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Effect of Homozygous Deletions at 22q13.1 on Alcohol Dependence Severity and Cue-Elicited BOLD Response in the Precuneus

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Abstract

Copy number variations (CNV) can alter the DNA sequence in blocks ranging from kilobases to megabases, involving more total nucleotides than single nucleotide polymorphisms. Yet, its impact in humans is far from fully understood. In this study, we investigate the relationship of genome wide CNVs with brain function elicited by an alcohol cue in 300 participants with alcohol use disorders. First, we extracted refined neurobiological phenotypes, the brain responses to an alcohol cue versus a juice cue in the precuneus, thalamus, and anterior cingulate cortex (ACC). Then, we correlated the CNVs with incidence frequency >1% to the neurobiological phenotypes. One CNV region at 22q13.1 was identified to be associated with alcohol dependence severity and the brain response to alcohol cues. Specifically, the 22k base-pair homozygous deletion at 22q13.1 affects genes APOBEC3a and APOBEC3b. Carriers of this homozygous deletion show a significantly higher score in the alcohol dependence severity ($p < 0.05$) and increased response to alcohol cues in the precuneus ($p < 10^{-12}$) than other participants. Tests of a mediation model indicate that the precuneus mediates the association between the homozygous deletions and alcohol dependence severity. Interestingly, the precuneus is not only anatomically and functionally connected to the ACC and thalamus (the main active regions to the alcohol cue), but also has the most predictive power to the alcohol dependence severity. These findings suggest that the homozygous deletion at 22q13.1 may have an important impact on the function of the precuneus with downstream implications for alcohol dependence.

Keywords

Alcohol use disorders; copy number variation; homozygous deletion; neurobiological phenotype; 22q13.1; precuneus; alcohol dependence

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1. Introduction

DNA copy number variation (CNV) is a large scale structural variation of DNA sequence, mainly caused by segmental deletion or insertion ranging from kilobases to several megabases. It accounts for most of the nucleotide sequence variation, involving up to 13% of the whole genome (Stankiewicz and Lupski, 2010). Reports have suggested that CNVs make a substantial contribution to the mechanisms underlying disease susceptibility and have already been associated with a wide range of diseases affecting multiple body systems, including neurodevelopmental disorders such as autism spectrum disorders (Pinto et al., 2010), bipolar disorder (Wilson et al., 2006), schizophrenia (Kirov et al., 2009; Walsh et al., 2008), Alzheimer's disease (Rovelet-Lecrux et al., 2006), and immunity, inflammation and rheumatoid arthritis (McKinney et al., 2008; Yang et al., 2007b). Despite such promising results, CNVs are still mainly understudied, especially with respect to their potential effect on substance abuse, in particular alcohol abuse. In this study, we examine the potential effect of CNVs as one specific type of genetic variations on alcohol use disorders (AUD).

The genetic influence on AUD has been studied for decades and abundant evidence has shown that genetic factors play a very important role (Dick et al., 2006; Heath et al., 1997). Candidate gene studies have established a number of associations between alcohol abuse and specific genes, which include the mu opiate receptor gene (*OPRM1*) (Filbey et al., 2008; Ray and Hutchison, 2004), the dopamine receptor D2 gene (*DRD2*) (Yang et al., 2007a), and the GABA receptor genes (*GABRG1* and *GABRA2*) (Enoch et al., 2009; Ray and Hutchison, 2009). A recent genomic SNP study identified fifteen SNP loci strongly related to alcohol dependence in a large sample (The Study of Addiction: Genetics and Environment), but the findings failed to replicate in other samples (Bierut et al., 2010). To an extent this is consistent with the model whereby multiple genes of small effect contribute to the vulnerability to alcohol dependence (Bierut et al., 2010). As large segmental variations, CNVs could alter multiple genes and loci and influence many aspects of gene function; thus, it may play an important role in conferring genetic susceptibility from diverse risk factors.

