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Exploring the role of low-frequency and rare exonic variants in alcohol and tobacco use

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ABSTRACT

Background: Alcohol and tobacco use are heritable phenotypes. However, only a small number of common genetic variants have been identified, and common variants account for a modest proportion of the heritability. Therefore, this study aims to investigate the role of low-frequency and rare variants in alcohol and tobacco use. *Methods:* We meta-analyzed ExomeChip association results from eight discovery cohorts and included 12,466 subjects and 7432 smokers in the analysis of alcohol consumption and tobacco use, respectively. The ExomeChip interrogates low-frequency and rare exonic variants, and in addition a small pool of common variants. We investigated top variants in an independent sample in which ICD-9 diagnoses of "alcoholism" (N = 25,508) and

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Pathway analysis

"tobacco use disorder" (N = 27,068) had been assessed. In addition to the single variant analysis, we performed gene-based, polygenic risk score (PRS), and pathway analyses.

Results: The meta-analysis did not yield exome-wide significant results. When we jointly analyzed our top results with the independent sample, no low-frequency or rare variants reached significance for alcohol consumption or tobacco use. However, two common variants that were present on the ExomeChip, rs16969968 ($p = 2.39 \times 10^{-7}$) and rs8034191 ($p = 6.31 \times 10^{-7}$) located in *CHRNA5* and *AGPHD1* at 15q25.1, showed evidence for association with tobacco use.

Discussion: Low-frequency and rare exonic variants with large effects do not play a major role in alcohol and tobacco use, nor does the aggregate effect of ExomeChip variants. However, our results confirmed the role of the *CHRNA5-CHRNA3-CHRNB4* cluster of nicotinic acetylcholine receptor subunit genes in tobacco use.

1. Introduction

Alcohol and tobacco use belong to the world's leading health risks and are responsible for the premature death of 3.3 million and 6 million people each year, respectively (World Health Organization, 2014; World Health Organization, 2015). Overall 5.1% of the global burden of disease and injury, measured in disability-adjusted life years (DALYs), is attributable to alcohol, and 3.7% is attributable to smoking (World Health Organization, 2009, 2014). Alcohol and tobacco use initiation and severity are influenced by a combination of genetic and environmental risk factors. Examples of environmental exposures that impact on substance use outcomes are social stress, traumatic life events, peer pressure, inadequate parenting, insufficient social control, and low socio-economic status (De Bellis and Zisk, 2014; Kendler et al., 2011; Lijffijt et al., 2014; Loke and Mak, 2013; Van Ryzin et al., 2012; Young-Wolff et al., 2011). Heritability estimates (i.e., the proportion of phenotypic variance attributable to genetic variance) of 40–60% and 45–86% (Broms et al., 2006; Mbarek et al., 2015; Verhulst et al., 2015; Vink et al., 2005) for alcohol and tobacco use-related traits, respectively, indicate a strong genetic component influencing these behaviors. Elucidating which genetic variants contribute to alcohol and tobacco use is an important step in unraveling the underlying biological mechanisms. Although current pharmacological treatments for alcohol and tobacco use disorders have demonstrated positive treatment outcome, the effects are moderate at best, and many patients do not benefit from these treatments (Goh and Morgan, 2017; Stead and Lancaster, 2012). An improved understanding of the biological mechanisms will aid the development of novel medications and prevention methods for substance use-related problems.

Various methods have been applied to identify genetic variants associated with substance use. During the last decade, Genome-Wide Association Studies (GWAS) have been the preferred study design due to the capacity to study single nucleotide polymorphisms (SNPs) in a genome-wide manner without the need for *a priori* hypotheses. GWAS usually captures common variants (i.e., variants with a minor allele frequency (MAF) larger than 5% in the population), but recent large-scale GWAS also included low-frequency variants (MAF 1–5%) and even variants that occur in 0.1% of the population.

