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RESEARCH ARTICLE
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GNPTAB AND NAGPA GENE POLYMORPHISM LINKED TO STUTTERING: A CASE CONTROL STUDY IN PAKISTANI POPULATION

Wajiha Naeem[1*], Bashir Ahmad[1], Bibi Hajira Ishaq[2], Farzana Hakim[3]

[1]Department of Biotechnology, University of Peshawar, Khyber Pakhtunkhwa, Pakistan
[2]Khyber Medical College, Peshawar, Khyber Pakhtunkhwa, Pakistan
[3]Foundation University Medical College, Islamabad, Pakistan

***Corresponding author:** Wajiha Naeem
*Email: wajiha.naeem9495@gmail.com

Abstract

Objective

This study examined GNPTAB and NAGPA gene polymorphisms associated with stuttering in Pakistani population.

Methodology

This case control study was initiated by screening stuttering in samples using SSI-4 tool. Individuals who suffered physical abnormalities, incomplete data of participants (SSI-4 and PCR bands) were excluded. Total 100 individuals including 69 stuttering and 31 non-stuttering participants data was recorded. Statistical analysis included Hardy-Weinberg Equilibrium, Chi square of homogeneity and odds ratios with 95% Confidence intervals.

Results

Results showed significant ($P<0.05$) association between family history and stuttering, with 88% of participants reporting a familial history of the condition. The GNPTAB SNP distribution for stuttering participants showed no deviation (via Hardy-Weinberg Equilibrium) in the Pakistani population. GNPTAB polymorphisms distribution showed a highly significant association with stuttering ($P<0.01$), with the AG genotype significantly increasing the risk (OR = 21, 95% CI: 2.66–165.37). In contrast, NAGPA genotypes showed no significant association ($P>0.05$) with stuttering. The chi-square test for homogeneity revealed a highly significant ($P<0.01$) difference in genotype between stuttering and non-stuttering groups. The dominant genotype (AA) reduced the risk of stuttering, whereas heterozygous genotypes GNPTAB SNP (A>G) increased the risk of stuttering. For NAGPA gene, the dominant genotype (CC) appeared protective.

Conclusion

Stuttering showed a strong association with family history and GNPTAB gene polymorphisms. Future research should validate the GNPTAB and NAGPA association across diverse populations and explore gene-environment interactions, epigenetics, neuroimaging and transcriptomics to refine stuttering models.

Keywords: GNPTAB, NAGPA, ARMS PCR, SNPs, Stuttering

Introduction

Globally, stuttering affects approximately 1% of the population, with significant proportion in children and fewer in adulthood[1]. Males being four times more likely to experience this disorder than females[2]. This gender disparity is commonly observed in both developmental and neurogenic stuttering cases. Developmental stuttering emerges as part of the normal speech development process. Neurogenic stuttering results from a lack of coordination between various brain regions involved in speech production.

Stuttering can affect individual social interactions, emotional well-being, and overall quality of life, as they often experience feelings of frustration, embarrassment, and anxiety related to their speech difficulties[3,4]. Stuttering decreases with altered auditory feedback in hearing-impaired individuals[5]. The difference between the prevalence and incidence rates of stuttering can be attributed to the fact that developmental stuttering resolves in approximately 80% of cases before adulthood[6].

Recent research has suggested that developmental stuttering is a multifactorial disorder, with both neurological and genetic factors playing significant roles[7]. Children with persistent stuttering often show reduced brain activity in areas crucial for motor planning and coordination, such as the left premotor cortex and basal ganglia, compared to those who do not stutter[8]. The genetic complexity of stuttering stems from its deviation from typical Mendelian inheritance patterns, involving multiple genetic loci and environmental factors[9,10,11].

Stuttering exhibits significant heritability, with inheritance patterns that may follow either a dominant or autosomal recessive mode, suggesting a complex interaction between genetic and environmental factors[12,13]. Various genetic analyses have identified loci associated with stuttering in genes like GNPTAB, GNPTG, NAGPA, and AP4E1, which are involved in protein trafficking and lysosomal function[14]. The GNPTAB and GNPTG encode subunits of N-acetylglucosamine-1-phosphotransferase, which initiates synthesis of the mannose 6-phosphate (M6P) signal, essential for directing enzymes to the lysosomes[15]. Using genome-wide linkage scans across 46 families, Kang[16] identified 87 candidate genes on chromosome 12q23.3 and pinpointed the mutation c.3598G>A (p.Glu1200Lys) in the GNPTAB gene. The NAGPA gene facilitates the second step of this process by exposing the M6P marker, allowing M6P receptors to recognize and properly direct lysosomal enzymes. Mutations in these genes can disrupts cellular processes, leading to lysosomal dysfunctions such as mucolipidosis types II and III, which may impair neural communication and contribute to stuttering[17]. In this study, we examined the association between the GNPTAB and NAGPA gene polymorphism with stuttering in Pakistani population.

Methodology

Participants in speech therapist clinics were screened for stuttering using SSI-4[18]. A stratified random sampling technique was employed. In total, 100 participants were selected for subsequent analysis, including 69 stuttering and 31 controls. Demographic characteristics including stuttering family history as well as SSI-4 data were collected from these participants. Those individuals who suffered some physical abnormalities were excluded from the study. Prior to data collection, ethical approval (10^{th} April, 2022), from University of Peshawar was granted as well as consent form from the participants/guardian of children was taken that were below 16 years age.