Despite the progress in identifying genetic susceptibility in AUD, studies have yet to identify the specific neurobiological mechanisms that more directly influence the development of AUD. To address the concerns, brain-based phenotypes related to alcohol dependence have emerged recently, which provide a translational framework that can be used to tightly link genetic variants to changes in neuronal function, and ultimately to changes in the clinical presentation of AUD (Hutchison, 2010). One of these brain-based phenotypes is derived from functional magnetic resonance imaging (fMRI) measured while exposing individuals to the taste of alcohol (Claus et al., 2011; Wrase et al., 2002). Consistent reliable results have reported that altered brain function in regions including the ventral tegmental area, striatum, precuneus, and prefrontal cortex is associated with severity of alcohol dependence (Filbey et al., 2008; Myrick et al., 2008; Tapert et al., 2004). Following this idea we utilize fMRI data elicited by an alcohol cue as the neurobiological phenotype to investigate the CNVs effect on brain function. More importantly, to further probe the underlying mechanism from the genetic variation to the abnormal behavior, we analyze a three-way association among CNV variations, brain function and alcohol dependence assessments, and hypothesize that brain function serves as a mediator, relaying the genetic variation impact to the downstream behavioral changes.

As for CNVs, to date, most findings are related to the effects of deletions, either from individual microdeletions or from the total deletion burden. For instance, deletions in regions of 1q21, 15q11, 15q13 and 22q11 have been identified to be associated with the risk of schizophrenia (Bassett et al., 2010), and some have been more broadly associated with

neurodegenerative disorders (Guilmatre et al., 2009). The CNV burden across the genome measured by the total CNV numbers and base pairs is elevated in patients with autism spectrum disorders and schizophrenia (Pinto et al., 2010; Walsh et al., 2008). The total number of rare deletions, in particular, has also associated with intelligence (Yeo et al., 2011).

Given that little is known about CNVs in the alcohol dependent population, we conducted a genome wide association study on CNVs extracted from high-density genotyping data from the Illumina Human 1M-duo assay. We specifically focused on CNVs and deletions with frequency >1%. To investigate the CNV effect, fMRI images reflecting the differential response to the taste of alcohol versus juice were used to extract neurobiological phenotypes to associate with CNVs. We hypothesized that the CNV effect on AUD is first manifested on the brain function, and then alters the downstream behavior.

2. Materials and Methods

2.1 Subjects and experiments

The study to investigate genetic effects on alcohol dependence was conducted according to the principles expressed in the declaration of Helsinki, approved by the institutional review board of University of New Mexico. Subjects between age 21 and 55 (right handed) with a minimum alcohol consumption of a regular pattern of binge drinking (5 or more drinks per episode for men, 4 or more drinks for women; these binge drinking episodes had to occur at least 5 times within the past month), otherwise healthy (no history of severe brain injury or brain related medical problems, no symptoms of psychosis during a diagnostic interview), were recruited (Claus et al., 2011). Three hundred and twenty-six participants provided written informed consent for the collection of samples and subsequent analyses. 61% of participants were diagnosed with alcohol dependence, 80% with alcohol abuse at the examining time. 70% had alcohol dependence and 93% had alcohol abuse at the life time. The demographic information and alcohol dependence level were assessed through self-report questionnaires during the interview session. Multiple alcohol dependence assessments were conducted, including the alcohol use disorder identification test (AUDIT) (Babor et al., 2006), alcohol dependence score (ADS) (Skinner and Allen, 1982), impaired control scale (ICS) (Heather et al., 1993), frequency of drinking and total drinking amount in the last 60 days, etc. We list 12 related variables used in the study in Table 1. All participants also provided saliva for DNA extraction for genotyping, and underwent a functional MRI scan. Good quality CNV data (from 305 participants) and fMRI images were obtained from 300 participants, including 90 females (mean age 32.37 ± 10.52) and 210 males (mean age 31.53 ± 9.26). Self-reported ethnicity was the following: Caucasian (148), Latino (79), African American (5), Asian (2), Native American (14) and mixed group (52).