The latest GWAS for alcohol consumption comprised 112,117 individuals and identified 14 significant loci (Clarke et al., 2017), including variants in the gene *KLB,* which had been identified previously by Schumann et al. (2016). The gene product of *KLB*, β-klotho, controls alcohol use in mice (Schumann et al., 2016). Other significant loci were found in the alcohol dehydrogenase (ADH) gene cluster, which has consistently been associated with alcohol consumption (Gelernter et al., 2014; Macgregor et al., 2009). SNPs identified in these genes alter alcohol metabolism (Harada et al., 1983; Thomasson et al., 1991; Yoshida et al., 1991). Furthermore, the authors reported multiple gene-based associations including *DRD2*, encoding a dopamine receptor, and *PDE4B,* which plays a role in signal transduction.

For tobacco use, SNPs located at the chromosomal region 15q25.1, which includes the *CHRNA5-CHRNA3-CHRNB4* cluster of nicotinic acetylcholine receptor subunit genes, show the most robust associations (David et al., 2012; Liu et al., 2010; Saccone et al., 2010a; Tobacco and Genetics, 2010). Other genes implicated in tobacco use are *HECTD2-AS1 (LOC100188947)*, *EGLN2*, *BDNF*, and *DBH* (Tobacco and Genetics, 2010). Although the identified loci and genes for tobacco use are different from alcohol consumption, twin studies suggest that some proportion of the heritability of alcohol and tobacco use is shared between the two traits (Koopmans et al., 1997).

Individual SNPs identified by GWAS explain only a modest proportion of the variation in complex traits (Manolio et al., 2009). In addition, the aggregate effect of all SNPs included in a GWAS does not fully capture the family-based heritability of traits. For alcohol consumption and tobacco use, GWAS SNPs generally capture less than 50% of the twin-based heritability (Lubke et al., 2012; Mbarek et al., 2015; Vrieze et al., 2013). The proportion of the genetic liability which has not yet been explained by the studied common variants is referred to as "the missing heritability" (Manolio et al., 2009). Part of the missing heritability may be explained by gene-environment (GxE) interaction, structural variants, and epistasis. Furthermore, rare genetic variation (MAF < 0.01), which is not captured in conventional GWAS arrays, may contribute substantially to the heritability of complex traits (Speed et al., 2017).

Genome-wide interrogations of rare variants that contribute to alcohol and tobacco use are scarce. Studies that instead investigated the role of rare variants in a limited number of candidate genes showed significant associations with substance dependence. For example, variants within the gene *CHRNB3* are associated with alcohol dependence (Haller et al., 2014) while rare variants in the genes *CHRNB4*, *NRXN1*, *CHRNA9*, *TAS2R38*, *CHRNA2*, *NTRK2*, *GABBR2*, *GRIN3A*, *DNM1*, *DBH*, *NRXN2*, *ANKK1/DRD2*, *NRXN3*, *CDH13*, and *ARRB2* were shown to influence the risk of nicotine dependence (Haller et al., 2012; Yang et al., 2015). However, the associations of these rare variants with alcohol and nicotine dependence have not been replicated, and are therefore suggestive in nature. The relevance of investigating rare variants is further demonstrated by an observed inverse relationship between a variant's effect size and its frequency in the population (Park et al., 2011). This relationship indicates that a genome-wide exploration of the impact of rare genetic variants may provide additional insights into the genetic etiology of substance use phenotypes.

The introduction of the Illumina Infinium HumanExome BeadChip array (Illumina Inc., San Diego, CA) enabled the study of rare genetic variants in a genome-wide and cost-effective way (Cirulli and Goldstein, 2010; Grove et al., 2013; Lee et al., 2014; Vrieze et al., 2014). The "ExomeChip" contains a dense amount of low-frequency and rare nonsynonymous SNPs, and an additional smaller set of common SNPs that aid quality control of the genotypes. The nonsynonymous SNPs change the protein sequence by coding for different amino acids. Their interrogation facilitates functional interpretation of causal variants or genes due to a clear mechanism through which they exert their effect on traits and diseases. Combined with potentially large effect sizes, rare nonsynonymous SNPs are attractive drug targets. For alcohol and nicotine dependence, a single ExomeChip study has been conducted in 7181 individuals. No significant SNP or gene associations were detected, probably due to the limited sample size (Vrieze et al.,