Blood was taken from antecubital vein from these participants. Genomic DNA was extracted using QAIGEN Flexi-Gene DNA kit Catalog # 51206 and Thermoscientific DNA extraction kit Catalog # K0721. High quality intact genomic DNA with an optical density ratio of $260/280 \leq 1.8$ and $260/230 \geq 1.5$ was further used for ARMS PCR.

The Ensembl Variant Effect Predictor (VEP) indicated that the queried variants are previously known polymorphism with established annotation records. The 8 ARMS' primers with wild and mutant types with one base mismatched at the 3′-end following the mutation type (synonymous SNP) of the GANTAB and NAGPA gene is given table 1.

Table 1: GANTAB and NAGPA genes SNPs primers profile.

GNPTAB/ Exon13/ p.Thr644Thr / rs10778148 /c.1932A > G				
	Sequence (5'->3')	**Length**	**Tm**	**GC%**
Fo	GTGGAAAACCATCCACCTCATA	22	58.04	45.45
Ro	ACTCAACTGGGCGTCTTTTGG	21	61.09	52.38
Fi	GAGGGACCAAAACTGAATTCTAC**G**	24	58.69	41.67
Ri	ATTTTCGTAACCCTTCTGGGCT	22	59.96	45.45
NAGPA /exon10/ p.Thr465Ile /rs7188856 /c.1394 C > T				
Fo	TATCTATGCCGGGTAGAGGGA	21	59.00	52.38
Ro	GAAGCCAGACCGTGGGGAA	19	61.89	63.16
Fi	CTTCCTCCTGCTGATCAGCA**T**	21	60.74	57.14
Ro	CAAGGACAGGTTTGCTGCAG	20	59.69	55.00

PCR was performed in a 25 µL reaction volume that comprised 1.5 µL DNA template, 0.7 µM primers, 12 µL 2X PCR Taq Master Mix, and 10 µL double distilled water. PCR cycle regimen was as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles for 30 s at 95°C, 30 s at Tm-5 °C, 1 minute at 72°C, and then a final extension for 10 min at 72°C, and finally the PCR products were maintained at 4°C in the end.

PCR products were resolved on a 2% agarose gel with 1x Tris–acetate ethylenediaminetetraacetic acid (TAE) buffer, and then subjected to electrophoresis at 110 V, 200 mA and for 30 min. The agarose gel was stained with 2µl ethidium bromide and photographed by UV trans-illuminator. DNA ladder of 1kb was also used to presume the size of intensified product.

Statistical Analysis

Statistical analysis was performed using SPSS ver.26 (statistical package for social sciences; Chicago, IL, USA). Chi square was used for Hardy-Weinberg Equilibrium analysis. Chi square test of homogeneity was used for genotype distribution between stuttering and non-stuttering group. Odds ratios with 95% confidence interval was used for the genetic predictive risk of stuttering.

Results

In all stuttering categories i.e., very mild, mild, moderate and severe the highest participants were male with resultant 16%, 6%, 31% and 7%, respectively, compared to female with 15%, 4%, 18% and 3%, respectively (Table 2). Among the age groups, the highest participants with 5 to 9 years aged were recorded with moderate stuttering (28%) followed by those that were 10 to 14 years age (16%). The school going students for all the categories of stuttering i.e., very mild, mild, moderate and severe with resultant 25%, 8%, 35% and 8%, respectively, comparatively was higher than preschool participants. As for family history for stuttering, highest participants had moderate stuttering (43%), followed by very mild (25%), severe (9%) and least with mild stuttering (5%). Statistically, except for family history being significant ($P<0.05$), all others demographic characteristics were non-significant ($P>0.05$).

Table 2: Demographic characteristics of participants categorized by different stuttering severity levels

Demographic characteristics		Stuttering			
		Very Mild	Mild	Moderate	Severe
Gender^{ns}	Female	15	4	18	3
	Male	16	6	31	7
Age^{ns}	5-9	16	6	28	4
	10-14	8	3	16	2
	15-19	5	1	2	3
	20-24	2		3	1

Schooling[ns]	Preschool	6	2	14	2
	School	25	8	35	8
Family History*	No	6	5	6	1
	Yes	25	5	43	9

ns: Non-significant (P >0.05), *: significant (P <0.05)

As shown in table 3, among GNPTAB genotypes in the stuttering participants, the AA genotype was observed in the highest number of participants (46.38%) of the stuttering cases, followed by AG (34.78%) and least with GG genotype (18.84%). The P value (P>0.05) indicated stuttering group follows Hardy-Weinberg equilibrium. In control (non-stuttering) group, the highest number of participants was observed with AA genotype (90.32%). Only 1 participant was observed with AG genotype (3.23%) followed by GG genotype (6.45%). The chi square (P<0.05) indicates the control (non-Stuttering) group genotypes distribution deviates from Hardy-Weinberg equilibrium.

As for the NAGPA genotype in the stuttering participants, the CC genotype was observed the highest (63.77%) for stuttering cases, followed by CT (24.64%) and least with TT genotype (11.59%). In control (non-stuttering) group, the highest number of participants was observed with CC genotype (83.87%), whereas the least participants was observed with TT genotype (6.45%) followed by CT genotype (9.68%). The chi square for NAGPA both case and control group showed P value less than 0.05, indicating these groups genotypes distribution in population deviates from Hardy-Weinberg equilibrium.

