Selection of variants in SNARE complex genes to study their impact on gene expression and migraine susceptibility

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Introduction

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. Some genes previously associated with migraine susceptibility in the Portuguese population are *SYN1*, *SNAP25*, *VAMP2*, *STXBP1*, *STXBP5*, *SYN2*, *UNC13B*, *GABRA3*, *GABRQ*, and *STX1A*.

Aim: select the best SNP candidates to study *cis*-regulation of genes previously associated with migraine susceptibility.

Methods

1. Pre-selection of variants: LD analysis

Haploview software
HapMap Project data
r2>=0.80
MAF>=0.10

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2. Prioritization of variants: annotation and prediction

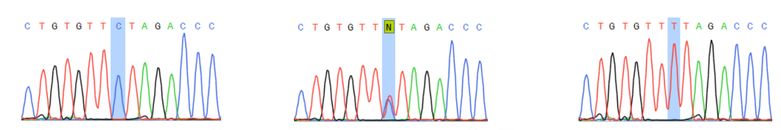
VEP, SNPInfo and SNPnexus

Regulatory annotation
HaploReg, GWAVA and RegulomeDB
→ Regulatory elements: promoters, enhancers, TFBS, and ncRNAs

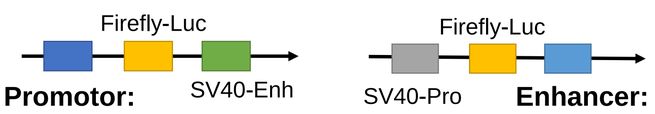
Functional Scoring
CADD, FATHMM, GWAVA, ReMM, DANN, FunSeq2, and RegulomeDB
→ ≥ 3 scores predicting deleteriousness

3. Functional validation: gene expression assays

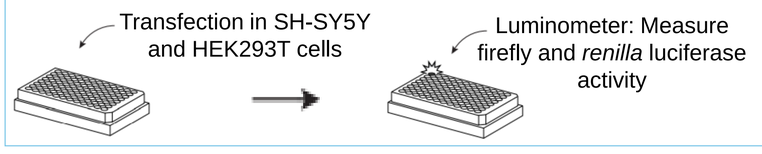
Detection of SNPs in migraineurs DNAs



Construction of luciferase reporter vectors



Luciferase reporter gene expression assays



Results

From the 22 *tagging* SNPs: 7 SNPs met our criteria, priority being given to the 3 SNPs located in major regulatory regions: **rs6951030** (*STX1A;* promotor), **rs2327264** (*SNAP25*; enhancer) and **rs1150** (*VAMP2*; 3'UTR).

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							

In silico* integrative *evaluation

Annotation: regulatory features

Gene	dbSNPs ID	Location	Regulation features	Chromatin State (brain)	Transcription Factors (ChIP)	Motifs
SNAP25	rs2327264	Intron1/7	ENSR00000645066 (enhancer); ENSR00000645067 (CTCF binding site)	Strong transcription	CTCF, MAFK, NFE2L2, NFE2, EZH2, NFE2L1, MAFF, EMSY, MAFG, ARID1B, SKI, RAD21, BACH1, SMC3, KDM1A, CTBP1	CEBPA
STX1A	rs6951030	Intron 1/9	ENSR00000213499 (promoter)	Active TSS	CTCF, ZEB2, EZH2, GLIS1, ZIC2, ZBTB20, ZNF740, MYC, SCRT2, CTBP1, KLF1, YY1, MAS, GABPB1, RNF2, ZBTB10, SRSF1, PATZ1, SCRT1, AGO2, TFAP4, KLF7, HNRNPLL, ZNF263	Rad21
VAMP2	rs1150	3'UTR	ENSR00000548703 (CTCF binding site)	Strong transcription	CTCF, VEZF1, ZNF629, NR2C2, MAZ, ZBTB11	GLIS2, OBOX4-PS35, ZFP281, Zbtb12

Final Remarks

In silico analyses suggested possible alterations in gene regulation of SNARE complex proteins implicated in exocytotic neurotransmitter release in migraine.
→ We are currently performing the functional validation of these SNPs through luciferase reporter gene expression assays.

References

1 - Neurogenetics. 2020; 21: 149–157. 2 - BMC Medical Genetics. 2010; 11(1): 103. 3 - Headache. 2020; 60(10): 2152-2165. 4 - PLoS ONE. 2013; 8(9): e74087. 5 - Arch Neurol. 2010; 67(4): 422-427.

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