Published in final edited form as:

Brain Lang. 2019 April ; 191: 28–30. doi:10.1016/j.bandl.2019.02.003.

Are variants in sex hormone metabolizing genes associated with stuttering?

Carlos E. Frigerio Domingues¹, Katherine Grainger¹, Hui Cheng¹, Danilo Moretti-Ferreira², Sheikh Riazuddin³, and Dennis Drayna¹

¹NIDCD/National Institutes of Health, Bethesda, Maryland 20892, USA

²Institute of Biosciences, Sao Paulo State University, Botucatu, Brazil

³Alama Iqbal Medical Research Center, Lahore 54550, Pakistan

1. Introduction

We read with interest the report by Mohammadi et al. in *Brain and Language* 175 (2017) 47–56, which addressed the sexual dimorphism that exists in stuttering. Their study of variants in two genes that encode sex hormone metabolizing enzymes is an interesting and novel approach to this question. Their report of a significant association between a genetic variant in the *CYP17* gene and stuttering warrants replication.

We sought to replicate their findings in our independently ascertained stuttering case and population-matched control groups enrolled with written informed consent under NIH IRB-approved protocol 97-DC-0057. These participants were all unrelated to each other, and were enrolled in the United States, Brazil, Pakistan, and Cameroon (West Africa) and have been well characterized both clinically and genetically (Domingues et al., 2014; Kang et al., 2010; M. H. Raza, Amjad, Riazuddin, & Drayna, 2011; M. Hashim Raza et al., 2012; M. H. Raza, Riazuddin, & Drayna, 2010; Riaz et al., 2005). Our data are summarized in Table 1.

2. Results

Our results for rs2236722 in *CYP19* closely matched those of Mohammadi et al. (2017), where we found no evidence of association with this variant and stuttering in any of our populations (data not shown). However, we note that the C allele of this SNP is uncommon in populations worldwide (gnomad.broadinstitute.org), and thus this SNP has low informativity for tests of association.

Regarding rs743572 in *CYP17*, our results differed markedly from those of Mohammadi et al. (2017). While they found both the CC genotype and the C allele more frequent in individuals who stutter, we failed to find a higher frequency of these in any of our stuttering subject populations. Overall in our combined sample, the frequency of the CC genotype was

almost identical in the two groups overall, 0.1664 in affected individuals (All-St), and 0.1599 in normally fluent controls (All-Co), and not significantly different in each sub-population ($p = 0.29$) (Table 1b, 1c), indicating no association between stuttering and the *CYP17* SNP.

3. Discussion

Several factors might contribute to the discrepancies between our results and those of Mohammadi et al. (2017). One element might be a difference in the study populations. While Mohammadi et al. (2017) studied exclusively Iranian Kurds, our analysis included four ethnically distinct populations. Of note, we found no difference between our Pakistani cases and controls, and the population of Pakistan has been shown to be genetically very similar to the population of neighboring Iran (Grugni et al., 2012; Nasidze, Quinque, Ozturk, Bendukidze, & Stoneking, 2005). Consistent with this, the overall Pakistani allele frequencies of both SNPs were very similar to (and statistically indistinguishable from) those of the Iranian Kurdish population reported by Mohammadi et al. (2017). Additionally, all of our cases had a family history of stuttering, while this was reported in some but not all of the stuttering cases studied by Mohammadi et al. (2017).

A second factor might be differences in genotyping methods. Mohammadi et al. (2017) employed a single genotyping method for each SNP, using PCR restriction fragment length polymorphism for *CYP17* and allele-specific PCR for *CYP19*. We used two quite different methods of DNA sequencing (dideoxy- Sanger and whole exome sequencing) to provide orthogonal approaches to genotyping both SNPs.

Overall, we hypothesize that a combination of the small sample size and genotype miscalls due to the methods employed by Mohammadi et al. (2017) resulted in a false positive finding of association between rs743572 in *CYP17* and stuttering.

Developmental stuttering clearly has a male preponderance, and some authors suggest that this male bias is associated with a difference in sex hormones (Selçuk, Erbay, Özcan, Kartalci, & Batcio lu, 2015). However, the published data from these studies has not been definitive. Our findings argue against the *CYP17* variant association and indicate that a role for sex hormones in developmental stuttering remains an open question.