It is important to note that the main effects of the alcohol vs. juice cue and associations with individual assessments of alcohol use for this sample were reported previously (Claus et al., 2011). The primary focus of this report is the effect of CNV on these measures.

2.2 Genotyping and CNV detection

Participants were instructed to deliver 5 ml of saliva into a sterile 50 ml conical centrifuge tube. DNA was then extracted, purified and ready for genotyping based on Illumina Human 1M-duo assay recommendations. 1,199,187 loci were genotyped and the intensity (LRR) and beta allele frequency values of loci in autosomes were used to identify CNVs. The details of CNV detection were described by Chen et al. (Chen et al., 2011). Briefly, outlier correction and principal component correction were first performed to eliminate variation induced by undesirable factors such as GC-content and DNA quality. Furthermore, one ethnicity related factor was also corrected in the LRR data to eliminate the influence of

population structure on CNV calls. Quality control based on LRR standard deviation (< 0.28) was then applied, which disqualified 21 subjects for further analyses. A circular binary segmentation algorithm and a hidden markov model algorithm (PennCNV: <http://www.openbioinformatics.org/penncnv/>) (Wang et al., 2007) were implemented to detect CNVs independently. Only CNV segments (telomere and centromere regions excluded) detected by both algorithms went through a quantitative check on the segment mean compared with neighbor LRRs, so that the final calls about copy number were made. If two CNVs from different subjects overlapped or the distance between CNVs was less than 3 markers (averaged 3kbp), we treated them as a common CNV region. The region with $>1\%$ CNV incidence frequency among all subjects was defined as a frequent CNV region. In this study we focus on the effect of frequent CNVs on alcohol dependence, so that only frequent CNVs were tested. At each frequent CNV region, subjects can have gain (insertion), neutral (normal), and loss (deletion) status. Additionally, we separated homozygous deletions (deletion at both alleles) from all other status.

2.3 fMRI data collected during the alcohol craving task

A well established alcohol cue exposure paradigm was used in this study to assess brain functional changes related to craving for alcohol (Claus et al., 2011). Participants were exposed to the taste of alcohol (subject's preferred alcoholic beverage) versus the taste of a control (litchi juice). fMRI images were collected during pseudo-randomized alcohol and control taste trials. Each trial consisted of a delivery period, followed by a washout period to allow the liquid taste to dissipate before the next trial. A Siemens trio 3T MRI scanner was used with configuration of TR=2s, TE=29ms, flip angle=75°, voxel size = 3.75mm $\times$ 3.75mm $\times$ 4.55 mm. The contrast images between alcohol trials versus control trials were extracted using FSL (<http://www.fmrib.ox.ac.uk/fsl/>) (Smith et al., 2004), after standard motion correction, normalization to the Montreal Neurological Institute space and smoothing with Gaussian model preprocessing steps. Three hundred subjects provided good quality fMRI data (at base line assessment) and their contrast images were admitted for CNV and brain activation association analyses.

2.4 Association and the mediation model tests

To fully investigate the relationship among the CNV, brain activation and drinking behavior, we examined all three pairs of associations, and tested a mediation model whereby the brain function mediates the effect of CNVs on drinking behavior. The analyzing steps are dissected into the following:

1. Due to the co-linearity among all the drinking assessments, we extracted the main factor using principal component analysis (PCA), presenting the overall alcohol dependence severity. PCA is a data decomposition procedure that uses an orthogonal transformation to convert a set of observations of possibly related variables into a set of uncorrelated variables called principal components. It can be described in a generic form as $S = W \cdot X$, where S is the extracted component matrix, X is the original measurement matrix, and W is the projection matrix. Each principal component (or called factor) is a linear combination (weighted sum) of multiple variables initially correlated to present a specific aspect of data. Principal components are ranked by the variance explained with the 1st component explaining the highest data variance. In this study, the 1st principal factor extracted from the 12 related alcohol use assessments explains 76% of total variance, mainly contributed to by ADS-loss of behavior control, AUDIT total score, ICS-failed control (ICS-fc), and ICS-perceived control (ICS-pc). The contribution weights from each assessment are plotted in Figure 2a, where ICS-fc and ICS-pc negatively contribute reflecting that the more severe the alcohol dependence is, the less

