A genetic variant in the glucocorticoid receptor co-chaperone *FKBP5*, associated with chronic pain vulnerability, changes RNA secondary structure and alters binding by miR-320a

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Trauma Recover**Y**: Understanding Mechanisms & Promoting Healing

INTRODUCTION

Genetic variants influencing vulnerability to chronic pain are being identified with increasing frequency. The utility of such findings is incomplete without determining whether the variant results in changes in cellular function, and, if so, the mechanisms by which this occurs. We previously found that the minor allele of glucocorticoid receptor co-chaperone *FKBP5* single nucleotide polymorphism (SNP) rs3800373 is associated with worse persistent pain outcomes after motor vehicle collision and sexual assault (Table 1).[1] Potential functional effects of the minor allele of SNP rs3800373 are unknown. microRNA (miRNA) have been shown to be important regulators of cellular function, and rs3800373 is located in the 3'UTR of the mRNA (Figure 1), where miRNA binding takes place.

HYPOTHESES

We hypothesized that the genetic variant rs3800373 in *FKBP5* alters miRNA binding. We also hypothesized that the mechanism by which it affects miRNA binding is not through direct disruption of seed binding but by altering the secondary structure of the *FKBP5* 3'UTR.

METHODS

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). 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. Luciferase activity was measured on a luminometer 72 hours after co-transfection of HEK293T cells with reporter constructs and a microRNA expression vector. The RNAsnp RNA folding algorithm (http://rth.dk/resources/rnasnp/)[2] was then used to determine whether the presence of the major vs minor allele of rs3800373 has an effect on RNA secondary structure within the SNP-related binding region.

TABLE 1. rs3800373 is associated with persistent pain 6 weeks following motor vehicle collision (MVC) and sexual assault (SA) trauma

		T/T Mean (95% CI)	T/G + G/G Mean (95% CI)	p-value
MVC (n = 949)	Overall pain	3.6 (3.3, 3.8)	4.1 (3.9, 4.4)	0.003
	Neck pain	2.4 (2.1, 2.7)	3.2 (2.9, 3.5)	0.0001
SA (n = 53)	Overall pain	2.1 (0.9, 3.3)	3.8 (2.7, 4.9)	0.029
	Neck Pain	1.2 (0.1, 2.3)	2.8 (1.8, 3.9)	0.035

FIGURE 1. rs3800373 is located in 3'UTR of *FKBP5*

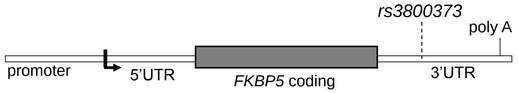


FIGURE 2. Three miRNAs are predicted to bind in close vicinity to SNP rs3800373, but the allele at rs3800373 is not predicted to directly affect seed region binding by any of these miRNA

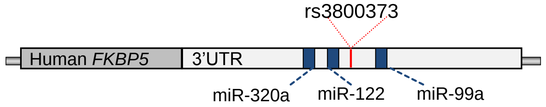


FIGURE 3. miRNA binding to *FKBP5* 3'UTR containing the Major vs. Minor allele at rs3800373. miR-320a represses the *FKBP5* 3'UTR containing the minor allele less efficiently than the 3'UTR containing the major allele. *p value = 0.025

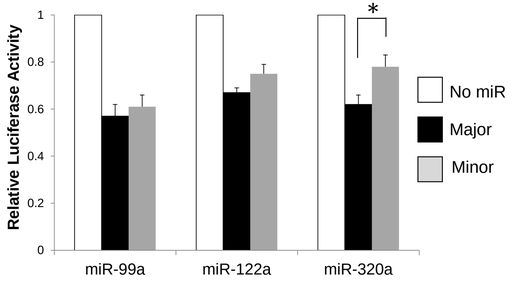


FIGURE 4. Multiple species conservation of nucleotides in the miR-320a binding domain of *FKBP5*

