Candidate regulatory variants in SNARE complex genes and their involvement in migraine susceptibility

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Migraine is a disabling and multifactorial neurological disease, remaining unexplained most of its heritability and susceptibility. Some migraine risk *loci* have been shown to reside in non-coding regions, which may alter gene expression and epigenetic regulation[1,2]. Genes previously associated with migraine susceptibility in the Portuguese population are *SYN1*, *SNAP25*, *VAMP2*, *STXBP1*, *STXBP5*, *SYN2*, *UNC13B*, *GABRA3*, *GABRQ*, and *STX1A*[3-5].

Aim: To select the best SNP candidates to study regulatory changes in genes previously associated with migraine susceptibility.

1. Pre-selection of variants: Linkage Desequilibrium (LD) analysis

Haploview software

HapMap Project data

r2 ≥ 0.80

MAF ≥ 0.10

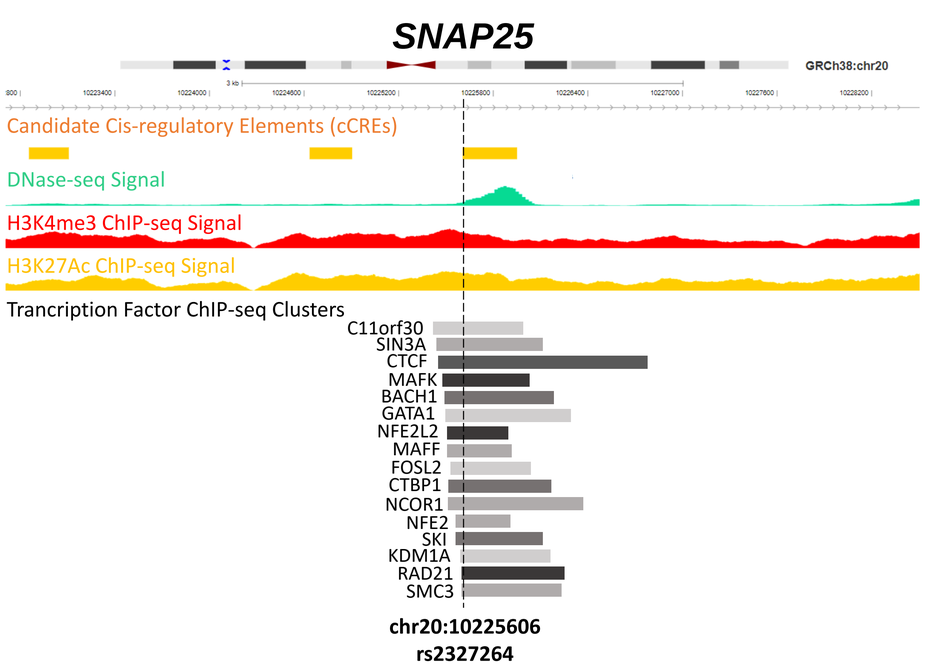
Non-coding *tag*SNPs from LD analysis								
SYN1	***SNAP25***	***VAMP2***	***STXBP1***	***STXBP5***	***SYN2***	***UNC13B***	***GABRA3***	***STX1A***
rs723556	rs3787303	rs1150	rs3780658	rs1765028	rs217049	rs7851161	rs3902802	rs941298
rs5906435	rs363050				rs307574		rs2131190	rs6951030
rs2239459	rs6108463				rs3773364			
rs5906437	rs363039				rs310763			
	rs2327264							
	rs362990							

