Author(s): Salvatore, Jessica E.; Savage, Jeanne E.; Barr, Peter; Wolen, Aaron R.; Aliev, Fazil; Vuoskimaa, Eero; Latvala, Antti; Pulkkinen, Lea; Rose, Richard J.; Kaprio, Jaakko; Dick, Danielle M.

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DR. JESSICA ELIZABETH SALVATORE (Orcid ID : 0000-0001-5504-5087)

DR. JEANNE E SAVAGE (Orcid ID : 0000-0002-2034-8341)

DR. DANIELLE DICK (Orcid ID : 0000-0002-1636-893X)

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Incorporating functional genomic information to enhance polygenic signal and identify variants involved in gene-by-environment interaction for young adult alcohol problems

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Jessica E. Salvatore, Ph.D.[1,2*], Jeanne E. Savage, Ph.D.[2*], Peter Barr, Ph.D.[1], Aaron R. Wolen, Ph.D.[3], Fazil Aliev, Ph.D.[1,4], Eero Vuoksimaa, Ph.D.[5], Antti Latvala, Ph.D.[5], Lea Pulkkinen, Ph.D.[6], Richard J. Rose, Ph.D.[7], Jaakko Kaprio, M.D.[5] & Danielle M. Dick, Ph.D.[1]

[1] Department of Psychology, Virginia Commonwealth University, PO Box 842018, Richmond, VA 23284-2018, United States
[2] Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, PO Box 980126, Richmond, VA 23298, United States
[3] Center for Clinical and Translational Research, Virginia Commonwealth University, P.O. Box 980261, Richmond, VA 23298-0261, United States
[4] Faculty of Business, Karabuk University, 78050 Karabuk, Turkey.
[5] Institute for Molecular Medicine FIMM, University of Helsinki, PO Box 20 (Tukholmankatu 8), FI-00014 Helsinki, Finland
[6] Department of Psychology, University of Jyväskylä, PO Box 35, 40014 University of Jyväskylä, Jyväskylä, Finland
[7] Department of Psychological & Brain Sciences, Indiana University, 1101 E. 10th St., Bloomington, IN 47405, United States
*Denotes shared first-authorship.

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Correspondence concerning this manuscript should be addressed to: Jessica E. Salvatore, VCU PO Box 842018, 806 West Franklin Street, Richmond, VA 23284-2018, USA. Phone: +1 (804) 828-8132; fax: +1 (804) 828-2237. email: jesalvatore@vcu.edu.

Abstract

Background. Characterizing aggregate genetic risk for alcohol misuse and identifying variants involved in gene-by-environment interaction (G×E) effects has so far been a major challenge. We hypothesized that functional genomic information could be used to enhance detection of polygenic signal underlying alcohol misuse, and to prioritize identification of single nucleotide polymorphisms (SNPs) most likely to exhibit G×E effects.

Methods. We examined these questions in the young adult FinnTwin12 sample (n=1170). We used genome-wide association estimates from an independent sample to derive two types of polygenic scores for alcohol problems in FinnTwin12. *Genome-wide polygenic scores* included all SNPs surpassing a designated p-value threshold. *DNase polygenic scores* were a subset of the genome-wide polygenic scores including only variants in DNase I hypersensitive sites (DHSs), which are open chromatin marks likely to index regions with a regulatory function. We conducted parallel analyses using height as a non-psychiatric model phenotype in order to evaluate the consistency of effects. For the G×E analyses, we examined whether SNPs in DHSs were overrepresented among SNPs demonstrating significant G × E effects in an interaction between romantic relationship status and intoxication frequency.

Results. Contrary to our expectations, we found that DNase polygenic scores were not more strongly predictive of alcohol problems than conventional polygenic scores. However, variants in DNase polygenic scores had per-SNP effects that were up to 1.4 times larger than variants in conventional polygenic scores. This same pattern of effects was also observed in supplementary analyses with height. In G×E models, SNPs in DHSs were modestly overrepresented among SNPs with significant interaction effects for intoxication frequency.

Conclusions. These findings highlight the potential utility of integrating functional genomic annotation information in order to increase the signal-to-noise ratio in polygenic scores and identify genetic variants that may be most susceptible to environmental modification.

Keywords: alcohol, functional genomics, gene-environment interplay, polygenic scores

Introduction

Alcohol misuse (i.e., risky drinking and alcohol use disorder) is a top public health problem worldwide (World Health Organization, 2014), and reflects a complex interplay of genetic and environmental influences across development (Pagan et al., 2006). Twin and adoption studies have been critical in demonstrating that genetic influences account for roughly half of the variation in the risk for alcohol use disorder (Verhulst et al., 2015) and other alcohol use behaviors (Dick et al., 2011). Translating findings from family-based research designs of unmeasured genetic variance (i.e., inferred based on resemblance among different types of relatives) into a measured genetic framework to identify the specific variants associated with alcohol outcomes has been challenging (Hart and Kranzler, 2015). Although a few individually important genes and genetic variants have been identified, results from genome-wide association studies (GWAS) of alcohol use disorder underscore its highly polygenic nature (Yang et al., 2014, Hart and Kranzler, 2015, Mbarek et al., 2015). This high level of polygenicity is consistent with emerging findings from GWAS of psychiatric disorders more broadly (Geschwind and Flint, 2015), as well as findings that psychiatric conditions also share much of their polygenic underpinnings (Anttila et al., available online April 2016).

