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Genome-wide linkage analysis of heroin dependence in Han Chinese: Results from Wave Two of a multi-stage study

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Abstract

Previously we reported the results of Wave One of a genome-wide search for heroin dependence susceptibility loci in Han Chinese families from Yunnan Province, China, near Asia's "Golden Triangle". Our initial analysis of 194 independent affected sibling-pairs from 192 families identified two regions with nonparametric linkage (NPL) Z-scores greater than 2.0, which were suggestive of linkage. Presently we have supplemented our sample with additional individuals and families, bringing the total number of genotyped individuals to 1513 and the number of independent sibling-pairs to 397. Upon repeating our analyses with this larger sample, we found that the evidence for linkage at our most strongly implicated locus from Wave One (marker D17S1880; 53.4 cM on 17q11.2; NPL $Z = 2.36$; uncorrected $p = 0.009$) was completely abolished ($Z = -1.13$; $p = 0.900$). In contrast, the evidence for linkage at the second-most strongly implicated locus from Wave One (D4S1644; 143.3 cM on 4q31.21; NPL $Z = 2.19$; uncorrected $p = 0.014$) increased in its magnitude

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Conflict of interest

All authors declare that they have no conflicts of interest.

and significance ($Z = 2.64$; uncorrected $p = 0.004$), becoming the most strongly implicated locus overall in our full sample. Other loci on chromosomes 1, 2, 4, 12, 16, and X also displayed nominally significant evidence for linkage ($p \leq 0.05$). These loci appear to be entirely distinct from opioid-linked loci reported by other groups; however, meta-analyses of all available linkage data may reveal common sites of interest and promising candidate genes that can be further evaluated as risk factors for the illness.

Keywords

Affected sibling-pairs; Chromosome 4; Genome-wide linkage analysis; Heroin dependence; Opioid dependence

1. Introduction

Heroin dependence is a major threat to global public health, and efforts to identify avenues for its prevention are sorely needed. Unfortunately, the specific factors that predispose individuals to become dependent on heroin are not yet understood well enough to target these for intervention and prevention efforts. Evidence from several twin studies (Karkowski et al., 2000; Kendler et al., 2000; Tsuang et al., 2001, 1996; van den Bree et al., 1998) suggests that genes account for between 23 and 54% of the total liability toward heroin dependence, with the remainder of variance in liability attributable to environmental factors; thus, genetic studies of the disorder are well warranted.

Candidate gene studies of opioid dependence have largely failed to robustly implicate any particular variants as risk genes for the disorder (Kreek et al., 2005), suggesting that the selection of positional candidate genes based on the results of genome-wide linkage analysis might be a beneficial alternate (or at least supplemental) strategy. Toward that end, we collected DNA and clinical data from Han Chinese sibling-pairs (both of whom were dependent on heroin), and collected DNA from their parents and additional family members from Yunnan Province, China, which borders an area of Myanmar and Laos known as the “Golden Triangle”. This region is the source of more than 20% of the world’s heroin (Cai, 1998), and increased heroin smuggling through Yunnan Province during the last two decades (Beyrer et al., 2000; Stimson, 1993, 1994) has produced a concomitant rise in heroin dependence among the Province’s 37 million residents (Beyrer et al., 2000; McCoy et al., 2001), especially the region’s youth (Kulsudjarit, 2004). These high levels of exposure to a necessary environmental factor (*i.e.*, heroin) make this geographical region ideal for investigating the genetic bases of heroin dependence.

In 2006, we published the results of a linkage analysis for heroin dependence conducted on 194 independent sibling-pairs from 192 Han Chinese families who were ascertained in Yunnan Province for Wave One of our multi-stage study (Glatt et al., 2006). The results from that preliminary analysis revealed two regions of the genome (on chromosomes 4 and 17) that showed some evidence of harboring potential susceptibility genes for the disorder. Since then, we have successfully completed ascertainment for this study, increasing the number of families informative for linkage analysis to 397. In this report, we document the results of our follow-up linkage analysis on this full sample, and compare and contrast the results with those previously obtained in our sample and by others (Gelernter et al., 2006; Lachman et al., 2007).

