Genetic mapping of habitual substance use, obesity-related traits, responses to mental and physical stress, and heart rate and blood pressure measurements reveals shared genes that are overrepresented in the neural synapse

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Links between substance use habits, obesity, stress and the related cardiovascular outcomes can be, in part, because of loci with pleiotropic effects. To investigate this hypothesis, we performed genome-wide mapping in 119 multigenerational families from a population in the Saguenay-Lac-St-Jean region with a known founder effect using 58,000 single-nucleotide polymorphisms and 437 microsatellite markers to identify genetic components of the following factors: habitual alcohol, tobacco and coffee use; response to mental and physical stress; obesity-related traits; and heart rate (HR) and blood pressure (BP) measures. Habitual alcohol and/or tobacco users had attenuated HR responses to mental stress compared with non-users, whereas hypertensive individuals had stronger HR and systolic BP responses to mental stress and a higher obesity index than normotensives. Genetic mappings uncovered numerous shared genes among substance use, stress response, obesity and hemodynamic traits, including CAMK4, CNTN4, DLG2, FHIT, GRID2, ITPR2, NOVA1 and PRKCE, forming network of interacting proteins, sharing synaptic function and display higher and patterned expression profiles in brain-related tissues; moreover, pathway analysis of shared genes pointed to long-term potentiation. Subgroup genetic mappings uncovered additional shared synaptic genes, including CAMK4, CNTN5 and DNM3 (hypertension-specific); CNTN4, DNM3, FHIT and ITPR1 (sex-specific), having protein interactions with genes driven from general analysis. In summary, consistent with the observed phenotypic correlations, we found substantial overlap among genomic determinants of these traits in synapse, which supports the notion that the neural synapse may be a shared interface behind substance use, stress, obesity, HR, BP as well as the observed sex- and hypertension-specific genetic differences.

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INTRODUCTION

Alcohol, tobacco and coffee are among the most commonly consumed psychoactive substances in the world; their concurrent use has been consistently shown across a wide variety of populations. Stress is also related to the initiation, intensification and relapse of substance use. In addition, both stress and substance use have been implicated in cardiovascular disease and cardiovascular risk factor phenotype, obesity. Substance use, obesity, stress and cardiovascular disorders are complex traits that have multifactorial etiologies with substantial genetic components. The HPA axis, the main component of body’s response to stress, has been implicated in the etiology of these traits. For example, variations in the mu opioid receptor, a regulator of the HPA axis, influence the response to stress, food intake and substance use. However, the genetic determinants of and the relationships between these traits are still largely elusive, and further studies are required.

We hypothesized that the connections between substance use habits, obesity, stress and cardiovascular outcomes are, in part, because of shared genetic factors. To investigate this hypothesis, we performed genome-wide linkage and association scans in a cohort of 119 families from the founder population of Saguenay-Lac-St-Jean, which was previously recruited to identify the genetic factors of hypertension, to
identify the genetic determinants of the following: habitual alcohol, coffee and tobacco use; obesity-related anthropometric data; the cardiovascular components of the behavioral response to the mental stress of mathematical tests and changes in plasma catecholamines after an orthostatic test as biomarkers of responses to mental and physical stress; and 24-hour heart rate (HR) and blood pressure (BP).9 Furthermore, the identified genes were subjected to functional annotation to explore the underlying biological meanings.

METHODS

Phenotypes
The details about the families and the results of extensive phenotyping were previously described.9 A total of 15% of the subjects (N = 135) had received antihypertensive and/or lipid-lowering medications, which were stopped before the start of phenotyping protocol.9 Phenotyping for habitual substance use was performed using standard questionnaires; those using a substance on regular basis were grouped as affected, and those who never or occasionally use a substance were considered to be unaffected. HR and BP changes during the mental stress of mathematical tests were used as markers of the response to mental stress.9 The subjects were asked to pass a 2-min arithmetic test; in parallel, the subjects’ HR, diastolic blood pressure (DBP) and systolic blood pressure (SBP) were measured every 5 min before the test (three times), every 2 min during the test (two times) and every 2 min after the test (six times). The stress response was defined as the difference between the first HR and BP values during the math test and the average-resting values before the test. For the orthostatic stress test, the plasma epinephrine (EP) and norepinephrine levels were monitored in blood samples taken from the subjects during 60 min in a supine position and 10 min in a standing position; these data along the difference standing and supine positions (delta) were used as biomarkers of response to physical stress. Phenotyping for obesity and the hemodynamic measures were previously described.9