Characterizing Aggregate Genetic Risk for Alcohol Outcomes

In recent years, polygenic approaches have emerged as one method to characterize aggregate measured genetic risk (Wray et al., 2014). These efforts were motivated by the growing recognition that many genes and genetic variants, each of small individual effects, contribute to complex disorders; as well as the practical, clinical goal of being able to accurately predict disease and disorder from genetic information. Most commonly, polygenic scores are

created by summing the number of "risk" alleles an individual carries across a selected set of single nucleotide polymorphisms (SNPs), weighted by empirical information from genetic association results obtained from an independent discovery sample. In effect, polygenic scores capture the composite additive effect of these multiple variants. This approach was initially used in the study of schizophrenia (The International Schizophrenia Consortium, 2009) and has since been applied to numerous complex traits (Dudbridge, 2013). As reviewed by Hart & Kranzler (2015), several recent studies have successfully used polygenic score approaches to predict alcohol-related outcomes (Yan et al., 2014, Vrieze et al., 2013, Frank et al., 2012, Kos et al., 2013, Levey et al., 2014).

Polygenic scores can encompass thousands of individual genetic variants spread throughout the genome and include a mixture of true genetic association signal and noise from statistical artifact and stochastic error (Maher, 2015). Conventional polygenic scoring methods have typically accounted for less than 2% of the genetic liability underlying complex traits, although this improves as the discovery sample sizes increases. Simulations indicate that tens of thousands of subjects may still be needed to achieve clinically meaningful prediction with these methods (Dudbridge, 2013). Efforts to amplify true genetic signal and reduce noise could enhance the predictive power of polygenic scores. Although some methods have been developed to improve polygenic scores, as of yet there has been no attempt to use information beyond the discovery GWAS (i.e. *p* value thresholds for filtering the inclusion of SNPs or linkage disequilibrium structure for weighting SNPs) to further refine the creation of such scores.

The past decade of genomic research has provided a wealth of information about the genetic variants that are being aggregated in these polygenic scores, including information about which variants are more or less likely *a priori* to have functional consequences on human traits and behaviors (ENCODE Project Consortium, 2012). In the same way that functional genomic information is important for understanding the biological coherence underlying GWAS results, it may also inform better ways to characterize individuals' aggregate genetic risk for alcohol outcomes. Recent large-scale efforts have established that genetic variants associated

with a variety of complex diseases and traits are not randomly distributed throughout the genome, but rather are stratified based on their genomic context (Schork et al., 2013, Finucane et al., 2015). Across many complex diseases and traits, there is modest evidence for an overrepresentation of SNPs with significant GWAS signals in or near protein-coding regions, and even stronger evidence for overrepresentation of SNPs in certain noncoding regions (Hindorff et al., 2009). Once considered "junk DNA", it is now known that many regions outside of the exons that code for proteins have an indirect biological effect through the regulation of when, in what tissue, and under what circumstances a gene is expressed (ENCODE Project Consortium, 2012). Epigenetic factors near the transcription start site of a gene and in other key regulatory regions can influence gene expression by changing the physical conformation of the DNA, thus changing how accessible the DNA is to the cellular machinery responsible for transcribing genes into proteins.

In particular, GWAS signals are enriched within regions of open chromatin identified by deoxyribonuclease I (DNase I) mapping (Maurano et al., 2012). These so-called DNase I hypersensitive sites (DHS) are regions where DNA is highly accessible (Bell et al., 2011), and likely serves some *cis*-regulatory function (Thurman et al., 2012). The location of DHS signals overlaps that of many other regulatory markers, indicating that they are a broad, non-specific marker of sites of active regulatory DNA, capturing many different ongoing biological processes affecting gene expression. The enrichment of significant GWAS associations in these regions provides some biological coherence for interpreting the functional impact of variation in these non-coding variants, and also suggest that SNPs located in DHSs (referred to as DHS SNPs) may be more likely to be “true” signals and less likely to be false positives. For this reason, we hypothesized that functional annotation information like DHS location could be used to improve the predictive ability of polygenic scores. Using alcohol problems as our primary outcome, we expected that polygenic scores based on SNPs in regulatory regions (DHSs) would provide stronger predictive power (i.e., account for more variance) compared to conventional, unselected, genome-wide polygenic scores that included a mixture of DHS SNPs and non-DHS

SNPs. We focus specifically on localization in DHSs in view of recent evidence that SNPs with lower *p* values in our discovery sample GWAS were more likely to be in DHS regions (versus non-DHS regions) (Edwards et al., 2015), as well as broader evidence from genomic partitioning analyses that DHS SNPs accounted for the majority (79%) of the heritability across 11 common diseases (Gusev et al., 2014). Additionally, in the absence of existing knowledge about what specific functional annotations would be most advantageous to inform polygenic scores, DHS status provides a non-specific tool for a first look into whether this approach holds promise.