4. Methods

We genotyped the previously studied SNPs rs2236722 in *CYP19* and rs743572 in *CYP17* using dideoxy- Sanger sequencing in total of 1765 individuals, which together represent a 9.6-fold larger sample size than that employed by Mohammadi et al. (2017). Our combined sample, (877 cases and 888 controls) had 99.9% statistical power to have 80% chance of detecting association, while that of Mohammadi et al. (2017) (85 cases and 85 controls) had a 34% chance of detecting association if it truly exists. Genotypes at these two SNPs were additionally assayed in 1471 of these individuals by whole exome sequencing using the Nextera Rapid Capture Exome kit (Illumina, San Diego, CA) followed by evaluation on an Illumina HiSeq 1500 sequence analyzer. The genotypes determined by these two methods

showed high correlations ($r=0.998$, $P<0.0001$), and genotype distributions for both SNPs did not deviate from Hardy-Weinberg equilibrium.

Acknowledgements

We thank Isabelle Roux, Melanie Barzik, and the anonymous reviewer for insightful comments on the manuscript. This work was supported in part by the intramural research program of the NIH, NIDCD, under grant Z1A-000046–18 to DD and grant ZIC DC000086 to the NIDCD Genomic & Computational Biology Core.

5. References

- Domingues CE, Olivera CM, Oliveira BV, Juste FS, Andrade CR, Giacheti CM, ... Drayna D (2014). A genetic linkage study in Brazil identifies a new locus for persistent developmental stuttering on chromosome 10. *Genet Mol Res*, 13(1), 2094–2101. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/24737434>. doi:10.4238/2014.March.24.13 [PubMed: 24737434]
- Grugni V, Battaglia V, Hooshyar Kashani B, Parolo S, Al-Zahery N, Achilli A, ... Semino O (2012). Ancient migratory events in the Middle East: new clues from the Y-chromosome variation of modern Iranians. *PLoS One*, 7(7), e41252 Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/22815981>. doi:10.1371/journal.pone.0041252 [PubMed: 22815981]
- Hong EP, & Park JW (2012). Sample size and statistical power calculation in genetic association studies. *Genomics Inform*, 10(2), 117–122. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/23105939>. doi:10.5808/GI.2012.10.2.117 [PubMed: 23105939]
- 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. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/20147709>. doi:10.1056/NEJMoa0902630 [PubMed: 20147709]
- 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 AI (–34T:C) and CYP19 codon 39 (Trp:Arg) variants in children with developmental stuttering. *Brain Lang*, 175, 47–56. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/28992603>. doi:10.1016/j.bandl.2017.09.004 [PubMed: 28992603]
- Nasidze I, Quinque D, Ozturk M, Bendukidze N, & Stoneking M (2005). MtDNA and Y-chromosome variation in Kurdish groups. *Ann Hum Genet*, 69(Pt 4), 401–412. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/15996169>. doi:10.1046/j.1529-8817.2005.00174.x [PubMed: 15996169]
- Purcell S, Cherny SS, & Sham PC (2003). Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics*, 19(1), 149–150. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/12499305>. [PubMed: 12499305]
- Raza MH, Amjad R, Riazuddin S, & Drayna D (2011). Studies in a consanguineous family reveal a novel locus for stuttering on chromosome 16q. *Hum Genet*, 131(2), 311–313. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=22205390. doi:10.1007/s00439-011-1134-2 [PubMed: 22205390]
- Raza MH, Gertz EM, Mundorff J, Lukong J, Kuster J, SchÄffer A, & Drayna D (2012). Linkage analysis of a large African family segregating stuttering suggests polygenic inheritance. *Human Genetics*, 1–12. Retrieved from 10.1007/s00439-012-1252-5. doi:10.1007/s00439-012-1252-5
- Raza MH, Riazuddin S, & Drayna D (2010). Identification of an autosomal recessive stuttering locus on chromosome 3q13.2–3q13.33. *Hum Genet*, 128(4), 461–463. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/20706738>. doi:10.1007/s00439-010-0871-y [PubMed: 20706738]
- Riaz N, Steinberg S, Ahmad J, Pluzhnikov A, Riazuddin S, Cox NJ, & Drayna D (2005). Genomewide significant linkage to stuttering on chromosome 12. *Am J Hum Genet*, 76(4), 647–651. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/15714404>. doi:10.1086/429226 [PubMed: 15714404]
- Selçuk EB, Erbay LG, Özcan Ö, Kartalci , & Batcio lu K (2015). Testosterone levels of children with a diagnosis of developmental stuttering. *Ther Clin Risk Manag*, 11, 793–798. Retrieved from