The generalized estimating equation (implemented in R statistical package version 2.6.1), which accounts for familial correlation using a sandwich estimator of the variance under exchangeable correlation, was used to measure the relatedness of the phenotypes.13 As significant portions of variation of studied traits were attributed to sex and hypertension status,16 sex- and hypertension-specific genetic mappings were also performed. In the general and hypertension-specific analysis, age and sex were included as covariates, and in the sex-specific analysis, only age was used as a covariate. Heritability was calculated using the SOLAR software version 4.2.0.17

Genotyping
Genotype information was previously described.9,16,18 In summary, 469 subjects were genotyped using the GeneChip Human Mapping 50 K Array Xba240, and 537 subjects were genotyped using 437 microsatellite markers. Altogether, the 719 subjects that were genotyped using microsatellites and/or single-nucleotide polymorphisms (SNPs) were analyzed in the present study. Genotyping errors were detected and filtered (~0.2%) using the MERLIN (version 1.1.0)19 and PLINK software (Boston, MA, USA; version 1.06). SNPs with the parameters \( r^2 \leq 0.08, \text{HWE} > 0.001 \) and MAF > 0.05 were included in analysis. The WGA-view software (Durham, NC, USA; version 1.52Z) was used to identify nearby genes for each SNP by specifying up- and downstream spans of 500 kb.20

Genetic mapping
Merging the stable markers, SNPs and potentially unstable but highly informative microsatellite markers can increase the power of genetic analysis.21 Hence, to increase the power of current study, the microsatellite and SNP genotype information were merged, and a haplotype map was created by specifying \( r^2 = 0.4.22 \) A multipoint linkage analysis was performed on the haplotype map using MERLIN and followed by the family-based association test (FBAT) in the FBAT software version v2.0.2.23 Unlike case–control studies, family-based association is designed to test for association in the presence of linkage. Hence, a positive finding implies both linkage and association. In addition, family-based association tests are more immune to spurious association caused by population stratification compared with case–control designs.23

To identify specific loci for each trait, loci that were within a 1 LOD-drop interval of the linkage peak and that had at least two identically annotated SNPs associated with \( P < 10^{-3} \) were preselected. Furthermore, for each SNP, \( P \)-values were corrected by the number of SNPs within the linkage area, and \( P \)-value-adjusted SNPs that were not significant (\( P > 0.05 \)) were excluded. For loci that were associated with multiple traits, SNPs with association \( P \)-value < 10\(^{-3} \) were preselected. Combined \( P \)-values were calculated using Fisher’s combined probability test, and \( P < 1.4 \times 10^{-6} \) was considered to be significant after a Bonferroni correction by the number of SNPs analyzed (\( N = 34741 \)).

Functional annotations
Protein interaction data were extracted from the Ingenuity Pathway Analysis database version 8.0, and DAVID Functional Annotation Tool24 version 6 was used to identify significantly enriched KEGG pathways from the list of shared genes. Genes involved in a biological process display higher expression and patterned expression profiles in the tissues in which the biological process takes place.25 Therefore; we compared the expression profiles of the shared genes across 65 normal human tissues using data from COXPRESdb database26 version c3.1. Hierarchical clustering of the expression data was performed using the GenePattern software27 version 3.1.1 based on the complete linkage method and using the Pearson’s correlation coefficient as a measure of distance. The synapse databases SynDB21 release 2006 and G2Cdb28 version 67 were used to query whether a gene has synaptic function.

RESULTS

General phenotypic characteristics
The prevalence of hypertension was significantly higher in those who had never used tobacco (\( P = 0.02 \)) compared with former and current smokers (Supplementary Table S1). Hypertensive subjects had a higher obesity index (\( P < 0.05 \)) and a stronger HR (\( P = 0.03 \)) and SBP (\( P = 0.02 \)) response to mental stress compared with normotensive subjects (Supplementary Table S2). In all, 80% of the studied traits exhibit sexual dimorphism (\( P < 0.05 \)). Norepinephrine levels and the hemodynamic responses to mental stress were not significantly different between males and females (Supplementary Table S2). In addition, although BP increased with age, HR changes remained insignificant (Supplementary Table S2).