Identifying Genetic Variants Involved in Gene-by-Environment Interaction Effects

Unlike Mendelian disorders such as cystic fibrosis or Huntington's disease, where a mutation in a single gene is sufficient to cause disorder, the pathway from genotype to phenotype for alcohol outcomes is not necessarily straightforward. Alongside advances in characterizing genetic risk, research has suggested that a number of environmental factors can alter the importance of genetic influences on alcohol outcomes (Young-Wolff et al., 2011), and it has also been suggested that G×E effects may harbor some of the 'hidden heritability' for complex behavioral outcomes (Manolio et al., 2009). Despite strong evidence for G×E effects from twin studies (Young-Wolff et al., 2011), the study of G×E using measured genotypes has been controversial (Duncan and Keller, 2011, Dick et al., 2015).

Among the major criticisms is the focus on "usual suspect" candidate genes in the serotonin or dopamine pathways (e.g., *SLC6A4* or *MAO-A*) (Dick et al., 2015). Thus, the field is in need of answers to the question of which SNPs are worth carrying forward into studies of G×E using measured genotypes. One way to answer this question is to examine whether certain types of SNPs (based on genomic information) are overrepresented among SNPs with G×E effects. Thus, in an effort to move away from the candidate gene approach, we tested the exploratory hypothesis that SNPs in regulatory regions would be more likely to have significant G×E effects. We believed DHS SNPs would be enriched for G×E interaction effects given that the DNA variants in DHS regions may be more likely to affect the chromatin structure around a gene that determines whether the DNA is accessible to transcription factors (i.e., the proteins

responsible for transcribing DNA to RNA and determining gene expression levels) (Cockerill, 2011). Environmental exposures are known to affect epigenetic processes, and can further drive gene expression or repression via alterations to the availability of transcription factors (Meaney, 2010, Lopez-Maury et al., 2008). For these reasons, we hypothesized that allelic variation in DHS SNPs may be particularly impactful for responsiveness to environmental cues that alter gene expression (Liu et al., 2008).

We examined romantic relationship status as the environmental moderator for these analyses in view of evidence that (1) involvement in a romantic relationship in young adulthood is associated with lower alcohol use (Fleming et al., 2010) and (2) that romantic relationship status changes the degree to which genetic influences are important for alcohol outcomes (Heath et al., 1989, Prescott and Kendler, 2001). Of particular relevance for this exploratory G×E hypothesis, recent analyses of the FinnTwin12 sample indicated that genetic variance for intoxication frequency was attenuated for those in a romantic relationship compared to those not in a romantic relationship (Barr et al., in press). This implies that genetic influences on intoxication frequency are less important for those who are in a relationship, and more important for those who are single. The results from these twin studies suggested that romantic relationship status would be a particularly good "candidate environment" when testing our hypothesis that SNPs in regulatory regions would be enriched for G×E effects. Twin studies of G×E effects using inferred genotypes typically show a fan-shaped pattern of effects, whereby additive genetic factors have more influence in certain environments, and less in others. Detecting a latent G×E effects with inferred genotypes implies that the majority of measured genes are likely to be moderated in the same way, such that the effect of measured genotypic influences on a phenotype varies across levels of the environment.

The Current Study

We examined two research questions related to the incorporation of functional genomic information to understand the genetic and G×E influences on alcohol use outcomes in a population-based sample of young adult Finnish twins (Kaprio, 2013, Kaprio et al., 2002): 1) Do

polygenic scores informed by DHS annotation predict lifetime alcohol problems better than conventional polygenic scores that include a mixture of DHS and non-DHS SNPs? And 2) Are DHS variants overrepresented among SNPs with G×E effects for alcohol misuse in a model where romantic relationship status is the environmental moderator? As a set, these questions contribute to efforts to enhance polygenic signal and empirically prioritize variants likely to be involved in G×E effects.