2. Methods

2.1. Ascertainment and clinical assessment

The ascertainment, clinical characterization, and sample acquisition for this study is as previously described (Glatt et al., 2006). Briefly, heroin-dependent probands and their affected siblings were recruited from the Yunnan Institute of Drug Abuse (YIDA), Yunnan Province, China, and diagnoses were confirmed using supplemental medical records, a semi-structured interview based on the Diagnostic and Statistical Manual, Fourth Edition (DSM-IV) (American Psychiatric Association, 1994), and the Diagnostic Interview for Genetic Studies (DIGS) (Faraone et al., 1996; Nurnberger et al., 1994; Wang et al., 2004). Best-estimate final diagnoses were made by two board-certified psychiatrists independently based on all the clinical information that was collected. When these psychiatrists disagreed, a third diagnostician was used as the tiebreaker. Parents and other siblings were also contacted, interviewed, and individually evaluated for inclusion in the study. All procedures used in this study were approved by the Institutional Review Boards of the participating institutions, including YIDA, Harvard Medical School, and the University of California, San Diego.

2.2. DNA sample acquisition, storage, and genotyping

Approximately 10 ml of blood was drawn from each subject and immediately shipped to the NIDA Center for Genetic Studies at the Rutgers University Cell and DNA Repository, where cells were immortalized via transformation with Epstein-Barr virus. DNA was extracted from these cell lines and sent to the Center for Inherited Disease Research (CIDR) for genotyping. CIDR genotyped all samples in Wave One on 386 microsatellite markers spaced at an average inter-marker distance of 9 cM, following their standard procedures, which are published on the web (<http://www.cidr.jhmi.edu/protocol.html>). No gap was greater than 20 cM between any two markers. Marker distances were generated using the sex-averaged Marshfield genetic map (Broman et al., 1998). Most markers used in Wave One were retained for analysis in Wave Two, although nine were removed and replaced by eight better-performing markers. A common genetic map was then constructed which included all markers used in either wave; thus, the present linkage analyses evaluated all individuals on a common genetic map.

2.3. Quality control

Pedigree inconsistencies were evaluated using RELCHCK (Boehnke and Cox, 1997) and GRR (Abecasis et al., 2001). Once the pedigree errors were identified, each error was examined manually and corrected accordingly or, if the source of the discrepancy could not be identified, the pedigree was eliminated from the analyses. Mendelian inconsistencies were checked and removed using PEDCHECK (O'Connell and Weeks, 1998). Additional unlikely genotypes were removed using *pedwipe*, a command available in Merlin (Abecasis et al., 2001).

2.4. Linkage analysis

Multipoint nonparametric linkage analysis was performed across all autosomes and the X chromosome using the computer program Merlin (Abecasis et al., 2001). Although all relative-pairs were included in the data analyses, only data from affected sibling-pairs contributed to the test statistics. This was due to unavoidable limitations on the data collection protocol, which prohibited the collection of detailed phenotypic information from family members other than probands and their affected siblings. Results are reported as nonparametric linkage (NPL) Z-scores calculated from Merlin, the method of which is described in detail by Whittemore and Halpern (1994). NPL Z-scores were calculated at each marker throughout the genome, as well as at each 2 cM interval.

3. Results

By the close of ascertainment, we had collected clinical and genetic marker data on 1513 individuals from 397 Han Chinese families. These 1513 subjects included 397 heroin-dependent probands and 397 of their heroin-dependent siblings (comprising 397 independent affected sibling-pairs), as well as 719 of their additional family members. 83.9% of the affected individuals in the sample were male, while 16.1% were female. The mean age-at-onset of heroin dependence in this sample was 24.6 years, with a standard deviation of 7.3 years. Rates of comorbid psychiatric and substance abuse disorders (occurring in more than 1% of cases) were as follows: sedative, hypnotic, or anxiolytic dependence, 8.8%; antisocial personality disorder, 5.1%; and pathological gambling, 2.7%.

