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Cytoplasmic FMR1-Interacting Protein 2 Is a Major Genetic Factor Underlying Binge Eating

Stacey L. Kirkpatrick, Lisa R. Goldberg, Neema Yazdani, R. Keith Babbs, Jiayi Wu, Eric R. Reed, David F. Jenkins, Amanda F. Bolgioni, Kelsey I. Landaverde, Kimberly P. Luttik, Karen S. Mitchell, Vivek Kumar, W. Evan Johnson, Megan K. Mulligan, Pietro Cottone, and Camron D. Bryant

ABSTRACT

BACKGROUND: Eating disorders are lethal and heritable; however, the underlying genetic factors are unknown. Binge eating is a highly heritable trait associated with eating disorders that is comorbid with mood and substance use disorders. Therefore, understanding its genetic basis will inform therapeutic development that could improve several comorbid neuropsychiatric conditions.

METHODS: We assessed binge eating in closely related C57BL/6 mouse substrains and in an F_2 cross to identify quantitative trait loci associated with binge eating. We used gene targeting to validate candidate genetic factors. Finally, we used transcriptome analysis of the striatum via messenger RNA sequencing to identify the premorbid transcriptome and the binge-induced transcriptome to inform molecular mechanisms mediating binge eating susceptibility and establishment.

RESULTS: C57BL/6NJ but not C57BL/6J mice showed rapid and robust escalation in palatable food consumption. We mapped a single genome-wide significant quantitative trait locus on chromosome 11 (logarithm of the odds = 7.4) to a missense mutation in cytoplasmic FMR1-interacting protein 2 (*Cyfip2*). We validated *Cyfip2* as a major genetic factor underlying binge eating in heterozygous knockout mice on a C57BL/6N background that showed reduced binge eating toward a wild-type C57BL/6J-like level. Transcriptome analysis of premorbid genetic risk identified the enrichment terms morphine addiction and retrograde endocannabinoid signaling, whereas binge eating resulted in the downregulation of a gene set enriched for decreased myelination, oligodendrocyte differentiation, and expression.

CONCLUSIONS: We identified *Cyfip2* as a major significant genetic factor underlying binge eating and provide a behavioral paradigm for future genome-wide association studies in populations with increased genetic complexity.

Keywords: Anxiety, Binge, Eating disorders, GWAS, Myelin, Prader-Willi syndrome

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Eating disorders (ED) are among the most lethal psychiatric disorders and exhibit a lifetime prevalence of 1–3% (1). Binge eating disorder (BED) is frequently associated with severe obesity, metabolic dysfunction, and increased mortality (1,2). Both genetic and environmental factors contribute to binge eating (BE) (3); however, genome-wide association studies in humans are currently limited in their power to detect the contribution of common variants (4). BE, defined by the uncontrolled overconsumption of a large amount of food within a brief time period (usually energy-dense palatable food [PF]), is one of the most highly heritable traits associated with ED, including BED (5), bulimia nervosa (6), and a subset of cases of anorexia nervosa (7). Focusing on the genetic and biological basis of a single trait such as BE that is presumed to comprise less genetic and biological complexity than an aggregate disorder could be a more tractable goal toward gene identification (5,8) and accelerate the development of new therapeutics.

ED are comorbid with anxiety traits (9), mood disorders, obsessions/compulsions, impulse control, and substance use disorders, suggesting shared genetic factors (1). Recent theories of BE have adopted theories of addiction to explain its compulsive basis and the underlying genetic and neural mechanisms (10–12). Compulsive BE shares several features with addiction, including an escalation in consumption, physiological and emotional-affective dependence, cue-induced craving, and relapse (12).

Neurochemical mechanisms of BE converge on activation of the mesocorticolimbic dopamine system (13,14). Cue-induced craving correlates with BE in humans (15) and changes in extracellular dopamine in the dorsal striatum in response to food stimuli correlate with scores of BE severity in patients with BED (16). The dorsal striatum processes food sensation and reward (17), for example the nutritional value of sugar (18) and enkephalin-mediated coding of sensory reward induced by PF consumption (19,20). Furthermore, the volume

SEE COMMENTARY ON PAGE e77

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of the dorsal striatum is reduced in patients with anorexia and bulimia and correlates with predicted sensitivity to reward (21). Finally, recruitment of the dorsal striatum combined with a loss of prefrontal cortical inhibition is hypothesized to mediate a shift to habitual, cue-responsive compulsive-like behaviors associated with addiction (22), including BE (10).

Quantitative trait locus (QTL) mapping is a genome-wide, discovery-based approach to uncover novel genetic and biological mechanisms of complex traits such as BE (23–25). In mice, millions of genetic variants typically distinguish commonly used inbred strains (26), highlighting one of the challenges to identifying causal genetic variants. To help overcome this particular challenge, closely related substrains exhibiting extreme phenotypic and little genetic diversity can be employed to facilitate gene mapping (27,28). C57BL/6 (B6) substrains show robust differences in behavioral responses to drugs of abuse (28) and whole-genome sequencing identified only approximately 10,000 single nucleotide polymorphisms that distinguish C57BL/6NJ (B6NJ) and C57BL/6J (B6J) strains (26). This drastically reduced genetic complexity can facilitate identification of causal genetic factors (29). Behavioral differences between B6J and B6NJ combined with whole-genome sequence information make these strains an attractive and accessible model for identifying the genetic basis of variation in complex traits. However, whether these strains differ in BE has not been examined.