Materials and Method

Sample

Our sample comes from the youngest cohort of the Finnish Twin Cohort Study (FinnTwin12), which was established to examine genetic and environmental factors influencing health-related behaviors (Kaprio, 2013), including the development of alcohol misuse. Participants were recruited from Finland's Population Registry, permitting comprehensive and unbiased nationwide ascertainment of all twins born across five birth cohorts in Finland from 1983 to 1987. Baseline collection occurred when twins were aged approximately 12 years old, with a sample of some 5600 twins and their families (Kaprio, 2013) and an overall participation rate of 87%. Follow-up surveys occurred at ages 14, 17.5, and 22 years. Of the original epidemiological sample, 1035 families were chosen as part of an intensive subsample, from which 1852 twins (89% participation) completed the adolescent version of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA; Bucholz et al., 1994) interview at age 14. Follow up of the intensive subsample when twins were, on average, age 22 (n=1347) included the adult SSAGA. DNA from blood or saliva samples was collected from 1295 twins. Data for the present study is drawn from the psychiatric assessment at age 22 among participants for whom genotypic data were available (n=1170). The sample was 53.6% female (N = 627) and the age range varied from 20-26, with a mean age of 22.42 years (SD=0.72). Participants were fully informed of study procedures and gave written consent to participate. The Helsinki University Central Hospital District's Ethical Committee and Indiana University's Institutional Review Board approved the FinnTwin12 study.

Measures

Lifetime alcohol dependence symptoms. The alcohol dependence symptoms (ADsx) measure was the count of the number of lifetime DSM-IV criteria that respondents endorsed from the SSAGA (Bucholz et al., 1994). Responses ranged from 0 to 7. ADsx was natural log-transformed after adding 1 to adjust for the positive skew and to retain participants who endorsed zero symptoms. Individuals who never initiated alcohol use were coded as missing (n = 35).

Frequency of intoxication. For the G×E analyses, we expected that the moderating effect of relationship status would be on a contemporaneous alcohol misuse outcome rather than a cumulative lifetime alcohol misuse outcome, such as ADsx. Accordingly, we used a time-delimited measure of frequency of intoxication for the G×E analyses. Frequency of intoxication was assessed at age 22 by the single item, "How often do you use alcohol in such a way that you get really drunk?" Response options included "never" (0) to "daily" (8). Response categories were transformed to reflect the number of days per month participants were intoxicated and natural log-transformed after adding 1 to adjust for the positive skew and to retain participants who reported “never” (Dick et al., 2001).

Relationship status. Participants were asked, “How long (in years) have you been together with your present partner?” Those who indicated they were not dating were coded as 0. Those who indicated they were in a romantic relationship (dating, married, or living in a common law relationship) of any length were coded as 1.

Genotyping

Genotyping was conducted using the Human670-QuadCustom Illumina BeadChip (Illumina, Inc., San Diego, CA, USA) at the Wellcome Trust Sanger Institute (Kaprio, 2013). Quality control steps included removing SNPs with minor allele frequency < 1%, genotyping success rate < 95%, or Hardy-Weinberg equilibrium $p < 1 \times 10^{-6}$, and removing individuals with genotyping success rate < 95%, a mismatch between phenotypic and genotypic gender, excess relatedness (outside of known families), and heterozygosity outliers. Genotypes were imputed

to the 1000 Genomes Phase I (v3) reference panel using ShapeIT (Delaneau et al., 2012) for phasing and IMPUTE2 (Howie et al., 2009) for imputation. Prior analyses indicated a single dimension of ancestry in the sample (Meyers, 2012). Although a single dimension of ancestry does not preclude variation along this dimension, we note that fine-scale population substructure is less of an issue for common variants (versus rare variants), especially in the present sample given the relatively longer LD blocks that make the Finnish population more homogenous than other populations of mixed European ancestry. We also note that in supplementary analyses of the first 10 ancestry principal components and the ADsx and frequency of intoxication measures, we found no substantial evidence of population stratification. Out of 20 possible associations, only a single PC had a *p*-value of less than 0.10 (PC3 for ADsx, $p = 0.04$). These converging pieces of evidence suggested that it was not necessary to correct for population stratification, and informed our decision to not include ancestry principal components in our analyses.

Analytic Plan

Polygenic score creation. Summary association statistics used to create the polygenic scores for alcohol problems come from a previously reported GWAS of an alcohol problems factor score conducted in 4,304 Caucasian young adults from the Avon Longitudinal Study of Parents and Children (Edwards et al., 2015); this is the largest GWAS to date of alcohol problems in European young adults. Genotypes in this discovery sample were also imputed to the 1000 Genomes Phase I (v3) reference panel. From this discovery sample, we selected a list of 4,415,289 SNPs also available in FinnTwin12 and with a minor allele frequency > 5% and imputation quality $R^2 > .90$ in both samples, and pruned this SNP set to obtain 212,718 autosomal SNPs (4.8% of the common SNPs) in approximate linkage equilibrium ($R^2 < .25$). This list was further filtered to create two sets of score SNPs with nominal GWAS association *p* values in the discovery GWAS (thresholds of $p < .05$ and $p < .01$, $N_{SNPs} = 10{,}693$ and 2,221, respectively), based on preliminary analyses as well as previous work showing these thresholds have the best signal-to-noise ratio/predictive power for polygenic scores (Yang et al., 2014).