successful the control of drinking and the perceived control of drinking are. It can be seen that this factor emphasizes the overall level of impaired control over alcohol use. While average alcohol consumption contributes to the factor score, but the alcohol dependence severity scores clearly contribute more. Thus, we define this factor as an indicator of alcohol dependence severity rather than drinking severity. This severity score is normalized with zero mean ranging from -29.18 to 38.13 and median as -0.26. The other 11 factors each explained from 7% to 0.01% of total variance.

2. We employed a whole brain voxel-wise correlation test on the fMRI contrast images and the alcohol dependence severity factor. A 5% false discovery rate (FDR) multiple comparison control was used to identify voxels significantly related to alcohol dependence severity, which then became the neurobiological phenotypes in this study.
3. To test the association of the CNVs with the alcohol dependence severity and brain function, we first used ANOVA to find the CNV regions showing modulation on the alcohol dependence severity. The CNV status (gain, neutral and loss) and homozygous deletion status were tested separately. For the CNVs modulating the alcohol dependence severity with $p < 0.05$ (uncorrected), we further tested their connections with brain function using the voxel-wise correlation test on contrast images (5% FDR correction, > 1000 voxels to eliminate sparse results). If the CNVs correlate with brain function in the voxels overlapping with the neurobiological phenotypes (overlapping > 100 voxels), then a three way connection was established.
4. To test the mediation effect, the CNV regions showing correlations with neurobiological phenotypes were first selected as the predictor. The mediator is the brain activation linked to the CNVs and the alcohol dependence severity, and the outcome variable is alcohol dependence severity. The mediation effect determines whether a significant portion of the relation between predictor and outcome is altered after introducing the mediator. As illustrated in Figure 1, the mediating effect is measured by $a \times b$, where a is the coefficient assessing directly the relation between the CNV and brain activation, b is the coefficient assessing the relation between the brain activation and alcohol dependence severity with the CNV considered. The coefficient between the CNV regions and alcohol dependence severity was assessed with and without considering the brain function. We utilized a bootstrapping method (Preacher and Hayes, 2004) to test the mean mediation effect and 95% confidence interval.
5. The lump sum features of CNVs, including total CNV number, total deletion number, total homozygous deletion number, and total insertion number, were also tested using steps 3 and 4.

3. Results

3.1. Total CNV regions identified

From 305 subjects' genome wide allelic intensity data, we identified a total of 5,229 CNV calls using a conservative CNV detection pipeline proposed by our group (Chen et al., 2011). Among them, we found 253 regions with CNV incidence frequency greater than 1%, termed frequent CNV regions. The information of the total CNVs and frequent CNVs is listed in Table 2, where we present the number of CNVs and the size of CNVs in base pairs (bp). The detected CNVs range from 514 bp to 2,978,538 bp, with 0.4% larger than 500K bp. Note that 28% of CNVs are rare, reflecting the high prevalence of rare CNVs. But in this study we focus on validating the hypothesis of common variation-common disorders for

alcohol use disorders. Thus, only the 253 frequent CNV regions (see supporting table 1 for details of the 253 CNV regions) are investigated. The related results are reported hereafter.

3.2 Drinking behavior and the neurobiological phenotypes

The 1st principal factor extracted by PCA from the 12 related alcohol use assessments presents the main alcohol dependence severity score. Since much more males exist in our sample, we further tested whether this severity factor is confounded by gender. The two-sample T-test on males vs. females showed that females and males demonstrate the same level of alcohol dependence severity ($p=0.83$), which can be ascribed to our strict subject recruitment criterion, targeting subjects with consistent heavy drinking, defined separately for males and females.

