LETTER TO THE EDITOR

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Increased Risk of Major Depression by Childhood Abuse Is Not Modified by *CNR1* Genotype

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Gene × environment (G × E) associations with psychiatric disorders, like all genetic associations, can give improved estimates of population wide risk upon replication [Ioannidis et al., 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 et al., 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 et al., 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 et al., 2012]. Rs1049353 is synonymous and located some 60 bp 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 et al., 1989; Fergusson and Horwood, 2001]. The CHDS has proven to be sufficiently powerful to detect gene × environment effects, [Fergusson et al., 2011a,b].

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-quad v1 chip (Illumina, San Diego, CA); 29,614 SNPs and 22 samples failed quality control (QC) and imputation was performed by MACH 1.0.16 using Hapmap3 CEU as a reference. Analysis was carried out in PLINK [Purcell et al., 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

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(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 G × E effect in a broader region of *CNR1* all 23 SNPs on the array within 30 kb of rs1049353 were examined with none showing a significant G × E interaction ($P = 0.05$) of childhood physical abuse on lifetime MDD while controlling for sex and ethnicity. The region was chosen as it includes two 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 G × E effect of physical abuse on lifetime MDD while controlling for sex and ancestry (Fig. 1). No SNP achieved genome-wide significance ($P < 5 \times 10^{-8}$). The most significant G × E associations were with five 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 G × E 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 $P = 6.7 \times 10^{-6}$. 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 et al., 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 et al., 2001; Munafo et al., 2009], with meta-analysis providing more reliable pooled estimates from all appropriate studies [Ioannidis et al., 2001; Kavvoura and Ioannidis, 2008]. Replicating gene × 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].

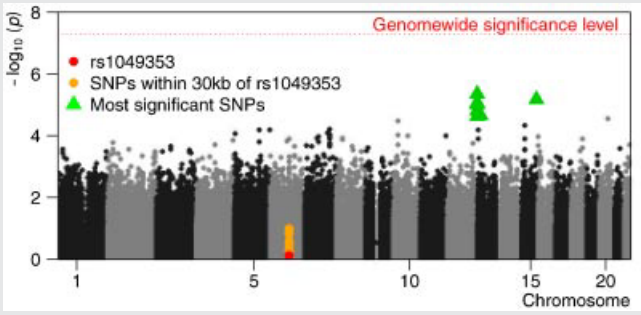

FIG. 1. Genome scan of the CHDS showing −log (*P*-value) for the interaction effect of each SNP and childhood physical abuse on MDD.

The CHDS has 75% power to detect the G × E effect of rs104393 by childhood physical abuse on lifetime depression reported from MOAFTS. Meta-analysis [Kavvoura and Ioannidis, 2008] of these three 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 [Juhasz et al., 2009; Munafo and Flint, 2009]. In conclusion, we do not find strong evidence in support of the reported G × E 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.

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