Scores were calculated in FinnTwin12 using the *score* procedure in PLINK version 1.9 (Chang et al., 2015) summing each individual's total number (imputed dosage) of minor alleles from the score SNPs, with each SNP weighted by the negative log of the GWAS association *p* value and sign of the association (beta) statistic. As illustrated in Figure 1, identical procedures were used to create a set of DNase I-restricted polygenic scores, except that the final list of LD-pruned SNPs described above was further restricted to SNPs located in DHS sites. The locations of DHSs were based on narrow peak hotspots identified across 53 consolidated epigenomes by the RoadMap Epigenomics Project (http://www.roadmapepigenomics.org). The 53 epigenomes are summarized in the Supplementary Information (Table S1). SNPs were considered to be DHS SNPs if they directly overlapped a DHS or were in perfect linkage disequilibrium (LD) with another SNP overlapping a DHS. Of the 212,718 genome-wide score SNPs, 78,948 (37%) were located in a DHS site, and 3,946 (37%) and 789 (36%) of the DHS score SNPs fell under the GWAS *p* value thresholds of $p < .05$ and $p < .01$, respectively. Hereafter we will use the terms "GW-scores" to refer to polygenic scores created from the genome-wide set of SNPs and "DHS-scores" to refer to polygenic scores from SNPs located in DHS sites only.

Polygenic scores for alcohol phenotypes have had very modest effects in previous studies. Thus, in an attempt to conceptually validate findings coming out of the primary alcohol analyses, we also compared the predictive power of DHS- and GW-scores for height as a secondary outcome. We selected height as a model phenotype given that its molecular genetic etiology is further advanced (relative to alcohol problems) and polygenic scoring methods have already demonstrated substantial success (Wood et al., 2014). We used the same procedure to calculate the polygenic scores for height, with discovery GWAS summary statistics coming from the GIANT Consortium meta-analysis results of ~250,000 adults of European ancestry (Wood et al., 2014; available at http://portals.broadinstitute.org/collaboration/giant). Genotypes from the GIANT study were imputed to the HapMap2 CEU reference population, so the LiftOver tool (http://genome.sph.umich.edu/wiki/LiftOver) was used to harmonize SNP IDs and genomic locations with those of the 1000 Genomes-imputed FinnTwin12 dataset. There were 1,831,837

SNPs in common after filtering, pruned for LD to 193,884 SNPs (31,358 and 15,239 below *p* thresholds of .05 and .01, respectively). Of these, 76,913 (39.7%) SNPs were located in a DHS, of which 13,593 (43.3% of GW) and 6,918 (45.4% of GW) met *p* value thresholds of .05 and .01. Height (in centimeters) was measured in the FinnTwin12 sample by a self-report survey item at age 22.

Predictive ability of DHS-scores versus GW-scores. In order to compare the relative strength of the DHS- and GW-scores, we fit a series of separate linear mixed-effects models incorporating each of the GW- and DHS-scores to predict ADsx and height. Each model also included sex (and, for height, age) as covariates. To account for clustering at the family level, we fit mixed models with random intercepts using the *lmer* function from the lme4 package (version 1.1.11) in *R* (version 3.2.3). Models were fit with risk scores calculated using SNPs at the $p < .01$ and $p < .05$ thresholds from the discovery GWAS. We examined the relative predictive ability of DHS- and GW-scores in two ways. First, we compared the significance of association and the overall variance accounted for (R^2) by each score. However, because the number of SNPs included in the polygenic scores differed substantially between the GW-scores and DHS-scores, a direct comparison of the magnitudes of their association statistics may not be meaningful. Thus, as a second approach we calculated an average "per-SNP" effect to facilitate comparisons on the same metric. To do this, we divided the variance accounted for each by each score (R^2) by the number of SNPs in that score.

G × E analyses. We examined whether SNPs in regulatory regions were enriched for G×E effects using a chi-square test that compared the proportion of DHS SNPs among the set of SNPs with significant ($p < .05$) G×E effects relative to the proportion of DHS SNPs in the full genome. For these analyses we used a contemporaneous measure of alcohol misuse, intoxication frequency. We focused on a contemporaneous measure of alcohol misuse in order to ensure that our romantic relationship status environmental moderator and alcohol misuse outcome were temporally matched[1]. For these analyses, we selected a set of top SNPs in the ALSPAC GWAS ($p < 0.005$) from the set of 212,718 LD-pruned autosomal SNPs common across

