Published in final edited form as:

Am J Med Genet B Neuropsychiatr Genet. 2013 March ; 0(2): 224–226. doi:10.1002/ajmg.b.32124.

Increased risk of major depression by childhood abuse is not modified by *CNR1* genotype

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Gene by environment (GxE) associations with psychiatric disorders, like all genetic associations, can give improved estimates of population wide risk upon replication (Ioannidis and others 2001; Munafo and Flint 2009).

The synonymous polymorphism, rs1049353, in the human cannabinoid receptor gene (*CNR1*) on chromosome 6q14-q15 has recently been reported to attenuate the effect of childhood physical abuse on lifetime major depressive disorder (MDD) with anhedonia in a cohort of 1,041 female twins from Missouri (MOAFTS) and in 1,428 heroin dependent individuals matched with 506 neighborhood controls (CATS) from Sydney, Australia (Agrawal and others 2012). Using MDD as the independent variable the CATS study did not replicate the significant result (OR=0.34, 95% CI 0.14-0.85, p=0.02) reported in the primary MOAFTS study. This SNP was chosen from some 1,350 on a custom genotyping array designed to haplotype tag 130 candidate genes for addictions, (Hodgkinson and others 2008). It is unclear why this SNP was selected of the 8 *CNR1* SNPS present on the array, but the argument for examining *CNR1* is based on prior implication of the endocannabinoid system in moderating chronic unpredictable stress in rodents, and some human genetic association studies (Agrawal and others 2012). Rs1049353 is synonymous and located some 60bp from the nearest splice site, so it does not constitute a strong *a priori* functional candidate.

We studied 739 individuals from the Christchurch Health and Development study (CHDS), a birth cohort collected in mid-1977 from urban Christchurch, New Zealand, studied at birth, 4 months, 1 year, annually to age 16 years, and at 18, 21, 25 and 30 years (Fergusson and Horwood 2001; Fergusson and others 1989). The CHDS has proven to be sufficiently powerful to detect gene by environment effects, (Fergusson and others 2011a; Fergusson and others 2011b).

Childhood physical and sexual abuse was assessed by retrospective reports at the age of 18 and 21. Sexual abuse was classified into four levels by severity of abuse reported about 15 abusive experiences prior to age 16 years. Physical abuse was defined by the parents' use of physical punishment prior to age 16 years. Genotyping was by Illumina Human660w-quadrant v1 chip (Illumina, San Diego, CA); 29,614 SNPs and 22 samples failed quality control (QC) and imputation was performed by MACH1.0.16 using Hapmap3 CEU as a reference.

Analysis was carried out in PLINK (Purcell and others 2007). We evaluated the same statistical model used by Agrawal *et al*; the dependent variable was lifetime DSM-IV MDD and the predictors were rs1049353 (coded for a dominant model), physical abuse (dichotomous), the interaction between rs1049353 and physical abuse, and covariates of sex and ancestry (first principal component). Unlike Agrawal *et al*, age was not a covariate as the CHDS is a birth cohort.

Physical abuse almost doubled the risk of MDD (OR=1.94, 95% CI 1.29-2.92, P=0.002) while the minor allele of rs1049353 was associated with moderate risk reduction (OR=0.7, 95% CI 0.52-0.95, P=0.02). However including an interaction term provided no evidence that the effect of physical abuse on MDD was attenuated by rs1049353 (OR=0.89, 95% CI 0.39-1.99, p=0.76). Similarly, there was strong evidence that childhood sexual abuse (dichotomous) increased the risk of lifetime MDD (OR=5.14, 95% CI 3.02-8.76, p<10⁻⁸) however there was no evidence that this effect was moderated by rs1049353 (OR=0.98, 95% CI 0.34-2.82, p=0.97).

To consider support for a GxE effect in a broader region of *CNR1* all 23 SNPs on the array within 30kb of rs1049353 were examined with none showing a significant GxE interaction (p=0.05) of childhood physical abuse on lifetime MDD while controlling for sex and ethnicity. The region was chosen as it includes 2 downstream SNPs with highest LD to rs1049353 in the CEU population, found using SNAP (Broad Institute, MA), and the promoter region. All 515,249 autosomal SNPs passing QC were analyzed for the GxE effect of physical abuse on lifetime MDD while controlling for sex and ancestry, figure 1. No SNP achieved genome-wide significance (p < 5×10⁻⁸). The most significant GxE associations were with 5 intronic SNPs in the EF-hand domain family member A1 (*EFHA1*) gene and an intronic SNP in the gene integrin alpha FG-GAP3 (*ITFG3*). The main effects for physical abuse for the *EFHA1* SNPs had ORs ranging from 3.3 to 3.5 and p values ranging from 1.1×10⁻⁶ to 2.7×10⁻⁶; while for the *ITFG3* SNP the effect was not significant at p = 0.05 (OR=1). The GxE effects for the *EFHA1* SNPs had ORs ranging from 0.08 to 0.11, p-values ranging from 4.4×10⁻⁶ to 2.4×10⁻⁵, and for the *ITFG3* SNP the OR was 5.7, with p=6.7×10⁻⁶. The evidence for these loci does not meet genome wide significance and the effects are much larger than typically observed on replication; if they replicate we would expect effect sizes to reduce following the process described by Ioannidis (Ioannidis and others 2001).