Here, we developed an intermittent, limited access procedure for BE in a conditioned place preference (CPP) paradigm using outbred Swiss Webster (CFW) (Charles River Laboratories, Wilmington, MA) mice. We identified a robust difference in BE between B6 substrains and used QTL mapping and gene targeting to identify a major genetic factor associated with BE. Finally, we used striatal transcriptome analysis via messenger RNA sequencing in a subset of F_2 mice and heterozygous gene knockout mice to gain insight into the premorbid neurobiological mechanisms that bridge genetic variation with BE susceptibility and the neurobiological adaptations induced by BE.

METHODS AND MATERIALS

Mice

All experiments were performed in accordance with the National Institutes of Health Guidelines for the Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Boston University. CFW mice (7 weeks old) were purchased from Charles River Laboratories (Crl: CFW [SW]) and were used to choose the PF diet (20-mg pellets; 5-TUL, TestDiet, St. Louis, MO) and design the BE and PF-CPP paradigm (Supplement). C57BL/6J (B6J) and C57BL/NJ (B6NJ) mice (7 weeks old) were purchased from the Jackson Laboratory (Bar Harbor, ME). Details on purchased mice and on B6J × B6NJ-F_1, -F_2, and heterozygous *Cyfip2* knockout mice (*Cyfip2*$^{N/-}$) are provided in the Supplement.

BE and PF-CPP

We chose a BE CPP design to examine both the consummatory and the conditioned motivated behaviors associated with BE. The long-term goal is to identify shared and divergent genetic factors that mediate these behaviors using forward genetics. We used a two-chamber CPP design (30) to measure BE and PF-CPP on day (D) 1–D22 (see Figure 1). On D23, F_2 mice received a final PF training session and on D24 mice were tested on the elevated plus maze for 5 minutes (Supplement) and sacrificed for collection of striatum punches (31). For the knockout study, *Cyfip2*$^{N/-}$ and *Cyfip2*$^{N/N}$ mice were tested for compulsive-like eating in the light/dark conflict test on D23 (see below) and sacrificed on D24 (24 hours later) for collection of striatum punches (31). Mice homozygous for the *Cyfip2* knockout allele are lethal at postnatal day 1.

Light/Dark Conflict Test

The light/dark conflict test was employed on D23 as a measure of compulsive-like eating (32) in *Cyfip2*$^{N/N}$ and *Cyfip2*$^{N/-}$ mice. One side is black opaque Plexiglas whereas the other side is transparent and light exposed with a small doorway allowing access to both sides. The light side is an aversive, bright compartment that the mice normally avoid. We operationalized an increase in PF consumption on the light side despite adverse conditions as a construct of compulsive-like eating (32). A porcelain bowl containing PF (5-TUL pellets) was placed into the center of the light side and each mouse was placed on the light side facing the food bowl and the doorway. Time on the light side, amount of PF consumed, and entries were recorded for 30 minutes.

Behavioral Analysis of BE in CFW, B6J, and B6NJ Mice

Behavioral analysis was conducted in R (https://www.r-project.org/) using mixed-model analyses of variance (genotype, treatment, and sex as independent variables; day as a repeated measure) with an alpha level of .05 to detect main effects and interactions. Primary outcome measures included percent body weight consumed and time spent on the PF-paired side. We also determined differences in the slope (rate) of escalation in PF consumption as an additional measure of BE (33). Post hoc analyses following main effects and interactions were performed using one- or two-way analyses of variance, and Welch's unequal variances t tests ($p < .05$, Bonferroni corrected for the number of comparisons made).

QTL Analysis of B6J × B6NJ-F_2 Mice

F_2 mice were genotyped using a custom Fluidigm genotyping array (South San Francisco, CA) with 96 single nucleotide polymorphisms (26,29). QTL analysis was conducted in R/qtl (scanone) using Haley-Knott regression and 1000 permutations to establish significance thresholds ($p < .05$, error probability = .0001) (34). We also analyzed female ($n = 78$) and male mice ($n = 78$) separately. The marker position (cM) was estimated using the sex-averaged position from the Mouse Map Converter (http://cgd.jax.org/mousemapconverter) (35).

Behavioral Analysis of *Cyfip2*$^{N/-}$ Mice

We used a 2 × 2 factorial design to measure PF versus chow consumption as a function of genotype (*Cyfip2*$^{N/N}$, *Cyfip2*$^{N/-}$; 59–61 days old on D1). Chow consisted of 20-mg pellets (TestDiet, St. Louis, MO) that contained a nearly identical nutritional profile to the PF with the exception that they lacked sucrose. A minimum sample size of $n = 20$ was employed based on 80% power ($p < .05$; Supplement). Mice were