The neurobiological phenotypes were identified by the voxel-wise correlation test on fMRI data and the alcohol dependence severity. The results are consistent with a previous analysis of these data that utilized each assessment score separately (Claus et al., 2011). Among the whole brain, 21,772 voxels in the region plotted in Figure 2b show significant correlations with the alcohol dependence severity level, after the 5% FDR multiple comparison correction. The correlation between these voxels and the alcohol dependence severity are positive from 0.15 to 0.30 (p -value from 8×10^{-3} to 1×10^{-7}). This significant positive relation indicates that the amount of increased response to the alcohol cue versus juice cue in these voxels is proportional to the subjects' alcohol dependence severity; *i.e.*, the more severe a subject's alcohol dependence is, the higher a subject's response to alcohol vs. juice. The top three regions are precuneus, thalamus, and anterior cingulate cortex (ACC) ordered by the correlation strength. It is important to note that precuneus is the strongest predictor of alcohol dependence severity.

3.3 The association of CNVs with the alcohol dependence and neurobiological phenotypes

For each CNV region, we studied two aspects: 1) copy number state (gain, neutral and loss), and 2) homozygous deletion only. First we identified the CNVs connected with the alcohol dependence level, and then tested these CNV associations with neurobiological phenotypes. The copy number state in 14 CNV regions demonstrated possible correlations with the alcohol dependence severity ($p<0.05$, not significant after correction), but none demonstrated a correlation with the brain-based phenotypes after FDR control. The homozygous deletion status at 4 CNV regions demonstrated possible correlations ($p<0.05$ uncorrected) to the alcohol dependence severity, and one CNV region at 22q13.1 (chromosome position 37,693,776 to 37,716,058) demonstrated a significant correlation with 10,203 voxels of the brain (mainly in the precuneus and ACC). Figure 3 shows the property of this CNV region, which includes 6 cases of homozygous deletion (5 Latinos and 1 mixed), 5 cases of hemizygous deletion (2 Caucasians, 2 Latinos and 1 Native American), and 5 cases of insertion (3 Caucasians, 1 Latino and 1 mixed) in 300 subjects. Even though each CNV case may have different starting and ending positions, they overlap with one another and are classified into one CNV region, as shown in Figure 3a. This CNV region encompasses 22k base pair nucleotides and two genes, APOBEC3A and APOBEC3B, where structural variations (deletions and insertions) have been previously reported in many studies (Conrad et al., 2010; McKernan et al., 2009; Park et al., 2010). The brain activations mainly from the precuneus and ACC, mapped in Figure 3b, are correlated with the homozygous deletions at this CNV region with the maximum correlation of -0.39 ($p<1 \times 10^{-12}$, passing Bonferroni correction on 253 CNV regions). Using the voxel in the precuneus with the strongest correlation as an example, the activation (t score in alcohol vs. juice contrast images) among 6 homozygous deletion carriers is about 57.10 ± 57.46 with a median of 29.90, while the activation of others is 1.47 ± 16.89 with a median of 0.57. It can be seen that homozygous deletions at 22q13.1 predict a significant increase in the brain

activation responding to the alcohol versus juice cue. Figure 3c shows the connection of homozygous deletion status with the alcohol dependence severity, where subjects carrying homozygous deletion at the 22q13.1 present higher alcohol dependence compared to others ($p = 0.04$).

Because 5 out of 6 homozygous deletions occur in Latinos, we performed additional association test on homozygous deletions at 22q13.1 with neurobiological phenotypes using only the 79 Latinos in the sample. The results showed that homozygous deletions among 79 Latinos are still significantly associated with 9,807 voxels in the brain, with the maximum correlation of -0.58 ($p = 1.69 \times 10^{-8}$) in the precuneus region.