Sex- and hypertension status-based differences in the heritabilities of the studied traits were observed (Supplementary Table S4). For instance, the heritabilities of average sitting HR (87% vs. 34%) and average sitting BP (0.69 vs. 33% for DBP and 48 vs. 10% for SBP) were higher in normotensive than in hypertensive subjects (Table 1). Moreover, although genetic factors appeared to be more important in the initiation (females = 81%, males = 56%) and persistence of tobacco use (females = 97%, males = 23%) in females, the heritability of alcohol use was higher in males (85%) compared with females (63%; Table 1).

Phenotypic relatedness
Alcohol and tobacco users had an attenuated HR response to mental stress compared with non-users (both \( P = 0.04 \); Supplementary Table S3). The analysis of both global and regional measures of obesity indicated that current tobacco users are slimmer compared with former users and those who had never used tobacco (Supplementary Table S3). In addition, tobacco users had higher EP levels compared with former smokers (Supplementary Table S1). Hypertensive subjects had a higher obesity index (\( P < 0.05 \)) and a stronger HR (\( P = 0.03 \)) and SBP (\( P = 0.02 \)) response to mental stress compared with normotensive subjects (Supplementary Table S2). In all, 80% of the studied traits exhibit sexual dimorphism (\( P < 0.05 \)). Norepinephrine levels and the hemodynamic responses to mental stress were not significantly different between males and females (Supplementary Table S2). In addition, although BP increased with age, HR changes remained insignificant (Supplementary Table S2).

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Genetic mappings and functional annotation

**General genetic mapping.** The functional annotation of the specific genes derived from the joint linkage and association mapping (Supplementary Table S5) indicated that a number of these genes share functions and interactions. Probing the protein interactions revealed that GRID2, HTR2A, LPHN2, LRPIB, MTPN and NETO1 share interactions with the synaptic protein DLG4 (Figure 3). Furthermore, a search of the synapse databases indicated that the CHRM2, DAB2, GRID2, HTR2A, ITPR2, LPHN2 and NETO1 genes have synaptic function (Figure 1 and Supplementary Table S10).

The family-based association scans identified genes commonly associated with smoking status, namely rs459985 (HWE p = 0.0006) downstream of the MKI67 gene, which was associated with average sitting BP (P = 0.0008), and rs1029819 (P = 0.0008) upstream of the CACN1C gene, which were associated with systolic blood pressure. The expression profiles of the shared genes derived from the general and subgroup mappings were compared across 65 normal human tissues. The results indicated that the shared genes that were identified through the sex-specific, hypertension-specific or general genetic mappings display higher expression values in brain-related tissues (Table 2 and Figure 2).

**Subgroup genetic mappings.** The hypertension-specific genetic analysis revealed that SNP rs4887150 (HWE P = 0.3, MAF = 0.3), which is inside the IL1RAP gene, is associated with coffee use with a negative association signal (Z = -3.5, P = 0.0004) in hypertensive subjects and a positive association signal (Z = +3.8 P = 0.0002) in normotensive subjects. The sex-specific genetic analysis revealed that SNP rs4888197 (HWE P = 1, MAF = 0.2), which is inside the PLGC2 gene, was associated with thigh obesity and responses to mental and physical stress and hemodynamic tissues (Table 2).

The functional annotation of the shared genes indicated that these genes are highly expressed and exhibit patterned expression in a cluster of brain-related tissues, including brain lobes, cerebral cortex, amygdala, hippocampus and putamen (Table 2 and Figure 2).

**Protein interaction analysis.** Candidate genes derived from the general (n = 76), sex-specific (n = 31) and hypertension-specific genetic mappings (n = 38) were pooled, and a connectivity diagram was built based on protein interactions. Figure 3 shows an overview of these interactions. Although these genes were derived from different analyses, we found numerous interactions among their encoded proteins. We also checked for the associations of SNPs within the missing genes of the constructed network (Figure 3). In this manner, we identified the following SNPs: rs6938572 (P = 0.0006) and rs2327017 (P = 0.0003) upstream of the BMP6 gene, which were associated with average sitting DBP; rs2370413 (P = 0.0003) and rs2887780 (P = 0.0004) inside the CAGNAC1 gene, which were associated with subcapsular skinfold measurements in males and the SBP response to mental stress, respectively; rs10492133 (P = 0.0009) inside GRIN2B, which was associated with tobacco use; rs10491321 (P = 0.00035) upstream of and rs7705319 (P = 0.0006) inside the PP2CA gene, which were associated with tobacco use; rs953944 (P = 0.0007) and rs1029819 (P = 0.0008), which were associated with tobacco use, and rs10485912 (P = 0.00075), which was associated with overall SBP, inside the MAG2L gene; rs93059 inside the NFKB1 gene, which was associated with the average sitting HR (P = 0.00098) and the DBP response to mental stress (P = 0.0006); and SNPs inside the ITPR1 gene, which were associated with (0.0004 ≤ P ≤ 0.0007) the BP and SBP response to mental stress.