Of the 397 included families, 358 families were retained for genetic linkage analyses after applying the various data-cleaning procedures. The notable loss of sample size was attributable to several factors, most notably including: (1) inadequate DNA quality; (2) unverified heroin dependence status; and (3) non-paternity. In Wave One, the strongest linkage signal was obtained at marker D17S1880, which maps to 53.4 cM on chromosome 17q11.2 (NPL $Z = 2.36$; uncorrected $p = 0.009$); however, after supplementing our sample with the additional families genotyped in Wave Two, all evidence for linkage at this locus was abolished (NPL $Z = -1.13$; $p = 0.900$). In contrast, the evidence for linkage at the second-most strongly implicated locus from Wave One (D4S1644; 143.3 cM on 4q31.21; NPL $Z = 2.19$; uncorrected $p = 0.014$) increased in its magnitude and significance (NPL $Z = 2.64$; uncorrected $p = 0.004$), becoming the most strongly implicated locus overall in our full sample (Fig. 1). This result corresponded to a LOD score of 1.95, and the 1-LOD drop-down region (analogous to the 95% confidence interval) surrounding this marker extended from 117-167 cM. Loci on chromosomes 1, 2, 12, 16, and X also displayed nominally significant evidence for linkage in the full data set, as did an additional marker at the telomeric end of chromosome 4q (Table 1). Based on its NPL Z -score of 2.26, this additional chromosome 4 marker (D4S1652) displayed the second-strongest evidence for linkage in our study; however, its LOD score of 2.2 was the highest detected genome-wide, surpassing even that observed at D4S1644. For brevity, only these nominally significant results have been described in the present report; however, to facilitate meta-analyses and comply with the data-sharing policies of the NIDA Genetics Consortium, all results and data will be made available to interested parties upon request.

4. Discussion

Based on the moderate heritability of opioid dependence that has been established by twin studies, several attempts have been made to relate the disorder to particular allelic variants of functional candidate genes by association mapping. The majority of genes that historically have been selected for examination of association with the illness can be roughly classified into two groups based on the functions of their proteins: (1) monoamine pathway genes, due to the known role of serotonin and dopamine neurotransmission in mediating the effects of several drugs of abuse; and (2) opioid pathway genes, due to their expression in or direct interaction with the opioid system, which is the primary transmitter system targeted by heroin. In the same sample examined presently, we have tested a candidate gene in the latter group (OPRM1, which codes for the μ opioid receptor), but found no evidence of association with the widely examined A118G (Asn40Asp) polymorphism or either of two other non-synonymous mutations (Val6Ala and Arg111His) (Glatt et al., 2007). Beyond our own study, we also failed to find any aggregate support for an effect of the Asn40Asp variant on risk for opioid dependence by meta-analysis (Glatt et al., 2007). Similarly, the collective evidence for an association between heroin dependence and this or any other functional candidate gene is quite limited (Kreek et al., 2005), suggesting that the selection of positional candidate genes

based on the results of genome-wide linkage analysis might be a beneficial alternate or adjunctive strategy.

In the linkage analyses presented here, several genomic loci (including two discrete regions on chromosome 4) showed some evidence for linkage with heroin dependence in our Han Chinese sample from Yunnan Province, China. However, the evidence for linkage at even our most strongly linked candidate regions is only suggestive (Lander and Kruglyak, 1995) and these regions are very broad, encompassing hundreds of genes; thus, any pursuit of candidate genes in these regions must proceed with due caution. Accordingly, these loci should first be examined by further fine-mapping so that the most likely positional candidate genes can be identified and evaluated in association analyses.

