GNPTAB: *N*-Acetylglucosamine-1-phosphate transferase subunits alpha and beta; GNPTG: *N*-Acetylglucosamine-1-phosphate transferase subunit gamma; NAGPA: *N*-Acetylglucosamine-1-phosphodiester alpha-*N*-acetylglucosaminidase; GWAS: Genome-wide association studies; iSNVs: Intronic single-nucleotide variants; SD: Standard deviation; SLC6A3: Solute carrier family 6 member 3; TMAP: Torrent mapping alignment program; ZMAT4: Zinc finger matrix-type 4.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42269-022-00971-1>.

Additional file 1. Table S1. Regulatory motifs identified using RegulomeDB. **Table S2.** Pathogenic impact of the intronic variants.

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Author contributions

CS performed the protocol, analyzed the data, and wrote the original article. RK collected the data. SM did the conception and design of the work, and make critical revisions to the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The authors declare that the data supporting the findings of this research are available within the article.

Declarations

Ethics approval and consent to participate

The study was carried out following the approval of the local Ethics Committee of the All India Institute of Speech and Hearing, Mysore India. All the cases provided their consent to participate in this study.

Consent for publication

Written consent to publish this information was obtained from study participants.

Competing interests

The authors declare that they have no competing interests.

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References

- Alm PA (2021) Stuttering: a disorder of energy supply to neurons? *Front Hum Neurosci* 15:662204
- Barnes DK, Neutel AM (2016) Severity of seabed spatial competition decreases towards the poles. *Curr Biol* 26(8):R317–R318
- Berk AJ (2016) Discovery of RNA splicing and genes in pieces. *Proc Natl Acad Sci USA* 113(4):801–805
- Bloodstein O, Ratner NB, Brundage SB (2021) A handbook on stuttering. Plural Publishing
- Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, Karczewski KJ, Park J, Hitz BC, Weng S, Cherry JM (2012) Annotation of functional variation in personal genomes using RegulomeDB. *Genom Res* 22(9):1790–1797