3.4 The mediation model of CNV, brain function and drinking behavior

Based on the association results, individuals carrying homo-deletions at 22q13.1 manifested increased activation to the alcohol cue and reported higher alcohol dependence. Consequently, we want to confirm whether the brain activation serves as a mediator directing the effect of the homozygous deletions on the alcohol dependence severity. The full mediation model is shown in Figure 1 using the precuneus region as the mediator (the strongest correlated voxel; the highlighted precuneus region in Figure 1 shows very homogenous functionality, which makes it reasonable to use a single voxel). We used the bootstrapping method proposed by Preacher and Hayes (Preacher and Hayes, 2004) to test the mediation effect, and found that the mean mediating effect is negative and significant ($a \times b = -7.38$), with a 95% confidential interval excluding zero (from -14.48 to -2.77). The rest of the effect, c is also negative but not significant ($c = -5.12$, 95% confidence interval from -13.68 to 6.23). The whole model accounts for 8% of the variance in the alcohol dependence severity, with 7% accounted for through brain function in the precuneus and 1% through the CNV directly or unknown mediators.

3.5 Lump sum effect of CNVs on the alcohol dependence and brain-based phenotypes

The lump sum effect of CNVs was tested using the total CNV number, total deletion number and total homozygous deletion number, and total insertion number. Only the total homozygous deletion showed significant correlation with the alcohol dependence severity with $p < 0.01$; *i.e.* more homozygous deletions were associated with severer alcohol dependence. However, no association with the neurobiological phenotypes was identified.

4. Discussions and Conclusion

Recent publications have suggested that rare CNVs ($< 1\%$) are associated with neurobiological disorders, and have reported important results about genetic risk for schizophrenia, autism, bipolar, etc. However, it is entirely possible that CNVs with frequencies greater than 1% influence disorders like alcohol abuse and dependence, given a prevalence of 17.8% for lifetime alcohol abuse, 4.7% for 12-month alcohol abuse, and 12.5% for lifetime alcohol dependence and 3.8% for 12-month alcohol dependence in United States (Hasin et al., 2007). As noted in the Table 2, the frequency of CNVs varies from $< 1\%$ to $> 50\%$. We focused on CNVs with frequency $> 1\%$. Additionally, we acknowledge that other types of genetic variation influence the susceptibility to alcohol use disorders, and hypothesize that different types of mutations (such as SNP and CNV) affecting the same genes might both predispose the mutation carriers to high risk for these disorders. Therefore, the CNVs' effect on alcohol use disorders reported here is not exclusive.

In this sample, the deletion to insertion ratio is 3:2. After we scrutinized the CNV data, we observed that the difference of the allelic intensity value between insertions and normal states is much less than that between deletions and normal states (3.44 for homozygous

deletion and 0.48 for hemizygous deletion versus 0.33 for single duplication and 0.69 for double duplication). It, on the one hand, reflects the difficulty in discovering insertions reported in other studies (Conrad et al.; Redon et al., 2006), and on the other hand, suggests that the method used in this study partially mitigates the difficulty. Despite less total insertion calls, high CNV incidence regions mostly resulted from high incidence of insertions. The median length of insertions, 44Kbp, is much larger than the median length of deletions (19Kbp), consistent with the observation that most of the large CNVs are insertions and that there were no homozygous deletion greater than 500Kbp. All together, the high frequency and large size of insertions imply that insertions in general have less deleterious effects than deletions, thus bear less selective pressure. In contrast, deletions are more interesting in terms of the potential effects on brain and behavior.

The neurobiological phenotypes are not only elicited by alcohol vs. juice cues, but also significantly related to alcohol dependence severity. Among the three top regions, thalamus and ACC are part of reward system, regulating and reinforcing the response to stimuli including pleasure. However, consistent with a previous analysis of these data (Claus et al., 2011), the precuneus was most significantly correlated with alcohol dependence severity level, even stronger than the thalamus and ACC. These results make the precuneus of particular interest. Situated in the superior-postero-medial portion of the parietal lobe, the precuneus has connections to cortical areas (adjacent parietal cortex, frontal cortex and ACC) and to subcortical areas (thalamus, striatum, claustrum and brainstem) as reviewed by Cavanna and Trimble (Cavanna and Trimble, 2006). Functionally, it is the region to integrate external and internal information and contributes to high order cognitive functioning. Even though the precuneus is seldom a targeted region in alcohol use disorder imaging studies, results from these studies consistently show that precuneus is related to alcohol craving status (Park et al., 2007), to alcohol use quantity (Tapert et al., 2003), and to alcohol sensation (Bragulat et al., 2008).

