miRNA-320a regulation of FKBP5 mediates chronic posttraumatic pain vulnerability in an allele-specific manner

INTRODUCTION

One of the most common causes of chronic pain development is exposure to traumatic or stressful events. Unfortunately, the molecular and genetic mechanisms driving posttraumatic chronic pain are poorly understood. We previously showed that: (1) A critical regulator of the stress axis, the glucocorticoid receptor co-chaperone (FKBP5), is a strong predictor of posttraumatic chronic pain development. (2) microRNA-320a directly regulates FKBP5 RNA and predicts chronic pain following trauma.2

HYPOTHESIS

In this study, we hypothesized that a variant in the 3’UTR of FKBP5 predicts chronic pain development following motor vehicle collision (MVC) trauma in both African American and European American individuals and that this variant is functional based on its ability to efficiently bind miR-320a.

METHODS

HUMAN: Two prospective longitudinal studies enrolling European or African American individuals ≥ 18 and ≥ 65 years of age presenting to the ED within 24 hours of MVC were used to study post-traumatic outcomes. The details of these study samples have been described previously.1, 4 MVC was defined as an overall MSP exceeding 10 points using MVC using the modified Regional Pain Scale. The relationship between rs3800373 and chronic MSP outcomes in European Americans and African Americans following MVC was assessed using general linear models. For RNA sample collection, research assistants collected blood samples in the ED at the time of enrollment using PAXgene RNA tubes. Total RNA (including miRNA) was isolated using the PAXgene blood RNA kit (QIAGEN). mRNA and miRNA were sequenced using Illumina technology.

BIOINFORMATICS: The miR$^\text{SNP}$ online database (http://mirsnps.ccr.buffalo.edu) was used to assess whether rs3800373 directly interferes with miRNA binding and to determine miRNA predicted to bind 200nt upstream or downstream of the SNP (SNP-regulated binding region). The RNA-RNA folding algorithm (http://r2d2.resources/marsi/) was used to determine whether the presence of major or minor allele of rs3800373 was likely to have an effect on RNA secondary structure within the SNP-regulated binding region. IN VITRO: To assess allele-specific mRNA binding in vitro, dual luciferase reporter assays were used in HEK293T cells. The reporter assay consisted of a miRNA expression construct and plasmids with the FKBP5 3’UTR inserted downstream of a firefly luciferase gene. mRNA binding was quantified by measuring the level of luciferase protein in cells and this level was compared between cells containing 3UTRs with major or minor allele at rs3800373 or between 3UTR constructs of wild type and SHAPE: Selective 2-hydroxy acylation analyzed by primer extension (SHAPE) data was obtained in vivo using the EOB immobilized lymphoblastoid cell line generated from Yoruban male 19082 (1000 Genomes - Coriell Institute for Medical Research), which is heterozygous for rs3800373.

RESULTS

FKBP5 allele rs3800373 is associated with chronic pain development following MVC in both European American (p=0.007, p=0.035) and African American (p=0.136, p=0.002) individuals (Figure 1). The relationship between FKBP5 mRNA expression and a) cortical or b) NGF1 miRNA (coronacoid receptor) expression is dependent on rs3800373 (Figure 2). These results suggest that rs3800373 is a functional allele and might affect glucocorticoid signaling

miRNA-3200373 is located in the 3’UTR of FKBP5 and is proximal to three predicted miRNA binding sites (Figure 3). Based on in vitro luciferase activity, three miRNAs can bind and regulate FKBP5 miRNA binding sites in an allele-dependent manner, with less efficient binding in the presence of the risk allele (Figure 3, left). Cell culture studies show that miR-320a regulates FKBP5 in an endogenous setting (Figure 3, right). The relationship between FKBP5 mRNA expression and miR-320a expression is allele dependent in MVC study participants. Consistent with in vitro data, individuals with the protective allele show a statistically significant negative correlation between these RNA molecules, suggesting FKBP5 regulation by miR-320a. No correlation was observed in individuals with the risk allele (Figure 4), suggesting inefficient regulation of FKBP5 by miR-320a.

miRNA-320a regulates FKBP5 in the vicinity of the miR-320a binding site (Figure 5). Previous literature demonstrates that miRNA bind to RNA in regions where the nucleotides are accessible.5 Thus, the structure formed in the presence of the major allele is more conducive with miR-320a binding. This data is consistent with data presented in Figures 3 and 4.

CONCLUSIONS

FKBP5 expression is regulated by miR-320a in a rs3800373 allele-dependent fashion. Identification of this functional SNP in FKBP5 increases understanding of specific molecular pathways of vulnerability to persistent treatment-related chronic pain, and suggests that the future exogenous methods to achieve targeted reduction in post-stress FKBP5 mRNA expression (e.g., via miRNA mimics or siRNA administration) in vulnerable individuals may constitute useful therapeutic strategies.

REFERENCES

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