- Chow HM, Garnett EO, Li H, Etchell A, Sepulcre J, Drayna D, Chugani D, Chang SE (2020) Linking lysosomal enzyme targeting genes and energy metabolism with altered gray matter volume in children with persistent stuttering. *Neurobiol Lang* 1(3):365–380
- Didirková I, Le Maguer S, Hirsch F (2021) An articulatory study of differences and similarities between stuttered disfluencies and non-pathological disfluencies. *Clin Linguist Phon* 35(3):201–221
- Drayna D, Kang C (2011) Genetic approaches to understanding the causes of stuttering. *J Neurodev Disord* 3(4):374–380
- Elliott K, Larsson E (2021) Non-coding driver mutations in human cancer. *Nat Rev Cancer* 21(8):500–509
- Frigerio-Domingues C, Drayna D (2017) Genetic contributions to stuttering: the current evidence. *Mol Genet Genom Med* 5(2):95–102
- Fedyna A, Drayna D, Kang C (2011) Characterization of a mutation commonly associated with persistent stuttering: evidence for a founder mutation. *J Hum Genet* 56(1):80–82
- Gunasekaran ND, Jayasankaran C, Justin Margret J, Krishnamoorthy M, Srisailapathy CS (2021) Evaluation of recurrent GNPTAB, GNPTG, and NAGPA variants associated with stuttering. *Adv Genet* 2(2):e10043
- IBM Corp (2012) IBM SPSS statistics for Windows, Version 21.0. IBM Corp, Armonk, NY
- Kang C, Riazuddin S, Mundorff J, Krasnewich D, Friedman P, Mullikin JC, Drayna D (2010) Mutations in the lysosomal enzyme–targeting pathway and persistent stuttering. *N Engl J Med* 362(8):677–685
- Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfoldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP, Gauthier LD (2020) The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 581(7809):434–443
- Kazemi N, Estiar MA, Fazilaty H, Sakhinia E (2018) Variants in GNPTAB, GNPTG and NAGPA genes are associated with stutterers. *Gene* 647:93–100
- Lan J, Song M, Pan C, Zhuang G, Wang Y, Ma W, Chu Q, Lai Q, Xu F, Li Y, Liu L (2009) Association between dopaminergic genes (SLC6A3 and DRD2) and stuttering among Han Chinese. *J Hum Genet* 54(8):457–460
- Liao X, Lan C, Liao D, Tian J, Huang X (2016) Exploration and detection of potential regulatory variants in refractive error GWAS. *Sci Rep* 6(1):1–9
- Lin H, Hargreaves KA, Li R, Reiter JL, Wang Y, Mort M, Cooper DN, Zhou Y, Zhang C, Eadon MT, Dolan ME (2019) RegSNPs-intron: a computational framework for predicting pathogenic impact of intronic single nucleotide variants. *Genom Biol* 20(1):1–6
- Mohammadi H, Joghataei MT, Rahimi Z, Faghihi F, Khazaie H, Farhangdoost H, Mehrpour M (2017) Sex steroid hormones and sex hormone binding globulin levels, CYP17 MSP A1 (– 34 T: C) and CYP19 codon 39 (Trp: Arg) variants in children with developmental stuttering. *Brain Lang* 175:47–56
- Nica AC, Dermitzakis ET (2013) Expression quantitative trait loci: present and future. *Philos Trans R Soc Lond B Biol Sci* 368(1620):20120362
- Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ (2008) Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nat Genet* 40(12):1413–1415
- Panaro MA, Calvillo R, Miniero DV, Mitolo V, Cianciulli A (2022) Imaging intron evolution. *Methods Protoc* 5(4):53
- Polikowsky HG, Shaw DM, Petty LE, Chen HH, Pruett DG, Linklater JP, Viljoen KZ, Beilby JM, Highland HM, Levitt B, Avery CL (2022) Population-based genetic effects for developmental stuttering. *HGG Adv* 3(1):173
- Raza MH, Gertz EM, Mundorff J, Lukong J, Kuster J, Schäffer AA, Drayna D (2013) Linkage analysis of a large African family segregating stuttering suggests polygenic inheritance. *Hum Genet* 132(4):385–396
- Raza MH, Mattera R, Morell R, Sainz E, Rahn R, Gutierrez J, Paris E, Root J, Solomon B, Brewer C, Basra MA (2015) Association between rare variants in AP4E1, a component of intracellular trafficking, and persistent stuttering. *Am J Hum Genet* 97(5):715–725
- Raza MH, Domingues CE, Webster R, Sainz E, Paris E, Rahn R, Gutierrez J, Chow HM, Mundorff J, Kang CS, Riaz N (2016) Mucopolipidosis types II and III and non-syndromic stuttering are associated with different variants in the same genes. *Eur J Hum Genet* 24(4):529–534
- Reilly S, Onslow M, Packman A, Cini E, Conway L, Ukoumunne OC, Bavin EL, Prior M, Eadie P, Block S, Wake M (2013) Natural history of stuttering to 4 years of age: a prospective community-based study. *Pediatrics* 132(3):460–467
- Reuter JA, Spacek DV, Snyder MP (2015) High-throughput sequencing technologies. *Mol Cell* 58(4):586–597
- Riaz N, Steinberg S, Ahmad J, Pluzhnikov A, Riazuddin S, Cox NJ, Drayna D (2005) Genome wide significant linkage to stuttering on chromosome 12. *Am J Hum Genet* 76(4):647–651
- Riley GD, Bakker K (2009) Stuttering severity instrument: SSI-4. Pro-Ed
- Rose AB (2019) Introns as gene regulators: a brick on the accelerator. *Front Genet* 9:672
- Shaw DM, Polikowsky HP, Pruett DG, Chen HH, Petty LE, Viljoen KZ, Beilby JM, Jones RM, Kraft SJ, Below JE (2021) Phenome risk classification enables phenotypic imputation and gene discovery in developmental stuttering. *Am J Hum Genet* 108(12):2271–2283
- Vaz-Drago R, Custódio N, Carmo-Fonseca M (2017) Deep intronic mutations and human disease. *Hum Genet* 136(9):1093–1111
- Wang Y, Liu J, Huang BO, Xu YM, Li J, Huang LF, Lin J, Zhang J, Min QH, Yang WM, Wang XZ (2015) Mechanism of alternative splicing and its regulation. *Biomed Rep* 3(2):152–158
- Yairi E, Ambrose N (2013) Epidemiology of stuttering: 21st century advances. *J Fluency Disord* 38(2):66–87
- Yang P, Wang D, Kang L (2021) Alternative splicing level related to intron size and organism complexity. *BMC Genom* 22(1):1–6

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