The additional model showed that odds ratios for AG genotype individuals significantly (OR=21, 95% Cl: 2.66-165.37) increased the risk of stuttering (Table 4). The odds ratios for the dominant model showed that dominant genotype (AA) was significantly (OR=0.09, 95% Cl: 0.02-0.33) less likely to develop stuttering compared to those with recessive genotype (AG + GG). As for the codominant model, in the stuttering group the AG genotype was observed in 24 stuttering participants whereas the homogenous genotypes (AA + GG) were found in 45 stuttering individuals. On the other hand, in non-stuttering participants, codominant heterogenous genotype (AG) was observed in only 1 individual and homogenous genotypes (AA + GG) was found in 30 non-stuttering individuals. Statistically, a highly significant (P<0.01) difference in genotype distribution was found between stuttering and non-stuttering groups. The odds ratios for codominant model showed significantly (OR=16, 95% CI: 2.05–124.66) increase of developing stuttering compared to homogenous genotypes (AA or GG). The chi-square (χ^2) test of homogeneity indicated a statistically high significant (P < 0.01) difference between stuttering and non-stuttering groups for distribution of genotypes in additive, dominant, codominant and allele distribution.

The odds ratios for NAGPA showed significant (OR=0.09, 95% Cl: 0.02-0.33) less development for dominant model (OR=0.33, 95% Cl: 0.22-0.99) and allele (OR=0.40, 95% Cl: 0.16-0.97) for stuttering. Similarly, the chi-square (χ^2) test of homogeneity indicated a statistically significant (P< 0.05) difference of allele and genotypes distribution between stuttering and non-stuttering groups.

Table 3. Hardy-Weinberg Equilibrium HWE of Allele and Genotype Frequencies among stuttering and control group

		Allele frequencies	Genotype	HW Observed frequency (%)	HW genotypes	HW expected frequency (%)	X^2 critical value	P-value HWE
GNPTAB	Case	A: 0.64	AA	32(46.38)	p2= 0.41	28.06(40.66)	4.22	P>0.05
		G: 0.36	AG	24(34.78)	2pq=0.46	31.88(46.21)		
			GG	13(18.84)	q2= 0.13	9.06(13.13)		
	Control	A: 0.92	AA	28(90.32)	p2= 0.85	26.20(84.52)	18.98	P<0.01
		G: 0.08	AG	1(3.23)	2pq=0.15	4.60(14.83)		
			GG	2(6.45)	q2= 0.01	0.20(0.65)		
N	Ca	C: 0.76	CC	44(63.77)	p2= 0.58	39.95(57.89)	7.20	P<0.05

		T: 0.24	CT	17(24.64)	2pq=0.36	25.11(36.38)		
			TT	8(11.59)	q2= 0.06	3.95(5.72)		
	Control	C: 0.89	CC	26(83.87)	p2= 0.79	24.40(78.69)		
		T: 0.11	CT	3(9.68)	2pq=0.20	6.21(20.03)	8.28	P<0.05
			TT	2(6.45)	q2= 0.01	0.40(1.27)		

Table 4. The GNPTAB and NAGPA genetic models (Additive, Dominant, Recessive, and Codominant) analysis of stuttering individuals

	GNPTAB Genotype	Stuttering		X^2 (P value)	OR (95% Cl)	**NAGPA Genotype**	Stuttering		X^2 (P value)	OR (95% Cl)
		Yes	No				Yes	No		
Additive	AA	32	28	0.00	-	CC	44	26	0.12	-
	AG	24	1		21 (2.66-165.37)	CT	17	3		3.35 (0.89-12.53)
	GG	13	2		0.27 (0.02-3.27)	TT	8	2		0.70 (0.09-5.09)
Dominant	AA	32	28	0.00	0.09 (0.02-0.33)	CC	44	26	0.04	0.33 (0.22-0.99)
	AG+GG	37	3			CT+TT	25	5		
Recessive	GG	13	2	0.10	3.36 (0.71-15.93)	TT	8	2	0.42	1.90 (0.38-9.52)
	AA+AG	56	29			CC+CT	61	29		
Codominant	AG	24	1	0.00	16 (2.05-124.66)	CT	17	3	0.08	3.05 (0.82-11.32)
	AA+GG	45	30			CC+TT	52	28		
Allele	A	88	57	0.00	0.15 (0.05-0.41)	C	105	55	0.04	0.40 (0.16-0.97)
	G	50	5			T	33	7		

Discussion

This study investigated the association between GNPTAB and NAGPA gene polymorphisms with stuttering in a Pakistani population. Descriptive analysis revealed that stuttering was more prevalent among males and younger age groups. Findings from the National Health Interview Survey indicated that the male-to-female prevalence ratio for stuttering was 2:1, with rates decreasing as age increased[19]. Samson et al.[20] and Boyce et al.[21] reported similar occurrences of stuttering in children aged 3 to 6 years. Among the participants in the current study, nearly half (49.9%) reported a family history of stuttering, reinforcing the notion that despite therapeutic interventions, stuttering is a complex trait that often persists. In the Indian population, the male-to-female ratio was found to be slightly higher, at 2.4:1[22]. Recent findings suggest that stuttering is now more prevalent among bilingual speakers than their monolingual counterparts[1]. In Pakistan, bilingual individuals, particularly children, demonstrate similar patterns of stuttering prevalence as observed in other bilingual populations[23].