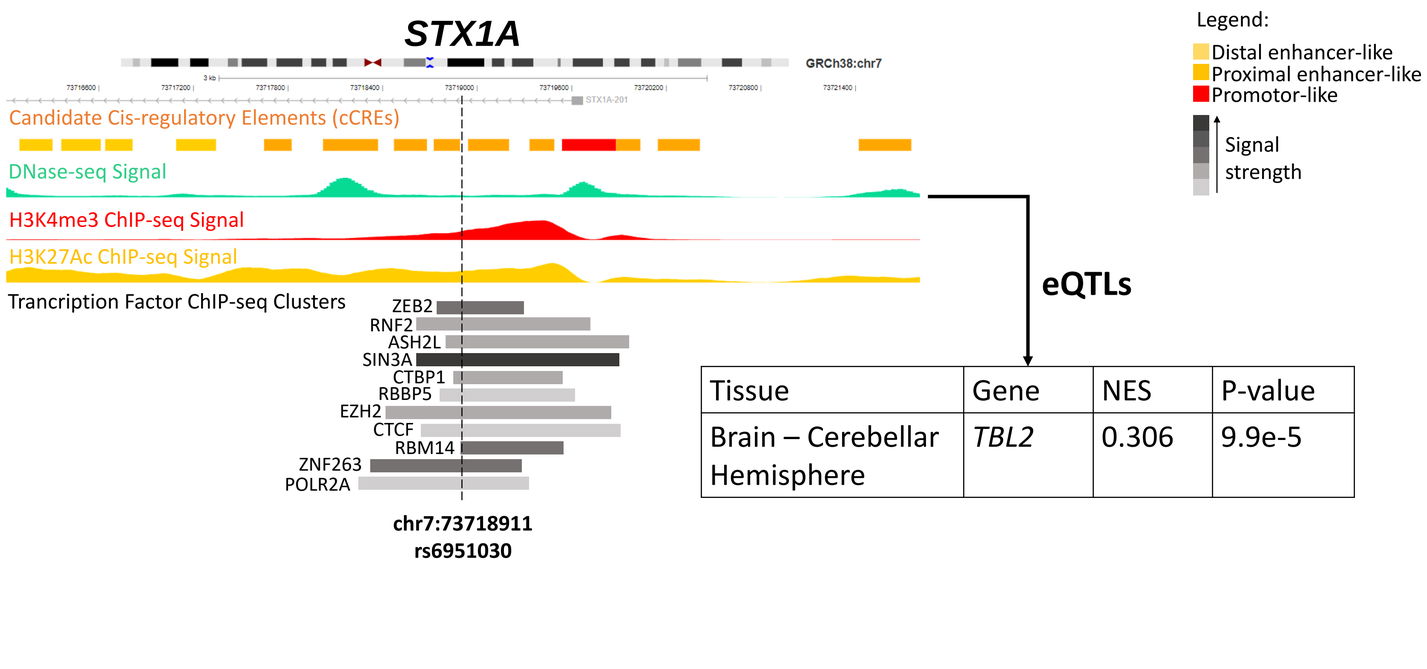
2. Prioritization of variants: prediction and annotation

Functional Scoring	Regulatory annotation
CADD, FATHMM, GWAVA, ReMM, DANN, FunSeq2, and RegulomeDB	HaploReg, GWAVA and RegulomeDB
≥ 3 scores predicting deleteriousness	DNA accessibility, eQTLs, histone marks and transcription factors domains from ENCODE/GTEx

In silico integrative evaluation of the 22 *tag*SNPs:

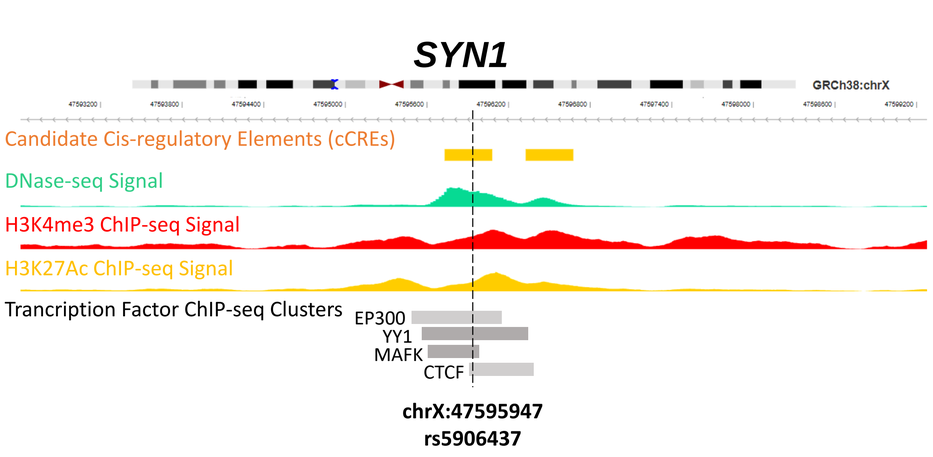
7 SNPs met our criteria, priority being given to the 4 SNPs located in major candidate regulatory regions: rs6951030 (***STX1A***), rs2327264 (***SNAP25***), rs5906437 (***SYN1***) and rs1150 (***VAMP2***).