**DISCUSSION**

In this study, we investigated the connections between substance use, obesity and responses to mental and physical stress and hemodynamic traits.
traits in families from the founder population of the SLS region. The founder effect provides several benefits for mapping the genomic determinants of complex traits, including limited heterogeneity, the low likelihood of population admixture and stratification, the large size of the LD blocks and good genealogical records.9,29

Our findings suggest that smoking may lower the BP through several mechanisms. First, obesity increases the risk of hypertension;7 therefore, the observed inverse correlation between obesity and tobacco use in our study could partly explain the lower BP in tobacco users. Second, stress is related to hypertension, which is consistent with the finding that hypertensive subjects had stronger HR and SBP responses to mental stress than normotensive subjects in our study. It has been already reported that a high-magnitude cardiovascular response to environmental stressors is a predictor of hypertension.4,12 Therefore, our finding that habitual tobacco users displayed significantly attenuated HR responses to mental stress compared with non-users suggest that the damping effect of tobacco use on response to environmental stress may also account for the lowering effect of tobacco use on BP.

In fact, the relief from stress and negative emotion and enhancing positive moods are among the main reasons for substance use.30,31 Physiological studies have also shown that both substance use and stress influence synaptic plasticity and that substance use can change the sensitivity of synaptic plasticity to stressors. For example, concurrent chronic nicotine treatment prevents the stress-induced impairment of the LTP pathway.32,33 Synaptic plasticity is also implicated in the regulation of energy homeostasis as an important pathway through which peripheral metabolic hormones influence brain functions.34 Moreover, synaptic sensitization is involved in both stress- and salt-related hypertension as well as obesity-related hypertension. The repeated stimulation of the

Table 2 Gene expression profile\textsuperscript{a} of shared genes driven from general, sex-specific and hypertension-specific genetic mapping

| Tissue                  | N  | Mean | s.e. | Tissue                  | N  | Mean | s.e. | Tissue                  | N  | Mean | s.e. |
|-------------------------|----|------|-----|-------------------------|----|------|-----|-------------------------|----|------|-----|
| Temporal lobe           | 60 | 0.33 | 0.1 | Cerebellum              | 27 | 0.31 | 0.2 | Cerebellum              | 16 | 0.62 | 0.3 |
| Occipital lobe          | 60 | 0.32 | 0.1 | Accumbens               | 27 | 0.31 | 0.1 | Temporal lobe           | 16 | 0.56 | 0.3 |
| Accumbens               | 60 | 0.32 | 0.1 | Occipital lobe          | 27 | 0.30 | 0.2 | Amygdala                | 16 | 0.52 | 0.2 |
| Frontal lobe            | 60 | 0.32 | 0.1 | Parietal lobe           | 27 | 0.30 | 0.2 | Accumbens               | 16 | 0.52 | 0.3 |
| Parietal lobe           | 60 | 0.32 | 0.1 | Frontal lobe            | 27 | 0.29 | 0.1 | Parietal lobe           | 16 | 0.52 | 0.3 |
| Amygdala                | 60 | 0.29 | 0.1 | Putamen                 | 27 | 0.29 | 0.1 | Cerebral cortex         | 16 | 0.50 | 0.3 |
| Cerebral cortex         | 60 | 0.29 | 0.1 | Temporal lobe           | 27 | 0.27 | 0.1 | Putamen                 | 16 | 0.50 | 0.3 |
| Dorsal root ganglia     | 60 | 0.28 | 0.1 | Amygdala                | 27 | 0.27 | 0.1 | Frontal lobe            | 16 | 0.50 | 0.2 |
| Putamen                 | 60 | 0.27 | 0.1 | Cerebral cortex         | 27 | 0.27 | 0.1 | Occipital lobe          | 16 | 0.49 | 0.3 |
| Hippocampus             | 60 | 0.26 | 0.1 | Hippocampus             | 27 | 0.25 | 0.1 | Hippocampus             | 16 | 0.49 | 0.2 |