The loci at which we presently observed the strongest evidence for linkage to heroin dependence do not intersect with those implicated in other recently completed genome-wide linkage scans of opioid dependence. For example, the strongest evidence for linkage to DSM-IV opioid dependence reported by Gelernter et al. (2006) was observed at 221.1 cM on chromosome 2q, where we see NPL Z-scores less than 1.0 and LOD scores less than 0.5. Other loci implicated in opioid dependence by Gelernter et al. included regions of chromosomes 5, 6, and 17 which again provided little or no evidence for linkage in our sample. The only other genome-wide linkage analysis of opioid dependence published to date is that of Lachman et al. (2007), which identified suggestive evidence for linkage on chromosome 14q and, to a lesser extent, on chromosome 10; neither of these loci showed any evidence for linkage to heroin dependence in our sample. These disparities may reflect the genetic heterogeneity of heroin dependence, or they may simply reflect methodological differences between the studies, including the ancestry of the examined samples, rates and types of psychiatric and substance dependence comorbidities, or the degree and nature of exposure to salient environmental factors that modulate the expression of opioid dependence in each sample. Because opioid dependence is considered a complex disorder, it is expected that many genes will make small contributions to the overall risk for the disorder. Thus, it is also possible that a meta-analysis of the available linkage data might identify a locus that is implicated more strongly in the collective data than in any particular study.

From our own collective body of work on heroin dependence in Yunnan Province, as well as the current state of the science, we can make several recommendations which may advance the field toward the discovery of opioid dependence risk genes and the translation of these discoveries into benefits for dependent individuals, their families, and society. First and foremost, it is likely that sharing and meta-analytic pooling of data from individual linkage and association studies will help enormously to overcome the power limitations of individual studies. Thus, meta-analysis will be necessary to maximize our power to detect genes that have very small or conditional effects on risk for the disorder; concurrently, meta-analysis will also afford ample power to rule out large effects of suspected candidate genes, such as the Asn40Asp variant of OPRM1.

Secondly, it is crucial to recognize opioid dependence as a complex disorder, with many genetic and environmental factors each making minor contributions toward overall susceptibility. It is likely that, apart from exposure to and use of heroin, no other factor (genetic or environmental) will prove either necessary or sufficient to cause the disorder; thus, large (pooled) samples will be required to test for gene-gene and gene-environment interactions that increase risk for the disorder. Lastly, it is abundantly clear that neither linkage nor association studies alone will succeed in identifying all loci that contribute to the susceptibility toward the disorder. Indeed, no genetic technique is likely to succeed in explaining large amounts of the variance in individual susceptibility toward heroin dependence, since the upper bounds on the heritability of the disorder are far from absolute (ranging from 13 to 77%) and some of this heritability

may well be accounted for by gene-environment interactions as well. Since gene expression can be influenced by *cis*- and *trans*- acting genetic polymorphisms as well as environmental factors, high-throughput gene expression microarrays may hold greater promise than genetic methods such as linkage or association in unraveling a greater portion of the etiology of gene-environment interaction disorders such as opioid dependence.

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Fig. 1. Evidence for linkage with heroin dependence on chromosome 4. The plot shows a curve of NPL Z-scores observed at markers mapping to the indicated centiMorgan (cM) positions on chromosome 4, including a peak NPL Z-score of 2.64 at marker D4S1644 (143.3 cM).

Table 1
Markers with nominally significant evidence for linkage with heroin dependence

Chromosome	Marker	Marshfield (cM)	NPL	p	LOD	p
1	D1S1653	164	2.20	0.014	1.41	0.005
2	D2S1400	28	1.91	0.030	1.40	0.006
4	D4S2394	130	2.22	0.013	1.59	0.003
4	D4S1644	143	2.64	0.004	1.95	0.001
4	D4S1625	146	1.83	0.030	0.94	0.020
4	D4S1629	158	1.73	0.040	1.01	0.020
4	D4S2368	168	1.67	0.050	0.92	0.020
4	D4S1652	208	2.26	0.012	2.22	0.001
12	PAH	109	1.65	0.050	0.77	0.030
12	D12S2070	125	2.05	0.020	1.42	0.005
12	D12S395	137	2.06	0.020	1.51	0.004
16	D16S2624	88	1.70	0.040	0.95	0.020
X	DXS9908	165	1.81	0.040	1.09	0.013
X	DXS998	173	1.81	0.040	1.30	0.007