METHYLATION AT A CPG SITE IN RAMP1 PROMOTER IS ASSOCIATED WITH MIGRAINE

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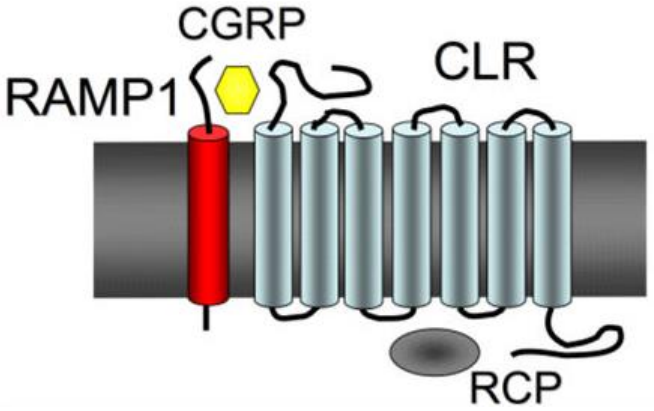
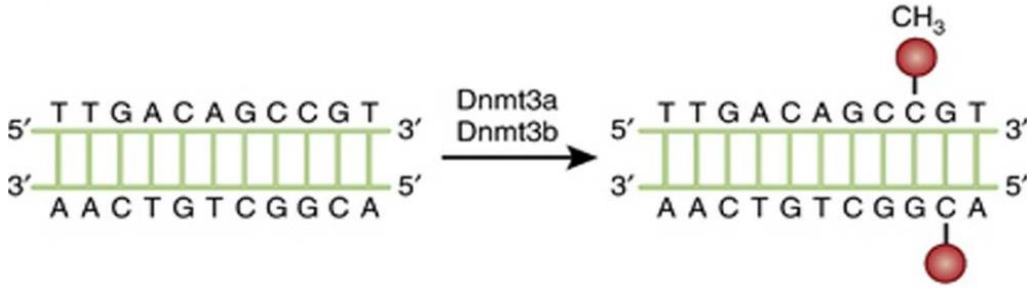
Introduction

- **Migraine** is a complex debilitating neurovascular disorder characterized by attacks of moderate to severe headache pain lasting 4 to 72h and symptoms may include photophobia, phonophobia, nausea and vomiting[1,2].
- Calcitonin Gene Related Peptide (**CGRP**) is frequently implicated in migraine pathophysiology and is a target for migraine treatment[3].
- CGRP receptor consists of three proteins: Calcitonin Receptor-Like Receptor (**CLR**); Receptor Activity Modifying Protein 1 (**RAMP1**); Receptor Component Protein (**RCP**)[4,5]
- **DNA methylation** occurs mostly at cytosine residues in CpG dinucleotides in the gene promoter. It can control gene expression by recruiting proteins involved in gene repression or by impeding the binding of transcription factors to DNA[6].



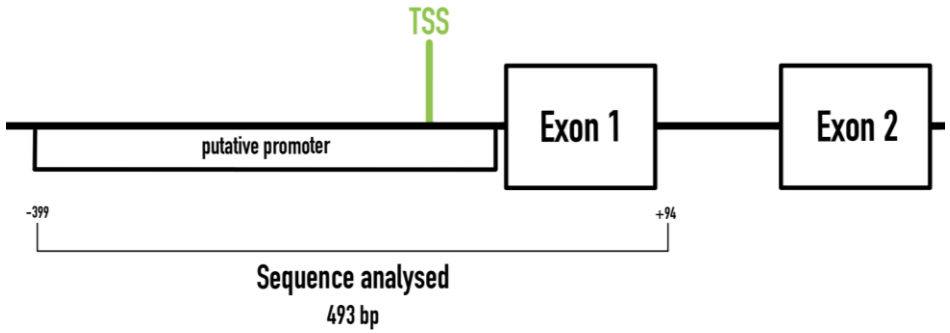


We **aim** to study the *RAMP1* promoter methylation status in order to find epigenetic biomarkers that predict female migraine risk in an accessible body fluid, such as blood.