Achieving effective therapeutic outcomes for stuttering in Pakistan presents numerous challenges. Speech-language pathologists face barriers such as divergent professional perspectives, clinical challenges, systemic and environmental constraints, and, most significantly, a lack of collaboration and institutional support[24]. Additionally, negative social reactions to stuttering, observed across

diverse cultural settings, underscore the urgent need for greater awareness, understanding, and acceptance of individuals with this speech disorder[25]. Addressing these societal attitudes, alongside clinical and systemic barriers, is imperative for fostering a more inclusive and supportive environment for individuals who stutter.

Over the past four decades, increasing evidence has supported a genetic basis for persistent developmental stuttering, with recent studies linking it to genes involved in intracellular trafficking deficits. In our study, in Pakistani population, GNPTAB polymorphisms was significantly associated with stuttering. These findings align with previous researches that have identify GNPTAB mutations as risk factors for stuttering. In Western populations, NAGPA variants have been significantly associated with speech disorders[12,16]. Mutations in the GNPTAB gene are associated with reduced astrocyte staining and structural changes in the corpus callosum, suggesting a crucial role of astrocytes in stuttering[26]. Our findings also align with Lehmann[27] who showed GNPTAB mutations, including Ser321Gly (S321G) and Ala455Ser (A455S), disrupt vocalization patterns in mice, mirroring speech deficits in humans who stutter. Our findings on GNPTAB mutation models contrast with mouse genetic studies on vocalization deficits[28], which associate GNPTAB variants—such as rs10778148—with dyslexia under a recessive model. Nonetheless, this suggests that GNPTAB dysfunction may underlie broader neurodevelopmental communication disorders, including both stuttering and dyslexia. A de novo variant (p. Ile268Leu) in GNPTG was previously reported[10], indicating GNPTG may contribute to stuttering in only a subset of cases rather than as a primary genetic factor. These findings reinforce the genetic link between GNPTAB and stuttering while highlighting the need for further investigation into the roles of GNPTG and NAGPA in different populations.

Conclusion

The study revealed a higher prevalence of stuttering in males compared to females, and statistically significant association between stuttering and family history. The Heterozygous GNAPTAB (A>G) increases stuttering risk, while dominant GNPTAB (AA) and NAGPA (CC) genotypes significantly reduce it. Further, large scale genetic studies related to stuttering, integration of stuttering risk screening in early childhood health policies, and increased awareness among healthcare professionals for early identification and intervention is needed.

