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SHORT COMMUNICATION

Epigenetic regulation of the kappa opioid receptor gene by an insertion-deletion in the promoter region

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Abstract
Preclinical and clinical studies have demonstrated that the kappa opioid receptor (KOR) regulates reward, hedonic tone and emotions. At therapeutic level, on-going clinical trials are assessing the potential of targeting the KOR for the management of depression, anxiety disorders and substance use disorders. However, genetic polymorphisms in the KOR gene that potentially contribute to its implication in these phenotypes have been poorly studied. Here we investigated an insertion-deletion in the promoter region of KOR (rs35566036), recently associated with alcohol addiction, in a cohort of depressed subjects who died by suicide, as well as psychiatrically healthy individuals. Focusing on 3 brain regions (anterior insula, anterior cingulate cortex, and mediodorsal thalamus), we characterized the functional impact of this structural variant on the expression and patterns of DNA methylation of the KOR gene, using qPCR and targeted Bisulfite-Sequencing, respectively. While there was no significant change in the expression of KOR as a function of the insertion-deletion, or as a function of disease status in any brain region, we found that this variant strongly determines DNA methylation in KOR promoter, leading to a significant decrease in methylation levels of 8 nearby CpG dinucleotides

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located approximately 500 base pairs upstream the transcription start site. In addition, our results suggest a possible association between the insertion-deletion and depression; however, this result should be tested in larger populations. In sum, in this study we uncovered an epigenetic mechanism potentially contributing to KOR dysfunction in carriers of the insertion-deletion.

1. Introduction

The kappa opioid receptor (KOR) belongs to the endogenous opioid system, and is known to tightly regulate essential physiological functions, including reward (Wee and Koob, 2010), mood (Lutz and Kieffer, 2013), and stress homeostasis (Bruchas et al., 2010). Over the last two decades, it has been demonstrated that dysregulation of KOR function is implicated in the pathophysiology of numerous psychiatric disorders such as anxiety, depression and addiction (Lalanne et al., 2014). Pharmacological research also suggests that KOR antagonists might have beneficial properties in the treatment of depressed mood (Falcon et al., 2016) and anxiety-related symptoms (Carlezon and Krystal, 2016), as well as in the prevention of stress-induced relapse in drug addicts (Lalanne et al., 2017, 2014), among others.

Genetic polymorphisms in the opioid system, including the KOR gene, have been largely investigated for their potential association with addiction to alcohol, heroin and cocaine (Butelman et al., 2012). Beyond substance use disorders, it is possible that KOR genetic variants may also impact on the risk of major depressive disorder. The later aspect, however, has been relatively poorly documented, with only limited evidence for an association between KOR polymorphisms and stress sensitivity (Xu et al., 2013).

An insertion-deletion was identified in 2008 by Edenberg et al. (2008) in the KOR promoter region (INDEL, rs35566036). This variant corresponds to the deletion of 11 base pairs (bp) from −1975 to −1985 upstream the transcription start site, and to the insertion of 841 bp at −1986, resulting in a net insertion of 830 bp. Luciferase assays provided *in vitro* evidence suggesting that the INDEL might lower the transcriptional activity of the KOR promoter. Surprisingly, to our knowledge, the *in vivo* relevance and potential implication in depression of this variant remains currently unexplored. Therefore, this study was designed to characterize how the expression and epigenetic landscape of the KOR locus might be affected in brain tissue by the INDEL. Focusing on a cohort of depressed subjects who died by suicide, and psychiatrically healthy individuals as control group, we genotyped the INDEL and quantified KOR expression in 3 brain regions implicated in mood control: the anterior insula (AI), the dorsal part of the anterior cingulate cortex (ACC), and the mediodorsal thalamus (MDT). We chose to focus our analyses on these three brain regions, due to their well-known involvement in emotional regulation and to remain consistent with a former study of ours (Lutz et al., 2015b). Furthermore, we characterized the impact of the INDEL on DNA methylation, a form of epigenetic plasticity that modulates gene activity, brain function and behaviour (Lutz et al., 2015a, 2017a).

Our results indicate that the INDEL does not directly affect KOR expression *in vivo*, at least in the brain regions examined, while it strikingly predicts DNA methylation within the KOR promoter. We propose that such major DNA methylation differences occurring as a function of the INDEL may contribute to epigenetic plasticity at the KOR locus, and help explain how this variant might be implicated in addictive or depressive conditions.

2. Experimental procedures

2.1. Cohort (Table 1)

Our cohort consisted of both males and females who were depressed and died by suicide, as well as psychiatrically healthy controls who died by accidental causes. Brain tissue was obtained from the Douglas-Bell Canada Brain Bank. Psychological autopsies were conducted for each subject as described elsewhere (Lutz et al., 2017b). Tissue samples from three brain regions (AI, ACC, MDT) were dissected as described in Supplementary Material (see also (Lutz et al., 2015b)).

2.2. Quantitative polymerase chain reaction (qPCR)

qPCR was performed as described previously (Lutz et al., 2015b) to quantify the expression of the *OPRK1* gene encoding the KOR, in quintuplicates, using 2 housekeeping genes (GAPDH, β-actin; see primers in Supplementary Table 1). Only samples with a RNA integrity number (RIN) >5 were used (5 out of 82 subjects discarded).

2.3. Genotyping

Allele frequencies for the INDEL were determined by PCR amplification of brain DNA (Table 1), using a Platinum *Taq* DNA Polymerase (Invitrogen®) and parameters and primers described by Edenberg et al. ((2008), and see Supplementary material).

2.4. DNA methylation

DNA methylation was analysed as described by Masser et al. (2013) and in Supplementary material. Briefly, genomic DNA was bisulfite converted, and then amplified by PCR (Supplementary Table 1). Libraries were prepared using the NEXTERA XT kit, indexed by PCR, and sequenced on an Illumina MiSeq.

2.5. Bioinformatic analysis

As described previously (Chen et al., 2017), adaptors were trimmed from sequencing reads, which were further trimmed when Phred quality scores dropped below 20. Bismark v0.14.4 was run with Bowtie 2 to align reads to the hg19 human genome. The extraction

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