Images adapted from Moore, L. 2012. Neuropsychopharmacology; and Russo, AF. 2009. Molecular and Cellular Pharmacology

Methodology

Bisulfite Sequencing of 104 samples: 54 cases + 50 controls	• Treatment with sodium bisulfate leads to the deamination of unmethylated cytosines converting them to uracil, while methylated cytosines are left intact.
Polymerase Chain Reaction (PCR)	• PCR amplification recognizes uracils as thymines and methylated cytosines as cytosines. • Bisulfite-specific primers designed to amplify the promoter region of RAMP1
Sanger Sequencing	• Using the same primers as those used for the PCR reaction
Epigenetic Sequencing Methylation (ESME) software analysis	• Software that performs quality control, corrects incomplete conversion, normalizes signals, and provides the measurement of cytosine methylation by comparing the C and T peaks at CpG sites
Statistical Analysis	• Chi-square test • Logistic regression



Results

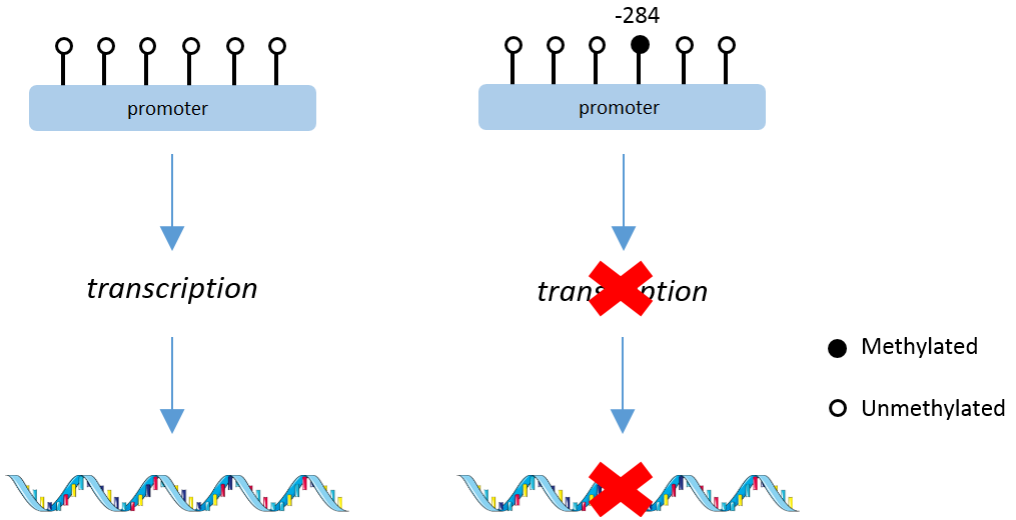
- **51 CpG** identified in our analysed sequence – only the first 5 showed variability
- Higher proportion of migraine cases with **all five CpG units methylated** compared to controls (26% vs 16%)
- **-284 CpG unit** (related to the Transcription Start Site) **showed significantly higher methylation** levels in cases when compared to controls

CpG UNIT	OR	95%C.I.	P-VALUE
-346	0.99	(0.95 - 1.03)	0.582
-334	1.02	(0.99 - 1.07)	0.509
-284	1.06	(1.01 - 1.12)	0.017*
-276	0.97	(0.92 - 1.02)	0.225
-234	0.98	(0.93 - 1.03)	0.411

OR- odds ratio; C.I.- confidence interval. *p<0.05

Discussion

- Only one study, relying on a small sample size, has analyzed the methylation of the human RAMP1 promoter in the context of migraine[7].
- Our preliminary results seem to contradict that study as we found that **female migraineurs generally tend to have higher methylation levels** than female controls.
- **We discovered a new CpG island potentially associated to migraine** which may disrupt the transcription of CGRP.



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