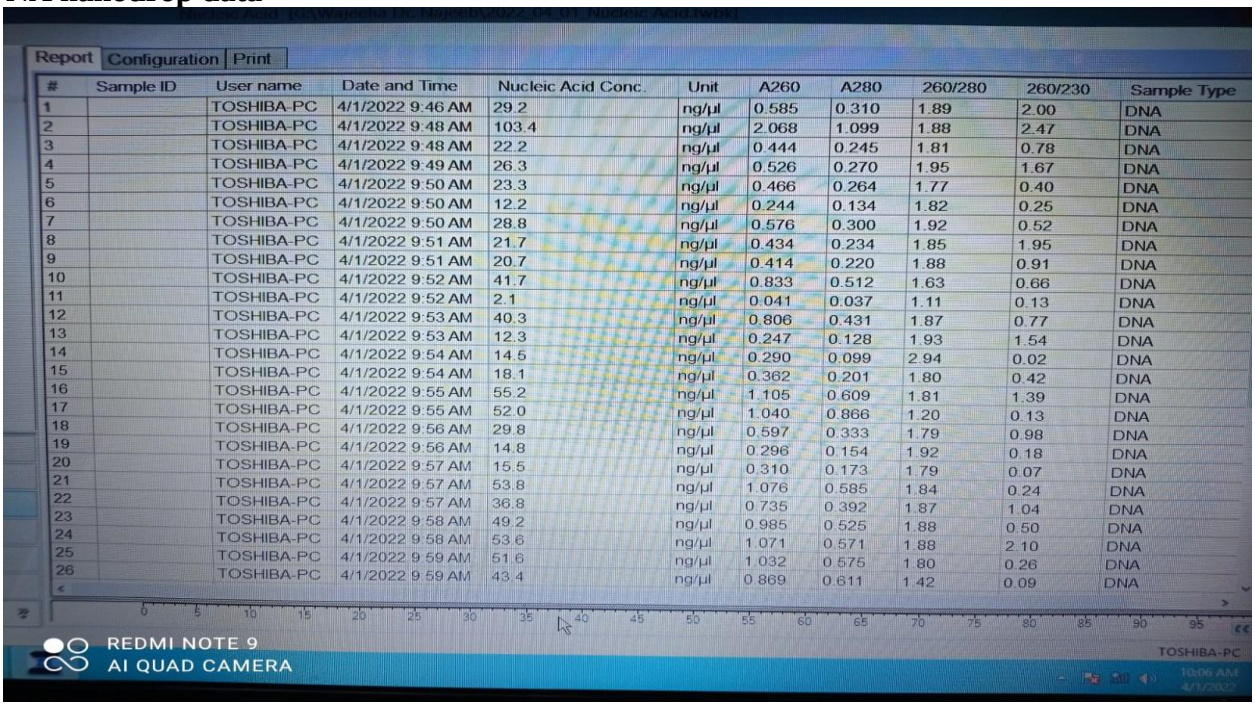
References

1. Choo AL, Smith SA. Bilingual children who stutter: Convergence, gaps and directions for research. Journal of Fluency Disorders. 2020, https://doi.org/10.1016/j.jfludis.2019.105741
2. Khasawneh MA. The level of stuttering severity among students with learning disabilities in English language. Science and Education. 2021 Sep;2(9):95-107.
3. Najafi S, Eshghizadeh M, Ganji Roudi M, Salehi Asl B, Ebrahimi N. The Comparison of anxiety, depression and self-esteem in healthy children and children with stuttering. Journal of Pediatric Nursing. 2020;6(4):1-0.
4. Gerlach-Houck H, Kubart K, Cage E. Concealing Stuttering at School:"When You Can't Fix It… the Only Alternative Is to Hide It". Language, Speech, and Hearing Services in Schools. 2023 Jan 17;54(1):96-113. https://doi.org/10.1044/2022_LSHSS-22-00029
5. Briley PM, Ellis C. Behavioral, social, and emotional well-being in children who stutter: the influence of race-ethnicity. Logopedics Phoniatrics Vocology. 2021 Oct 2;46(4):171-9. https://doi.org/10.1080/14015439.2020.1801833
6. Perez HR, Stoeckle JH. Stuttering: clinical and research update. Canadian family physician. 2016 Jun 1;62(6):479-84.
7. SheikhBahaei S, Millwater M, Maguire GA. Stuttering as a spectrum disorder: A hypothesis. Current Research in Neurobiology. 2023 https://doi.org/10.1016/j.crneur.2023.100116
8. Garnett EO, Chow HM, Nieto-Castañón A, Tourville JA, Guenther FH, Chang SE. Anomalous morphology in left hemisphere motor and premotor cortex of children who stutter. Brain. 2018 Sep 1;141(9):2670-84. https://doi.org/10.1093/brain/awy199