the ALSPAC and FinnTwin12 samples, resulting in 1137 SNPs. We focused on the set of the more highly associated SNPs in view of evidence that G×E effects are most likely to be observed for SNPs with smaller p-values (Thomas, 2010). This threshold was arbitrary, but was selected in an attempt to balance testing a large enough number of "top" SNPs with the computational resources required for such tests. For each SNP, we then examined gene-environment interaction effects in a linear model where relationship status was the moderator and frequency of alcohol intoxication was the outcome. G×E models were run using the lme4 package in R in order to account for familial nesting. G×E was tested using a parameterization method that takes into account effects between three gene levels in order to accurately capture interactions that can otherwise be misrepresented when using a single cross-product term (Aliev et al., 2014). The method checks the additive interaction between any two of the three gene levels and corrects for the number of tests. The resulting *p*-value corresponds to the difference between at least two of the gene levels. Sex and age were included as covariates. Preliminary analyses indicated only a modest association between relationship status and intoxication frequency (r = -.10), and no association between the GW- and DHS-scores and relationship status (range r_{pb} = -0.04 to 0.004, all p > 0.14). The latter null associations, in particular, minimized concerns about gene-environment correlation as a potential confounder when testing G×E effects. Regarding multiple testing concerns, we note that the inferential test of interest for this research question was a chi-square test of the proportion of DHS SNPs among the set of SNPs with a significant (p < .05) G×E effects relative to the overall proportion of DHS SNPs, thus representing a single statistical test for enrichment.

Results

Descriptive Statistics

Table 1 provides an overview of the distributions of the key measures in the FinnTwin12 sample. On average, participants endorsed 1.03 ADsx criteria, and reported being intoxicated 1.52 days per month (SD= 1.79). With respect to relationship status, of the 1,148

nonmissing responses, 58% (n=664) of the twins reported being involved in a relationship, of which 567 (84.8%) had been involved in that relationship for one year or more.

Do DHS- Scores Predict Outcomes Better than GW-scores?

ADsx. Regression results for GW-scores and DHS-scores predicting ADsx are shown in Table 2. The GW-scores predicted ADsx at both the $p<.01$ and $p<.05$ inclusion thresholds ($\beta = .0006$, $p<.001$ and $\beta = .0003$, $p<.001$, respectively). The DHS-scores also predicted ADsx at the $p<.01$ and $p<.05$ inclusion thresholds ($\beta = .0008$, $p=.009$ and $\beta = .0004$, $p=.022$, respectively). The overall effect sizes were relatively small, with each GW-score explaining about 1% of the variance in ADsx. Effect sizes for the DHS-scores were also modest, with each explaining ~0.5% of the variation in ADsx. The GW- and DHS-scores included different numbers of SNPs; accordingly, we compared the per-SNP effect sizes for the two types of polygenic scores. Ratios of the DHS to GW per-SNP effect were 1.1 to 1.4 for the per-SNP variance accounted for (R^2). This indicates that, on average, each SNP in DHS-scores accounted for 1.1-1.4 times more variance in ADsx compared to each SNP included in the GW-scores.

Height. Regression results for GW-scores and DHS-scores predicting height are shown in Table 3. The GW-scores significantly predicted height at both the $p<.01$ and $p<.05$ inclusion thresholds ($\beta = 0.0028$, $p=1\times10^{-47}$ and $\beta = 0.0025$, $p=3\times10^{-48}$, respectively). Likewise, the DHS-scores significantly predicted height at the $p<.01$ and $p<.05$ inclusion thresholds ($\beta = 0.0042$, $p=1\times10^{-37}$ and $\beta = 0.0040$, $p=3\times10^{-39}$, respectively). Compared to polygenic prediction of ADsx, the predictive power of polygenic scores for height was much stronger and the total phenotypic variance accounted for was considerably larger, ranging from 8.6 – 8.9% for GW scores and 6.4 – 6.9% for DHS scores. The per-SNP ratios for DHS to GW effects ranged from 1.6 to 1.8 for R^2, indicating that each SNP included in the DHS-scores accounted for, on average, 1.6-1.8 times the variance in height compared to SNPs in GW-scores.

Are DHS SNPs Enriched for Significant G × E Effects?

Of the top independent 1137 SNPs, 55 (4.8%) showed significant evidence for interaction ($p < .05$ in the interaction model). In total, 27 of the 409 DHS SNPs showed

significant G×E effects (7%) compared to 28 of 728 non-DHS SNPs (4%). A chi-square test of independence indicated that DHS SNPs were overrepresented among significant G×E effects relative to expectation, $\chi^2(1) = 3.92$, $p = 0.05$. This indicates that gene-environment interaction effects for this particular environment were modestly enriched for DHS SNPs.

In supplementary analyses, we also examined whether relationship status moderated the main effect of the aggregate GW- and DHS-scores in predicting alcohol intoxication. None of these interaction effects were significant (all $p > 0.05$).

Discussion

We tested two hypotheses related to the incorporation of functional genomic information to understand genetic and G ×E effects on alcohol use outcomes. We found that DHS-scores were more parsimonious compared to the GW-scores while capturing the majority of the same signal. The per-SNP effects for variants in the DHS-scores were 1.1 to 1.4 times larger than the per-SNP effect for variants in the GW-scores. We found a similar pattern of effects for a second non-psychiatric phenotype, height. We also found that DHS SNPs were modestly enriched for G×E effects compared to non-DHS SNPs in a model looking at romantic relationship status as the moderator.