Genetic associations often show more modest effect sizes in subsequent replications than in their initial reports due in part to genuine population diversity and reporting bias, (Ioannidis and others 2001; Munafo and others 2009), with meta-analysis providing more reliable pooled estimates from all appropriate studies (Ioannidis and others 2001; Kavvoura and

Ioannidis 2008). Replicating gene by environment studies has these same issues with heterogeneity and reporting bias and additionally suffers from the greater number of possible statistical tests that may be used when interactions are included in analyses, (Munafo and Flint 2009).

The CHDS has 75% power to detect the GxE effect of rs104393 by childhood physical abuse on lifetime depression reported from MOAFTS. Meta-analysis (Kavvoura and Ioannidis 2008) of these 3 studies yields an OR of 0.80 (95% CI 0.55-1.17, $p=0.25$), a small but not significant decrease of the effect of childhood physical abuse on risk of lifetime depression for carriers of the rs1049353 minor allele. The restriction of the phenotype to MDD with anhedonia appears to be critical to a significant inference at the sample sizes available in the current data, consistent with previous observations (Juhász and others 2009; Munafo and Flint 2009). In conclusion, we do not find strong evidence in support of the reported GxE effect of rs1049353 and childhood physical abuse on MDD in *CNR1*, either in the CHDS or in the combined datasets. The equivalent result for lifetime depression with anhedonia also requires replication and examination in a genome wide context.

Acknowledgments

This work was supported by the NIDA grant “A developmental model of gene-environment interplay in SUDs”. (R01DA024413) 2007-2012. The NIDA had no other role in the study. All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

- Agrawal A, Nelson EC, Littlefield AK, Bucholz KK, Degenhardt L, Henders AK, Madden PA, Martin NG, Montgomery GW, Pergadia ML, et al. Cannabinoid Receptor Genotype Moderation of the Effects of Childhood Physical Abuse on Anhedonia and Depression. *Arch Gen Psychiatry*. 2012
- Fergusson DM, Boden JM, Horwood LJ, Miller AL, Kennedy MA. MAOA, abuse exposure and antisocial behaviour: 30-year longitudinal study. *Br J Psychiatry*. 2011a; 198(6):457–63. [PubMed: 21628708]
- Fergusson DM, Horwood LJ. The Christchurch Health and Development Study: review of findings on child and adolescent mental health. *Aust N Z J Psychiatry*. 2001; 35(3):287–96. [PubMed: 11437801]
- Fergusson DM, Horwood LJ, Miller AL, Kennedy MA. Life stress, 5-HTTLPR and mental disorder: findings from a 30-year longitudinal study. *Br J Psychiatry*. 2011b; 198(2):129–35. [PubMed: 21282783]
- Fergusson DM, Horwood LJ, Shannon FT, Lawton JM. The Christchurch Child Development Study: a review of epidemiological findings. *Paediatr Perinat Epidemiol*. 1989; 3(3):302–25. [PubMed: 2671961]
- Hodgkinson CA, Yuan Q, Xu K, Shen PH, Heinz E, Lobos EA, Binder EB, Cubells J, Ehlers CL, Gelernter J, et al. Addictions biology: haplotype-based analysis for 130 candidate genes on a single array. *Alcohol Alcohol*. 2008; 43(5):505–15. [PubMed: 18477577]
- Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. *Nat Genet*. 2001; 29(3):306–9. [PubMed: 11600885]
- Juhász G, Chase D, Pegg E, Downey D, Toth ZG, Stones K, Platt H, Mekli K, Payton A, Elliott R, et al. *CNR1* gene is associated with high neuroticism and low agreeableness and interacts with recent negative life events to predict current depressive symptoms. *Neuropsychopharmacology*. 2009; 34(8):2019–27. [PubMed: 19242408]
- Kavvoura FK, Ioannidis JP. Methods for meta-analysis in genetic association studies: a review of their potential and pitfalls. *Hum Genet*. 2008; 123(1):1–14. [PubMed: 18026754]

- Munafo MR, Flint J. Replication and heterogeneity in gene x environment interaction studies. *Int J Neuropsychopharmacol.* 2009; 12(6):727–9. [PubMed: 19476681]
- Munafo MR, Stothart G, Flint J. Bias in genetic association studies and impact factor. *Mol Psychiatry.* 2009; 14(2):119–20. [PubMed: 19156153]
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007; 81(3):559–75. [PubMed: 17701901]

Figure 1.

Genome scan of the CHDS showing $-\log(p \text{ value})$ for the interaction effect of each SNP and childhood physical abuse on MDD.