From the CNV association tests, we identified that homozygous deletions at 22q13.1 that correlate with the neurobiological phenotype, as well as alcohol dependence severity level. The fact that the CNVs are more strongly correlated to brain function ($p < 1.98 \times 10^{-4}$, the 5% Bonferroni correction threshold) than drinking scores ($p < 0.05$ uncorrected, yet $p > 1.98 \times 10^{-4}$ Bonferroni correction), suggests that neurobiological phenotypes provide more statistical power, likely because they are intermediate between behavior and genetics (Hutchison, 2010). The deletion effects on the neurobiological phenotypes were true even in a subset of sample with 79 Latinos. As shown in Figure 2a, the CNVs in this region have slightly different beginning and ending positions, but all together they influence the genes APOBEC3A and APOBEC3B. These two genes are members of the cytidine deaminase gene family. This family plays a role in innate cellular immunity by inhibiting retroviral infection, hepatitis B virus propagation, and the retrotransposition of endogenous elements, where clear evidence can be found from specific studies on HIV-1, hepatitis B virus, and retroviral inhibition (Doehle et al., 2005; Esnault et al., 2006; Suspene et al., 2005).

More importantly, structural variations occurring at this region have been reported in many previous studies (Conrad et al., 2010; McKernan et al., 2009; Park et al., 2010), further supporting a functional role of this CNV. In particular, Kidd et al. have investigated the population stratification on the deletions at this region, and reported Africans and Europeans have frequencies of 0.9% and 6%, and it is more common in East Asians (Kidd et al., 2007). This reported frequency is compatible with what we observed (5% frequency) in our sample, comprised mostly of Caucasians and Latinos. How the homozygous deletions at this region pathologically impact the brain function will require further investigation, but others have encountered deletions in this general region, 22q11-13, for multiple neuropsychiatric disorders. For example the 22q12.2 deletion syndrome is associated with a 3-Mbp

microdeletion, and 25% of patients with 22q12.1 deletion have psychiatric disorders that include schizophrenia, attention-deficit hyperactivity disorder and autism spectrum disorders (Cook and Scherer, 2008). Another microdeletion at 22q13 causes the Phelan-McDermid Syndrome, and is also associated with autism spectrum disorders (Durand et al., 2007). The deletions we found in this study only affect 22Kbp, which might explain the less severe effect and high frequency.

Since carriers of homozygous deletions at 22q13.1 present increased activation in the precuneus during the presentation of an alcohol cue, and report a high score in the alcohol dependence severity, we tested whether the precuneus serves as a mediator between the CNVs and the behavior. The mediation model test confirmed our hypothesis that neural responses significantly mediate relationships between CNV markers and alcohol dependence severity. When not considering the brain mediation effect, 1% of the variance in alcohol dependence severity was explained by the homozygous deletions; after introducing brain function as a mediator, 8% was explained. This finding suggests that homozygous deletions can influence behavior through brain function. Additional tests on lump sum features of CNVs show that the total number of homozygous deletions carried by individuals was correlated to the individual alcohol dependence severity level, but not brain function. It suggests that overall homozygous deletion burden can influence behavior but may not be specific to any brain function. Nevertheless, all these findings about homozygous deletions are in fact encouraging to us, since they are in line with the most recent reports of genetic structural variations, where frequent homozygous deletions have been related to various diseases (Abbaszadegan et al., 2011; Gill et al., 2011; Van Ziffle et al., 2011).

Despite the strong evidence in our results, little is known about the underlying mechanism by which homozygous deletions at 22q13.1 influence brain function. Specific studies focusing on gene function and regulation in 22q13.1 region at the cellular and molecular levels are crucial. In this study we only investigated the CNV effect from the genotype-phenotype association viewpoint, the findings of which provide direction for further in-depth studies.

