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

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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.[1] (2) microRNA-320a directly regulates *FKBP5* RNA and predicts chronic pain following trauma.[2]

HYPOTHESIS

In this study, we evaluated the hypothesis that a genetic 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 it's 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 sister studies have been described previously.[3,4] MVC-related overall MSP in the past week was assessed six weeks following MVC using the modified Regional Pain Scale. The relationship between rs3800373 and chronic MSP outcomes in European Americans and African Americans following MVC were 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 miRNA kit (QIAGEN). mRNA and microRNA were sequenced using Illumina methodology.

BIOINFORMATICS: The miRdSNP online database (http://mirdsnp.ccr.buffalo.edu/) was used to assess whether rs3800373 directly interferes with miRNA binding and to determine miRNAs predicted to bind 200nt upstream or downstream of the SNP (SNP-related binding region). The RNAsnp RNA folding algorithm (http://rth.dk/resources/rnasnp/) was used to determine whether the presence of the major vs minor allele of rs3800373 was likely to have an effect on RNA secondary structure within the SNP-related binding region.

IN VITRO: To assess allele-specific miRNA 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. miRNA binding was quantified by measuring the level of luciferase protein in cells and this level was compared between cells containing 3'UTRs with major vs minor alleles at rs3800373 or between 3'UTR mutants.

SHAPE: Selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) data was obtained in vivo using the EBV immortalized lymphoblastoid cell line generated from Yoruban male 19098 (1000 Genomes - Coriell Institute for Medical Research), which is heterozygous for rs3800373.

FIGURE 1. FKBP5 rs3800373 allele predicts chronic pain severity in a stress-dependent manner following motor vehicle collision in European American (n = 844) and African American (n = 763) individuals.

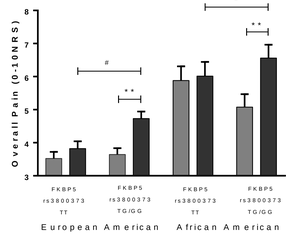


FIGURE 2. The *FKBP5* rs3800373 allele is functional. Shown is the relationship between circulating *FKBP5* mRNA expression levels and circulating cortisol levels (top) and circulating *FKBP5* mRNA versus *NR3C1* mRNA (bottom) in the early aftermath of MVC in individuals with the rs3800373 protective (left) versus risk (right) allele.

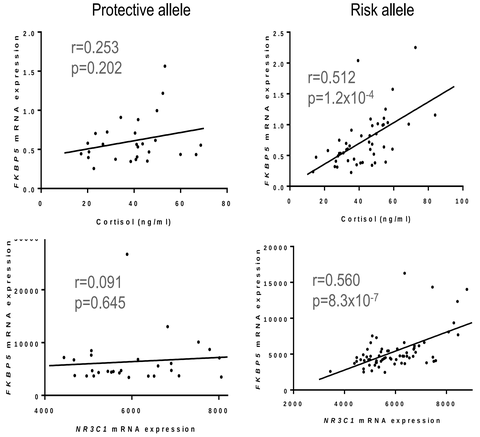


FIGURE 3. In vitro analyses show that miR-320a binds to the *FKBP5* 3'UTR and does so in an allele dependent manner (left). Additional cell culture data (right) shows that miR-320a regulates FKBP5 in an endogenous setting.

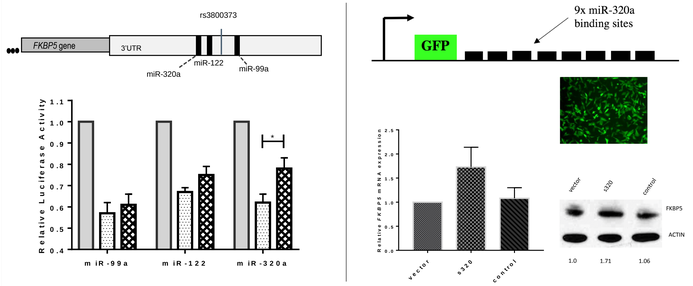


FIGURE 4. The relationship between circulating *FKBP5* mRNA levels and miR-320a levels is allele dependent

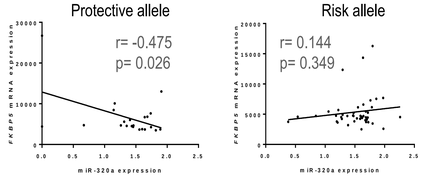
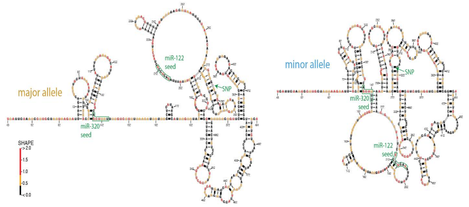


FIGURE 5. rs3800373 influences the RNA secondary structure of the *FKBP5* 3' UTR. Shown are the RNA structural models derived from *in vivo* SHAPE data of an ~500nt region encompassing rs3800373 and the miR-320a binding site.



RESULTS

FKBP5 allele rs3800373 is associated with chronic pain development following MVC in both European American (β=0.787, p=0.035) and African American (β=1.36, p=0.002) individuals (Figure 1).

The relationship between *FKBP5* mRNA expression and a) cortisol or b) NR3C1 (glucocorticoid receptor) expression is dependent on rs3800373 (Figure 2). These results suggest that rs3800373 is a functional allele and might affect glucocorticoid signaling.

rs3800373 is located in the 3'UTR of *FKBP5* and is proximal to three predicted miRNA binding sites (Figure 3). Based on *in vitro* binding studies, all three miRNA can bind and regulate *FKBP5.* miR-320a binds 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.

rs3800373 affects the RNA secondary structure of *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 i*ncreases understanding of specific molecular pathways of vulnerability to persistent trauma/stress-related chronic pain, and suggests that in 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.

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