\textsuperscript{a}Gene expression values (normalized log\textsubscript{2} ratios) were extracted from COXPRESdb database.25 For each group of genes 65 human tissues were tested and the first 10 tissues with the highest mean expression are presented here. The entire data set is available in Supplementary Table S9.
defense pathway sensitizes the synapse to respond to ever milder stressors such that hypertension eventually becomes permanent. In agreement with the above findings, our genetic analyses indicated that the neural synapse is a shared interface behind the studied traits. Genetic mapping uncovered numerous common genes among substance use, obesity, the response to mental and physical stress and HR and BP that had synaptic function. Furthermore, we found that the identified genes form a network of interacting proteins. The pathway analysis identified the LTP pathway, which is important in synaptic plasticity. In addition, gene expression findings revealed that these genes tend to be highly expressed and have patterned expression profiles in brain-related tissues.

Sex-specific and hypertension-specific genetic mappings identified IL1RAP, PLCG2 and SCIN as genes that were significantly negatively associated with a trait in one group and significantly positively associated with the same trait in the opposite group. All three genes had synaptic function, and in agreement with the identification of the SCIN gene as associated with tobacco use, it has been reported that nicotinic receptor stimulation induces the redistribution of the SCIN protein. The results of the pathway analysis and the comparison of the gene expression profiles of the shared genes derived from the sex-specific and hypertension-specific genetic mappings were similar to those from the general analysis. Moreover, we identified protein interactions and similar genes among the gene sets derived from the general and sex- and hypertension-specific genetic mappings, suggesting that sex-specific and hypertension-specific genetic differences are based on similar biological process to those identified by the general analysis. Therefore, by increasing genetic homogeneity, subgroup genetic mapping can uncover additional components of functional modules that otherwise remain obscure.

Figure 2 Gene expression heat map across 65 normal human tissues of the shared genes derived from the general genetic mapping. The shared genes tend to have higher expression and patterned expression profiles in the cluster of brain-related tissues, including the nucleus accumbens, putamen, brain lobes, cerebral cortex, hippocampus and amygdala. The expression values are presented in shades of red (high expression) to blue (low expression). The gene expression values were extracted from the COXPRESdb database. A full color version of this figure is available at the Hypertension Research journal online.

Figure 3 Overview of the protein interactions of genes derived from the general, sex-specific and hypertension-specific genetic mappings. The encoded proteins of the genes derived from the general, sex-specific or hypertension-specific genetic mappings share numerous protein interactions. The genes identified through two analyses (e.g., CAMK4) are displayed twice with different colors. A full color version of this figure is available at the Hypertension Research journal online.
Stressors

Mental processes as synaptic plasticity

Habits and lifestyle

Cardiovascular phenotypes and risk factors

Other paths

The synapse is a shared interface of HTN-related traits

On the basis of above findings, we propose a model that (Figure 4) once an environmental stressor is perceived at synapses, it can alter the efficacy of synaptic plasticity; however, other environmental factors as substance use may modify the sensitivity of synaptic plasticity to the stressors. In the long term, the combined effects of genetic variations in the synapses and environmental factors shape an individual’s lifestyles and habits. Conversely, the established habits and lifestyle influence the body and eventually modify health status outcomes, including cardiovascular disease and cardiovascular risk factors phenotypes as obesity. Nonetheless, there are other factors that modify cardiovascular outcomes through different mechanisms. A full color version of this figure is available at the Hypertension Research journal online.

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The identification of shared genetic factors among the studied traits suggests that complex phenotypes may not be entirely independent of one another and has implications for the design of gene-mapping studies that jointly examine complex traits. Such studies can provide a basis for disease classification and profiling as well as creating a map of human diseases based on underlying pleiotropic genetic factors. The constructed disease map can subsequently be overlaid with drug-targeted networks to explore potential therapeutic avenues.

The substantial overlap between the genomic determinants of substance use, stress, obesity and hemodynamic traits in genes related to synapses provides evidence to support additional studies on the involvement of genetic variations in the synapses in the etiology of complex traits in which stress and lifestyle are contributing factors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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