<https://www.ncbi.nlm.nih.gov/pubmed/25999727>. doi:10.2147/TCRM.S83129 [PubMed: 25999727]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights

- We sought to replicate the results reported by Mohammadi et al. in *Brain and Language* 175 (2017) 47–56.
- We employed a 9.6-fold larger sample size and two independent methods of genotyping for each sample
- We found no association between the variant rs743572 in the *CYP17* gene and stuttering

Table 1.*CYP17* gene SNP rs743572 genotypes.

Genotype Distribution						Genotype Distribution					
Group		Male	Female	Total	Freq. (Total)	Group		Male	Female	Total	Freq. (Total)
a.											
Br-St (n=150)	TT	36	9	45	0.3000	Cam-St (n=106)	TT	34	8	42	0.3962
	TC	57	16	73	0.4866		TC	32	12	44	0.4150
	CC	22	10	32	0.2133		CC	14	6	20	0.1886
Br-Co (n=204)	TT	37	38	75	0.3676	Cam-Co (n=73)	TT	12	15	27	0.3698
	TC	48	46	94	0.4607		TC	19	10	29	0.3972
	CC	16	19	35	0.1715		CC	6	11	17	0.2328
EurAm-St (n=474)	TT	158	45	203	0.4282	Pk-St (n=147)	TT	44	8	52	0.3537
	TC	144	52	196	0.4135		TC	69	7	76	0.5170
	CC	55	20	75	0.1583		CC	18	1	19	0.1292
EurAm-Co (n=434)	TT	68	92	160	0.3686	Pk-Co (n=177)	TT	58	11	69	0.3898
	TC	84	127	211	0.4861		TC	71	10	81	0.4576
	CC	32	31	63	0.1451		CC	22	5	27	0.1525
b.											
All-St (n=877)	TT	272	70	342	0.3899	All-Co (n=888)	TT	175	156	331	0.3727
	TC	302	87	389	0.4435		TC	222	193	415	0.4673
	CC	109	37	146	0.1664		CC	76	66	142	0.1599
Allele C freq. = 0.3882						Allele C freq. = 0.3935					
Allele T freq. = 0.6117						Allele T freq. = 0.6064					

c.						
<i>P values</i>						
Male+Female						
Males	Br-St	EurAm-St	Cam-St	Pk-St	All-St	
Females						
	0.3523					
Br-Co	0.6608					
	0.3264					
		0.0847				
EurAm-Co		0.2643				
		0.3711				
			0.771			
Cam-Co			0.4906			
			0.3283			
				0.5568		
Pk-Co				0.6261		
				0.5050		
All-Co					0.6042	

0.5961
0.6234

NIH, NIDCD, under grant Z1A-000046-18 to DD and grant ZIC DC000086 to the NIDCD Genomic & Computational Biology Core. a. Genotypes in stuttering case and controls separated by groups and gender. b. Total genotypes in stuttering case and controls, genotype and allele frequency. c. Chi-square *P* values comparing frequency in cases vs. controls. *Legend:* Genotypes determined by dideoxy- Sanger DNA sequencing in case and control populations. Br-St, Brazilian stuttering cases, Br-Co, Brazil controls, EurAm-St, European American stuttering cases, EurAm-Co, European American controls, Cam-St, Cameroonian stuttering cases, Cam-Co, Cameroonian controls, Pk-St, Pakistani stuttering cases, Pk-Co, Pakistani controls. All-St, all stuttering cases combined, All-Co, all controls combined.