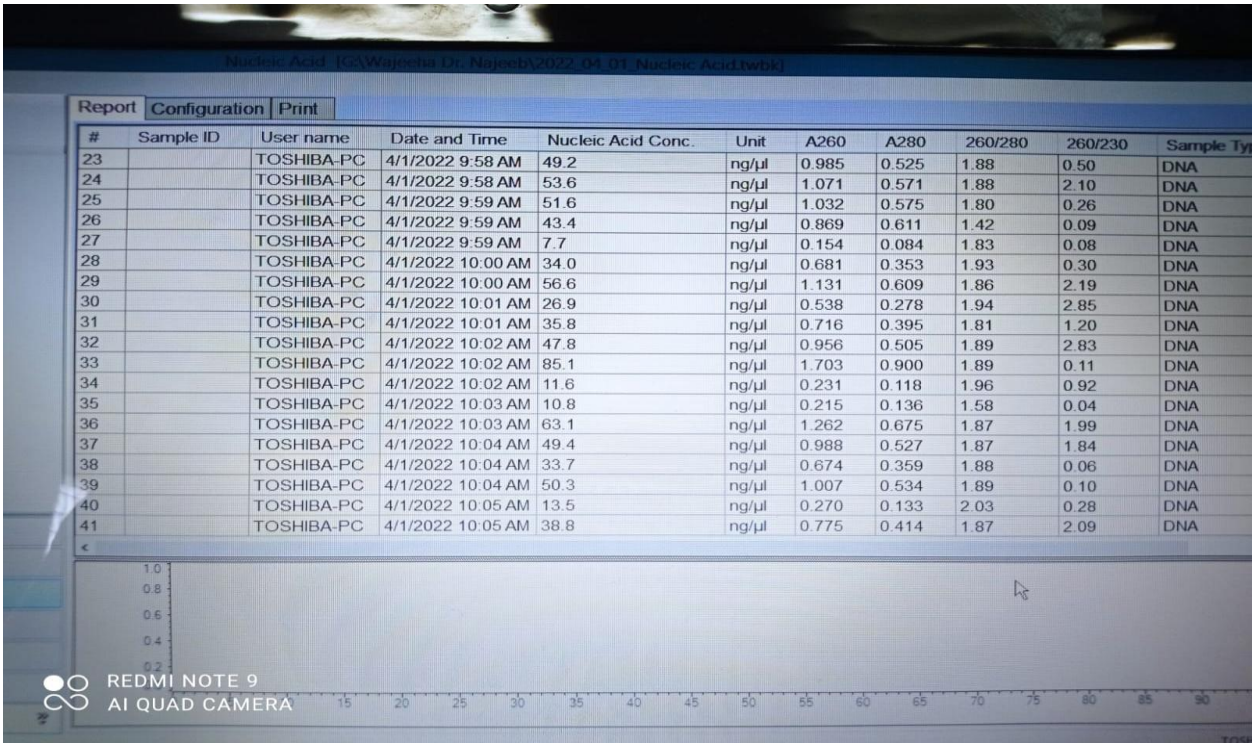
9. Srikumari CR, Nandhini G, Jeffrey JM, KRISHNAMOORTHY M. Recurrence of reported GNPTAB, GNPTG and NAGPA gene variations associated with stuttering-an evaluation. Authorea Preprints. 2020 Oct 8. 10.22541/au.160218237.79315446/v1
10. Gunasekaran ND, Jayasankaran C, Justin Margret J, Krishnamoorthy M, Srisailapathy CS. Evaluation of recurrent GNPTAB, GNPTG, and NAGPA variants associated with stuttering. Advanced Genetics. 2021 Jun;2(2):e10043. https://doi.org/10.1002/ggn2.10043
11. Polikowsky HG, Shaw DM, Petty LE, Chen HH, Pruett DG, Linklater JP, Viljoen KZ, Beilby JM, Highland HM, Levitt B, Avery CL. Population-based genetic effects for developmental stuttering. Human Genetics and Genomics Advances. 2022 Jan 13;3(1).
12. Frigerio-Domingues C, Drayna D. Genetic contributions to stuttering: the current evidence. Molecular genetics & genomic medicine. 2017 Feb 19;5(2):95. 10.1002/mgg3.276
13. Manoochehri J, Goodarzi HR, Tabei SM. Spastic paraplegia 51: phenotypic spectrum related to novel homozygous AP4E1 mutation. Journal of Genetics. 2022 Sep https://doi.org/10.1007/s12041-022-01385-8
14. Miyamoto S, Tsuge M. Treating stuttering in children with autism spectrum disorder. InEducation and technology support for children and young adults with ASD and learning disabilities 2021 (pp. 208-222). IGI Global Scientific Publishing. 10.4018/978-1-7998-7053-1.ch011
15. Kazemi N, Estiar MA, Fazilaty H, Sakhinia E. Variants in GNPTAB, GNPTG and NAGPA genes are associated with stutterers. Gene. 2018 Mar 20;647:93-100. https://doi.org/10.1016/j.gene.2017.12.054
16. Kang C. Progress, challenges, and future perspectives in genetic researches of stuttering. Journal of Genetic Medicine. 2021;18(2):75-82.
17. Chow HM, Garnett EO, Li H, Etchell A, Sepulcre J, Drayna D, Chugani D, Chang SE. Linking lysosomal enzyme targeting genes and energy metabolism with altered gray matter volume in children with persistent stuttering. Neurobiology of Language. 2020 Aug 1;1(3):365-80. https://doi.org/10.1162/nol_a_00017
18. Riley, G. D. (2009). Stuttering Severity Instrument–Fourth Edition. Austin, TX: Pro-Ed.
19. Briley PM, Merlo S, Ellis C. Sex differences in childhood stuttering and coexisting developmental disorders. Journal of Developmental and Physical Disabilities. 2022 Jun;34(3):505-27. https://doi.org/10.1007/s10882-021-09811-y
20. Samson I, Schalling E, Herlitz A, Lindström E, Sand A. A cross-sectional investigation of the impact of stuttering on Swedish females and males in childhood, adolescence, and young adulthood. Journal of Speech, Language, and Hearing Research. 2022 Dec 12;65(12):4608-22. https://doi.org/10.1044/2022_JSLHR-22-000
21. Boyce JO, Jackson VE, Van Reyk O, Parker R, Vogel AP, Eising E, Horton SE, Gillespie NA, Scheffer IE, Amor DJ, Hildebrand MS. Self-reported impact of developmental stuttering across the lifespan. Developmental Medicine & Child Neurology. 2022 Oct;64(10):1297-306. https://doi.org/10.1111/dmcn.15211
22. Varghese AL, Devani P, Kunnath L, Balasubramanium RK, Bajaj G, Karuppali S, Bhaskaran U. Prevalence of stuttering among school age children in Dakshina Kannada district of Karnataka, India. Clinical Epidemiology and Global Health. 2023 Jul 1;22: https://doi.org/10.1016/j.cegh.2023.101327
23. Naqvi S. A Preliminary Investigation of Speech Disfluencies in Bilingual Urdu-English Children. Senior Honors Theses. 2019.
24. Hasan A, Shah ST, Saqulain G. Potential Obstacles to achieve successful outcomes following stuttering interventions. Pakistan Journal of Health Sciences, 6(1), 58-63. https://doi.org/10.54393/pjhs.v6i1.1899
25. Veerabhadrappa RC, Dhrruvakumar S, Manjunatha C, Sulthana H, Maruthy S, St. Louis KO. Attitudes of Teachers, Students, and the Public Toward Stuttering in India: A Comparison. American Journal of Speech-Language Pathology. 2025 Jan 7;34(1):261-80. https://doi.org/10.1044/2024_AJSLP-24-002

26. Han TU, Root J, Reyes LD, Huchinson EB, Hoffmann JD, Lee WS, Barnes TD, Drayna D. Human GNPTAB stuttering mutations engineered into mice cause vocalization deficits and astrocyte pathology in the corpus callosum. Proceedings of the National Academy of Sciences. 2019 Aug 27;116(35):17515-24. https://doi.org/10.1073/pnas.1901480116
27. Lehmann C. A mouse model offers new clues to the underlying mechanism at work in stuttering. Neurology Today. 2019 Oct 3;19(19):48-55.
28. Chen H, Xu J, Zhou Y, Gao Y, Wang G, Xia J, Huen MS, Siok WT, Jiang Y, Tan LH, Sun Y. Association study of stuttering candidate genes GNPTAB, GNPTG and NAGPA with dyslexia in Chinese population. BMC genetics. 2015 Dec;16:1-7. https://doi.org/10.1186/s12863-015-0172-5