In summary, we scanned genome wide frequent CNV regions (>1%) in attempt to identify their associations with drinking related brain function and behaviors. We found that deletions, in particular homozygous deletions, more likely affect behavior. One CNV region at 22q13.1 demonstrated that homozygous deletions are significantly correlated with brain function in the precuneus and the alcohol dependence severity. A further mediation model test confirms that the precuneus serves as the mediator between the CNVs and alcohol dependence. Our findings not only support the relationship between the precuneus and the alcohol use disorders, but also suggest that the frequent CNVs, in particular homozygous deletions, can be partially responsible for it. The results prove the concept that frequent CNVs can influence behaviors through brain function, in particular alcohol use disorders. It is important to address that despite the strong results from the three-way connections and mediation model, a large sample replication test is necessary due to the relatively low frequency of the CNVs (e.g. only 6 subjects have homozygous deletions in CNVs at 22q13.1). Given the population stratification on deletions at 22q13.1 (Kidd et al., 2007), large samples from a homogenous ethnic cohort is desired to investigate the specific effect of homozygous deletions at 22q13.1 in the future.

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Figure 1.

The mediation model among CNV, brain function and behavior. In the mediation model, CNV is the predictor, brain function is the mediator and alcohol dependence level is the outcome variable. The mediation effect is measured by $a \times b$, and the direct effect from the predictor to outcome is measured by c . Our data show that brain function in the precuneus (yellow highlighted region) mediates the effect from homozygous deletion at 22q13.1 to the alcohol dependence severity, where 7% of variance is explained through mediator, and 1% is directly from the CNV.

Figure 2.

Alcohol use phenotype and neurobiological phenotype. a) The main alcohol dependence severity level comprising mainly ADS-con, AUDIT total score, ICS-fc and ICS-pc. b) Brain regions are significantly, and positively correlated to the alcohol dependence severity, passing 0.05 FDR control. The top three regions are precuneus, thalamus and ACC, ordered by the correlation strength. The activation of these regions comprises the neurobiological phenotypes.

Figure 3.

The CNVs at 22q13.1 and their association with brain function and alcohol dependence severity. a) The distribution of the CNVs in this region includes 6 homozygous deletions in brown, 5 single deletions in red, 5 insertions in green and 289 neutral in blue. This region codes for many genes close to one other, the main affected gene is CTH4. b) The brain regions correlated with the CNVs in this region, mostly in the precuneus with the minimum p value < 1E-12. c) The modulation of homozygous deletion status on alcohol dependence severity level. For each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually as cross markers.

Table 1
Categories of alcohol dependence assessments

AUDIT	ADS	ICS
AUDIT-1: How often do you have a drink containing alcohol?	ADS-con: Loss of behavior control	ICS-ac: Attempted control
AUDIT-2: How many drinks do you have on a typical day when you are drinking?	ADS-ods: Obsessive drinking style ADS-per: Psychoperceptual withdrawal	ICS-fc: Failed control ICS-pc: Perceived control
AUDIT-3: How often do you have 6 or more drinks on one occasion?	ADS-phy: Psychophysical withdrawal	Avg. Drinking:
AUDIT-tot: Total raw score from 10 questions.		Average number of drinks per drinking day

Table 2
Statistics of the total CNVs and frequent CNVs

	Total CNV statistics	Frequent CNVs
Deletion/insertion calls	3009/2220	1937/1850 CNV calls from 253 regions
length (bp)	514bp (smallest), 2,978,538 bp (largest) 19Kbp (median deletion), 44Kbp (median insertion), 21 CNVs > 500Kbp (5 deletions and 16 insertions)	514bp to 2,978,538bp, 19Kbp (median deletion), 43Kbp (median insertion), 15 CNVs > 500Kbp (2 deletions and 13 insertions)
Frequency	min (1/305), max(55%), median (4%)	min (1%), max (55%), median (10%)
CNV per subject	min (2), max (70), median (19)	min (0), max (40), median (12)
Homozygous deletions	1043, median size of 17Kbp, no CNV > 500Kbp	986, median size 17Kbp, no CNV > 500Kbp