These findings add to a growing literature demonstrating that incorporation of functional information about SNPs can advance our understanding of genetic contributions to complex diseases and disorders (Schork et al., 2013, Edwards et al., 2015, Maurano et al., 2012, Finucane et al., 2015). There was minimal loss in predictive power when polygenic scores were limited to variants in DHS regions, which is an encouraging sign that the included variants may be etiologically relevant given their higher *a priori* probability of having functional consequences. These results also provide some evidence that, like other complex traits (Maurano et al., 2012, Gusev et al., 2014), regulatory mechanisms appear to play a large role in the genetic factors impacting alcohol use outcomes. We should note, however, that the clinical utility of polygenic scoring methods for alcohol problems remains modest: both DHS- and GW-polygenic scores accounted for < 1% of the variance in alcohol dependence symptoms. It was for

this reason that we repeated the analyses with height, where there is greater predictive ability associated with polygenic risk scores calculated from large meta-analyses. We were encouraged to find a parallel pattern of results.

Our findings also provide initial evidence that DHS variants are more likely (compared to non-DHS variants) to be involved in gene-by-environment interaction effects. The study of measured gene-by-environment interaction is controversial, in part owing to problems surrounding the selection of the handful of SNPs in "usual suspect" candidate genes commonly examined (Dick et al., 2015, Duncan and Keller, 2011). Our results provide evidence for an empirically based approach that builds on findings from twin studies, GWAS, and functional genomics to select SNPs for studies of measured G×E. Thus, there may be a biologically and empirically justifiable way forward to identify the variants likely to be moderated by environmental factors. Important questions remain about the specific mechanisms underlying these statistical interactions, and where in the pathway from genes to behavior an environmental factor is likely to exert its moderating effect (Moore and Thoemmes, 2016). We speculate that DHS SNPs may be especially responsive to environmental inputs given their involvement in gene regulation (Liu et al., 2008).

Limitations

Our results should be considered in the context of several limitations. First, there was imperfect correspondence between the study populations and alcohol problems measures across the ALSPAC and FinnTwin12 samples. This concern is lessened in view of the genetic overlap evident between multiple dimensions of alcohol use (Dick et al., 2011). Second, the sample sizes of ALSPAC and FinnTwin12 are relatively small given the growing recognition of the large sample sizes needed to precisely estimate small effect sizes. We recognize that there are larger GWAS of alcohol-related behaviors (e.g., Schumann et al., 2016). Several factors guided our choice to use ALSPAC as our discovery sample, including the greater similarity between the ALSPAC and FinnTwin12 sample populations and alcohol problems phenotypes (in contrast to the aging-related cohorts included in the Schumann et al. (2016) study, as well as

that study's focus on an alcohol consumption phenotype). Third, the polygenic scores derived here include only common variants in regions well tagged by the variants in the 1000 Genomes panel. Fourth, there are alternative enrichment (Finucane et al., 2015) and alternative polygenic scoring methods (e.g., LDpred; Vilhjalmsson et al., 2015). Some combination of these may provide additional avenues for optimizing the predictive ability of polygenic scores in the future.

Fifth, we did not take into account the tissue specificity of regulatory markers when delineating DHS SNPs, as all variants located in (or in perfect LD with) a DHS site in any of the RoadMap tissue lines was considered a DHS SNP. Therefore, SNPs that have only a regulatory function in tissues that are not relevant to alcohol use would have been included in the DHS-scores along with true important functional variants, diluting the magnitude of the per-SNP association and the difference in association between SNPs included in the DHS versus non-DHS scores. We performed supplementary analyses using scores that included DHS SNPs limited to brain tissue samples and DHS SNPs present in two or more tissue samples to determine whether SNPs from certain samples were more relevant. Neither of these scores at either *p*-value threshold predicted ADsx. This may be due to the very small number of markers included in both the brain tissue score ($p<.01 = 139$; $p<.05 = 653$) or the two tissue score ($p<.01 = 495$; $p<.05 = 2{,}571$).

Sixth, our environmental moderator (romantic relationship status) captures only one of many relationship features previously implicated in studies of gene-environment interplay for alcohol use and problems (Jarnecke and South, 2014), and our measure of it was rather crude. Although we detect modest evidence that SNPs in DHSs were enriched for G × E effects, we note that these statistical interactions do not in themselves illuminate the biological processes through which these effects occur. Furthermore, as with all studies of G × E with measured genotypes, power is a concern and the results should be interpreted with appropriate caution. We conducted post-hoc power analyses using Quanto (Gauderman, 2002), and when the G × E effect was very small ($R^2 = 0.0005$), we had very low power to detect effects (12%). However,

when the G × E effect was somewhat larger (R^2 = 0.005) we had 72% power to detect interactions. On a more conceptual level, we note that previous analyses using the FinnTwin12 data have established latent G × E effects for relationship status and intoxication frequency (Barr et al., in press). This gives us more confidence in the G × E results using measured genotypes. Finally, as with other tests of enrichment, the focus of this analysis was not on interpreting the direction of any of the SNP × Relationship Status effects themselves, but rather examining whether there was overall enrichment to inform future studies about "which SNPs and which genes" are worth carrying forward into studies of GxE using measured genotypes.