DNA nanodrop data

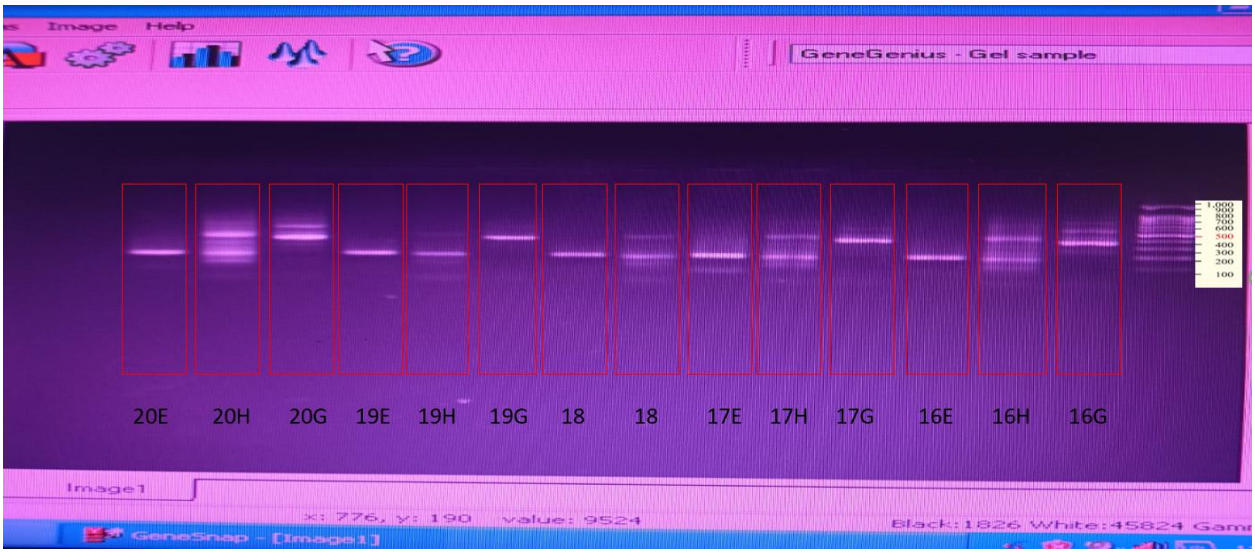


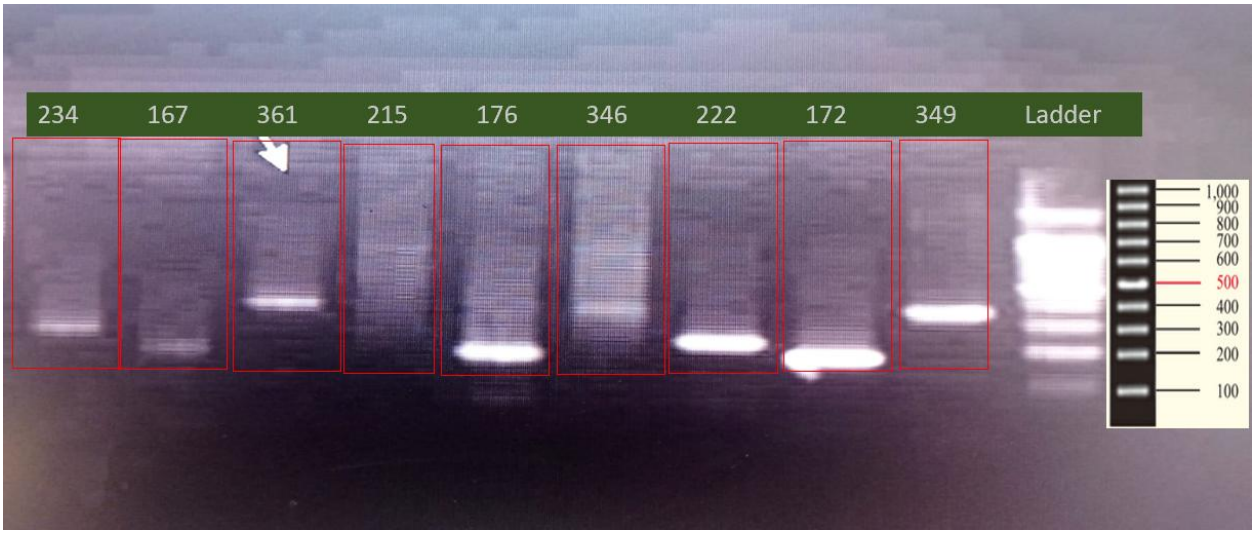
#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type
1		TOSHIBA-PC	4/1/2022 9:46 AM	29.2	ng/µl	0.585	0.310	1.89	2.00	DNA
2		TOSHIBA-PC	4/1/2022 9:48 AM	103.4	ng/µl	2.068	1.099	1.88	2.47	DNA
3		TOSHIBA-PC	4/1/2022 9:48 AM	22.2	ng/µl	0.444	0.245	1.81	0.78	DNA
4		TOSHIBA-PC	4/1/2022 9:49 AM	26.3	ng/µl	0.526	0.270	1.95	1.67	DNA
5		TOSHIBA-PC	4/1/2022 9:50 AM	23.3	ng/µl	0.466	0.264	1.77	0.40	DNA
6		TOSHIBA-PC	4/1/2022 9:50 AM	12.2	ng/µl	0.244	0.134	1.82	0.25	DNA
7		TOSHIBA-PC	4/1/2022 9:50 AM	28.8	ng/µl	0.576	0.300	1.92	0.52	DNA
8		TOSHIBA-PC	4/1/2022 9:51 AM	21.7	ng/µl	0.434	0.234	1.85	1.95	DNA
9		TOSHIBA-PC	4/1/2022 9:51 AM	20.7	ng/µl	0.414	0.220	1.88	0.91	DNA
10		TOSHIBA-PC	4/1/2022 9:52 AM	41.7	ng/µl	0.833	0.512	1.63	0.66	DNA
11		TOSHIBA-PC	4/1/2022 9:52 AM	2.1	ng/µl	0.041	0.037	1.11	0.13	DNA
12		TOSHIBA-PC	4/1/2022 9:53 AM	40.3	ng/µl	0.806	0.431	1.87	0.77	DNA
13		TOSHIBA-PC	4/1/2022 9:53 AM	12.3	ng/µl	0.247	0.128	1.93	1.54	DNA
14		TOSHIBA-PC	4/1/2022 9:54 AM	14.5	ng/µl	0.290	0.099	2.94	0.02	DNA
15		TOSHIBA-PC	4/1/2022 9:54 AM	18.1	ng/µl	0.362	0.201	1.80	0.42	DNA
16		TOSHIBA-PC	4/1/2022 9:55 AM	55.2	ng/µl	1.105	0.609	1.81	1.39	DNA
17		TOSHIBA-PC	4/1/2022 9:55 AM	52.0	ng/µl	1.040	0.866	1.20	0.13	DNA
18		TOSHIBA-PC	4/1/2022 9:56 AM	29.8	ng/µl	0.597	0.333	1.79	0.98	DNA
19		TOSHIBA-PC	4/1/2022 9:56 AM	14.8	ng/µl	0.296	0.154	1.92	0.18	DNA
20		TOSHIBA-PC	4/1/2022 9:57 AM	15.5	ng/µl	0.310	0.173	1.79	0.07	DNA
21		TOSHIBA-PC	4/1/2022 9:57 AM	53.8	ng/µl	1.076	0.585	1.84	0.24	DNA
22		TOSHIBA-PC	4/1/2022 9:57 AM	36.8	ng/µl	0.735	0.392	1.87	1.04	DNA
23		TOSHIBA-PC	4/1/2022 9:58 AM	49.2	ng/µl	0.985	0.525	1.88	0.50	DNA
24		TOSHIBA-PC	4/1/2022 9:58 AM	53.6	ng/µl	1.071	0.571	1.88	2.10	DNA
25		TOSHIBA-PC	4/1/2022 9:59 AM	51.6	ng/µl	1.032	0.575	1.80	0.26	DNA
26		TOSHIBA-PC	4/1/2022 9:59 AM	43.4	ng/µl	0.869	0.611	1.42	0.09	DNA