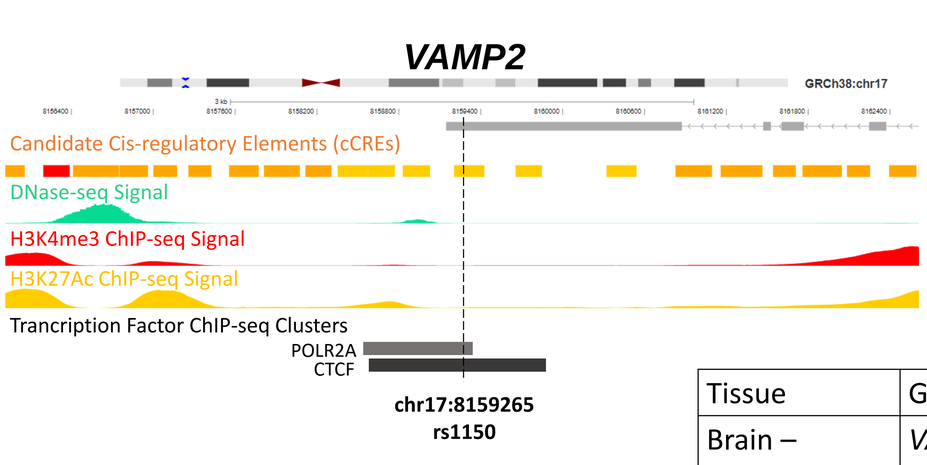




eQTLs

Tissue	Gene	NES	P-value
Brain – Cerebellar Hemisphere	*TBL2*	0.306	9.9e-5





eQTLs

Tissue	Gene	NES	P-value
Brain – putamen	*VAMP2*	0.424	1.4e-13

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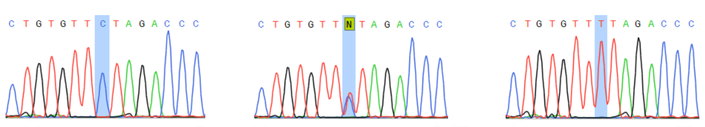
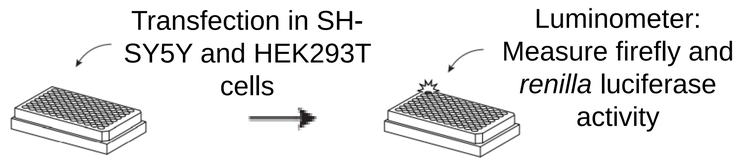
3. Functional validation: gene expression assay

Genotyping of SNPs in migraineurs → **Construction of luciferase reporter vectors** → **Luciferase reporter gene expression assays**





In silico analyses suggested possible alterations in gene regulation of SNARE proteins implicated in exocytotic neurotransmitter release in migraine, as well as modulation of gene expression related to cellular stress.
→ We are currently performing the functional validation of these SNPs through luciferase reporter assays.

References: 1- Neurogenetics. 2020; 21: 149–157. 2- BMC Medical Genetics. 2010; 11: 103. 3- Headache. 2020; 60: 2152-2165. 4- PLoS ONE. 2013; 8: e74087. 5- Arch Neurol. 2010; 67: 422-427.

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