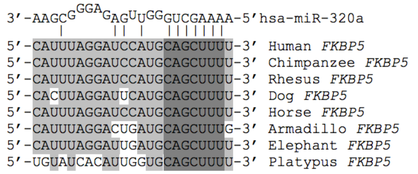


FIGURE 5. Mutations to the 3'UTR of FKBP5 demonstrate that the miR-320a site closest to rs3800373 confers most of the miR-320a knock-down activity

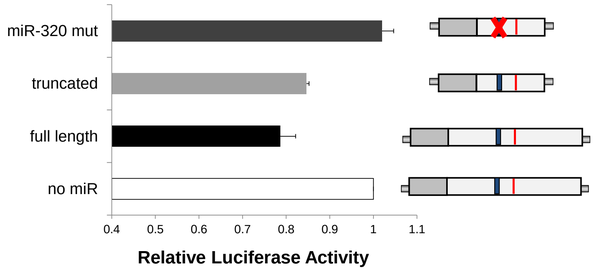
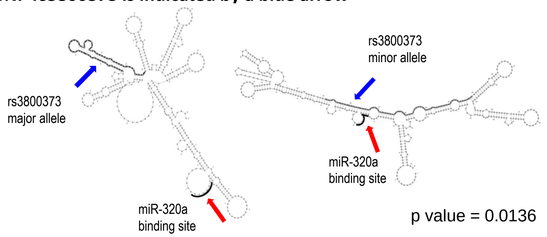


FIGURE 6. The predicted RNA secondary structure is different depending on the identity of the allele at rs3800373. The secondary structure with the major allele (left) has more loop regions than the structure with the minor allele (right). Loop regions have been shown to be more favorable for miRNA binding than paired regions. The miR-320a binding site is indicated in black (with a red arrow) and SNP rs3800373 is indicated by a blue arrow



RESULTS

- While the minor allele was not predicted to directly affect miRNA binding, three miRNAs, miR-99a, miR-122, and miR-320a are predicted to bind in the SNP-related binding region. (Figure 2)
- Luciferase reporter assays determined that miR-320a binds less efficiently to the 3'UTR of FKBP5 (p = 0.025) when the minor allele is present vs when the major allele is present. (Figure 3)
- The potential binding site for miR-320a within the *FKBP5* 3'UTR is highly conserved across eight species (Figure 4), supporting its potentially important role in biologic function.
- Mutational analyses rule out the possibility that other binding sites apart from the one in the SNP-related binding region of the FKBP5 3'UTR are responsible for miR-320a binding (Figure 5).
- RNAsnp RNA folding algorithm results indicate that the rs3800373 allele determines RNA secondary structure. When the major allele is present, the structure has multiple single stranded loop regions, including one where miR-320a binds. Loop regions are known to facilitate miRNA binding.[3] (p value for structural difference = 0.0136) (Figure 6).

CONCLUSIONS

The minor allele of rs3800373 in the glucocorticoid receptor co-chaperone *FKBP5*, which has been associated in increased vulnerability to persistent pain after multiple stress exposures, reduces miR-320a binding to the FKBP5 3'UTR. This loss of translational repression by miR-320a would result in increased FKBP5 protein levels and greater glucocorticoid resistance. These results suggest that the influence of *FKBP5* SNP rs3800373 on pain outcomes is mediated, at least in part, by effects on miRNA function. In future studies we will examine allele-specific differences in FKBP5 expression in vivo, and will explore miR-320a regulation of FKBP5 in more detail.

REFERENCES

1. Bortsov et al. Polymorphisms in the glucocorticoid receptor co-chaperone FKBP5 predict persistent musculoskeletal pain after traumatic stress exposure. Pain 2013
2. Sabarinathan, Tafer, Seemann, Hofacker, Stadler, Gorodkin. RNAsnp: efficient detection of local RNA secondary structure changes induced by SNPs. Human Mutation 2012
3. Kertesz, Iovino, Unnerstall, Gaul, Segal. The role of site accessibility in microRNA target recognition. Nature Genetics 2007

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