Conclusions and Future Directions

These findings highlight the potential utility of integrating genomic annotation information in order to increase the signal-to-noise ratio in polygenic scores, and identify genetic variants that may be most susceptible to environmental modification. This work can be expanded in several ways, including extensions to jointly consider multiple annotation categories (Pickrell, 2014) and to make use of alternative weighting schemes to up- and down-weight variants across a range of regulatory marks rather than the blunt filtering tool applied here. Such advancements, in conjunction with ongoing efforts to increase power in gene identification studies, have the potential not only to provide biological insights into the etiology of alcohol misuse and other complex psychiatric disorders, but also to one day provide clinical utility to identify and treat at-risk individuals.

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Figure Legend

Figure 1. Schematic of DHS- and GW-polygenic score creation in the FinnTwin12 sample using an illustrative p-value of $p < 0.01$. The GW-score is the weighted linear combination of all SNPs meeting $p < 0.01$ in the discovery sample (ALSPAC) GWAS (Edwards et al., 2015). The DHS-score is the weighted linear combination of the subset of SNPs meeting $p < 0.01$ in the discovery sample GWAS that were also located in a DHS site. Abbreviations: DHS = DNase I hypersensitive site; GWAS = Genome-wide association study; LD = linkage disequilibrium; SNP = single nucleotide polymorphism.

Footnote

1. Our measure of intoxication frequency was moderately correlated with ADsx ($r = .42$ $p < .001$). Comparisons of GW and DHS scores in predicting intoxication frequency showed that GW scores at the $p < .001$, $p < .05$, and $p < .01$ thresholds were significantly associated with intoxication frequency in the expected direction (i.e., higher polygenic score associated with more frequent intoxication). DHS-scores at the $p < .001$ and $p < .01$ thresholds were significantly associated with intoxication frequency. Overall, both GW- and DHS-scores predicted intoxication frequency, though less strongly than ADsx (GW-scores: $R^2 = .000$ - $.008$; DHS-scores: $R^2 = .000$ - $.004$).

Table 1

Descriptive statistics for FinnTwin12 sample

Measure	N / Mean	% / SD	Range	
Alcohol dependence symptoms	1.03	1.31	0	7
Frequency of intoxication (days per month)	1.52	1.79	0	30
Height (cm)	172	9.38	145	207
In a Romantic Relationship	664	58.0%	-	-
Relationship lasting > 1 year	567	49.4%	-	-

Note. All percentages based on valid responses.

Table 2

Linear mixed-effects models for alcohol dependence symptoms (natural log transformed) across two GW and DHS polygenic score thresholds (*N* = 1,098)

	GW scores					
Threshold	Beta	95% CI	P	Pseudo-R^2	SNP Count	Per-SNP R^2
P<.01	0.0006	0.0003 - 0.0010	0.0003	0.011	2,221	4.96E-06
P<.05	0.0003	0.0001 - 0.0006	0.0006	0.010	10,694	9.57E-07
	DHS scores					
Threshold	Beta	95% CI	P	Pseudo-R^2	SNP Count	Per-SNP R^2
P<.01	0.0008	0.0001 - 0.0014	0.0094	0.005	789	6.76E-06
P<.05	0.0004	0.00001 - 0.0008	0.0220	0.004	3,947	1.03E-06

Notes. All models include sex as a covariate. Pseudo-R^2 calculated using the method outlined by Nakagawa et al. (2013). Abbreviations: GW, genome-wide; DHS, DNase I hypersensitive sites.

Table 3

Linear mixed-effects models for height across two GW and DHS polygenic score thresholds (N = 1151)

	GW scores					
Threshold	Beta	95% CI	P	Pseudo-R^2	SNP Count	Per-SNP R^2
P<.01	0.0028	0.0024 - 0.0031	1.15E-47	0.086	15,239	5.63E-06
P<.05	0.0025	0.0022 - 0.0029	2.88E-48	0.089	31,358	2.85E-06
	DHS scores					
Threshold	Beta	95% CI	P	Pseudo-R^2	SNP Count	Per-SNP R^2
P<.01	0.0042	0.0036 - 0.0049	1.24E-37	0.064	6,918	9.27E-06
P<.05	0.0040	0.0035 - 0.0046	2.98E-39	0.069	13,593	5.05E-06

Notes. All models include age and sex as covariates. Pseudo-R^2 calculated using the method outlined by Nakagawa et al. (2013).
Abbreviations: GW, genome-wide; DHS, DNase I hypersensitive sites.