#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Ty
23		TOSHIBA-PC	4/1/2022 9:58 AM	49.2	ng/µl	0.985	0.525	1.88	0.50	DNA
24		TOSHIBA-PC	4/1/2022 9:58 AM	53.6	ng/µl	1.071	0.571	1.88	2.10	DNA
25		TOSHIBA-PC	4/1/2022 9:59 AM	51.6	ng/µl	1.032	0.575	1.80	0.26	DNA
26		TOSHIBA-PC	4/1/2022 9:59 AM	43.4	ng/µl	0.869	0.611	1.42	0.09	DNA
27		TOSHIBA-PC	4/1/2022 9:59 AM	7.7	ng/µl	0.154	0.084	1.83	0.08	DNA
28		TOSHIBA-PC	4/1/2022 10:00 AM	34.0	ng/µl	0.681	0.353	1.93	0.30	DNA
29		TOSHIBA-PC	4/1/2022 10:00 AM	56.6	ng/µl	1.131	0.609	1.86	2.19	DNA
30		TOSHIBA-PC	4/1/2022 10:01 AM	26.9	ng/µl	0.538	0.278	1.94	2.85	DNA
31		TOSHIBA-PC	4/1/2022 10:01 AM	35.8	ng/µl	0.716	0.395	1.81	1.20	DNA
32		TOSHIBA-PC	4/1/2022 10:02 AM	47.8	ng/µl	0.956	0.505	1.89	2.83	DNA
33		TOSHIBA-PC	4/1/2022 10:02 AM	85.1	ng/µl	1.703	0.900	1.89	0.11	DNA
34		TOSHIBA-PC	4/1/2022 10:02 AM	11.6	ng/µl	0.231	0.118	1.96	0.92	DNA
35		TOSHIBA-PC	4/1/2022 10:03 AM	10.8	ng/µl	0.215	0.136	1.58	0.04	DNA
36		TOSHIBA-PC	4/1/2022 10:03 AM	63.1	ng/µl	1.262	0.675	1.87	1.99	DNA
37		TOSHIBA-PC	4/1/2022 10:04 AM	49.4	ng/µl	0.988	0.527	1.87	1.84	DNA
38		TOSHIBA-PC	4/1/2022 10:04 AM	33.7	ng/µl	0.674	0.359	1.88	0.06	DNA
39		TOSHIBA-PC	4/1/2022 10:04 AM	50.3	ng/µl	1.007	0.534	1.89	0.10	DNA
40		TOSHIBA-PC	4/1/2022 10:05 AM	13.5	ng/µl	0.270	0.133	2.03	0.28	DNA
41		TOSHIBA-PC	4/1/2022 10:05 AM	38.8	ng/µl	0.775	0.414	1.87	2.09	DNA

ARMS PCR


GeneGenius - Gel sample
20E
20H
20G
19E
19H
19G
18
18
17E
17H
17G
16E
16H
16G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222
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349
Ladder
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300
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