

## A genomic approach to alcoholism

### *Un abordaje genómico del alcoholismo*

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Genomic technologies based on DNA chips (microarrays) permit the simultaneous analysis of hundreds of thousands of single nucleotide polymorphisms (SNP), and this has revolutionized case-control association studies. Research has moved on from studies of candidate genes, in accordance with previous etiopathogenic hypotheses, to genome-wide association studies (GWAS), which analyze the vast majority of the common variation throughout the genome. The impact of this on our knowledge about the genetic bases of predisposition to complex multifactorial diseases has been substantial.

Psychiatric disorders have not been immune to these advances, and this applies especially to schizophrenia, with 37,000 cases and 113,000 controls analyzed through GWAS by the Psychiatric Genomics Consortium (PGC) (Schizophrenia Working Group PGC, 2014). Even so, in the case of other psychiatric disorders, including alcohol dependence, the sample sizes of GWAS are much more modest, though the pioneering studies in schizophrenia are showing the potential contributions of GWAS, which are now being confirmed with other disorders. The most obvious of such contributions concerns the ability to detect common variants associated with the disorder under study,

employing the established criterion for genomic significance ( $p < 5 \times 10^{-8}$ , equivalent to a Bonferroni correction for a million tests). In alcohol dependence, GWAS have confirmed the already-known involvement of *ALDH2* or the *ADH* family, and have identified a few additional genes, such as *PECR* or *NKAIN1-SERINC2* (Frank et al., 2012; Treutlein et al., 2009; Zuo et al., 2013). Experience with other complex disorders indicates that these studies, which analyze fewer than 2000 patients and a similar number of controls in the GWAS phase, have very limited power for identifying vulnerability SNPs, since their individual effect is very small (odds ratio  $< 1.25$ ). Thus, in schizophrenia, we have moved on from identifying a maximum of three independent significant associations in analyses with sample sizes similar to those currently employed in alcohol dependence research, to 108 in the latest GWAS by the PGC, referred to above (Schizophrenia Working Group PGC, 2014; Stefansson et al., 2009). Hence, the significant results at the genome level represent just the tip of the iceberg. For example, the gene *SLC39A8*, initially associated with schizophrenia in a study led by our group and based on 4545 patients and 15,575 controls ( $p = 2.7 \times 10^{-6}$ ), reached the level of genomic significance in the PGC mega-GWAS ( $p = 7.98 \times 10^{-15}$ ) (Carrera et al., 2012; Schizophrenia Working Group PGC, 2014).

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Given that numerous variants of vulnerability fail to reach significance at the genome level, the next logical step would be the detection of groups of functionally related genes that are over-represented among the most significant values of a GWAS. By way of example, Biernacka et al. (2013) identified a possible association (pending confirmation in new samples) with the pathway of synthesis and degradation of ketone bodies. Excessive alcohol use can increase levels of ketone bodies in the blood, leading to alcoholic ketoacidosis. Ketoacidosis involves symptoms such as nausea, vomiting, or abdominal pain, which can cause aversion to heavy drinking.

In addition to advancing our understanding of the biological bases that predispose people to the onset of a disorder, GWAS could be applied for estimating individual genetic risk of developing a disorder. Such estimates have clear limitations, since they are based solely on common variants (though this may improve as new-generation sequencing studies begin to identify rare variants with larger effects). Moreover, genetic risk explains only a part of total risk. In the case of alcoholism, it is estimated that genetic factors explain 40-60% of populational variation in risk (heritability). But despite these limitations, the data for schizophrenia (heritability ~65%) suggest that the calculation of individual genetic risk from GWAS data may be useful for stratification of risk groups. Thus, the decile at highest risk presents an odds ratio of between 8 and 20 with reference to that of lowest risk (Schizophrenia Working Group PGC, 2014).

Currently, the estimate of polygenic risk is made in simple way. From the GWAS data of a discovery sample, a risk model is generated that includes all the independent SNPs below a lax significance threshold and their effect (logarithm of odds ratio). In each individual in a target sample, the number of risk alleles for each SNP of the model is count, weighted by their effect. The sum of this value for all the SNPs considered constitutes the polygenic risk score of a target individual. Frank et al. (2012) were the first to apply this method to alcohol dependence, based on 1333 cases and 2168 controls divided at random into discovery and target samples of equal size. Using a significance threshold of  $p < 0.5$  in the GWAS of the discovery sample (equivalent to analyzing the ~84.000 most significant independent SNPs), they found a difference in the polygenic risk score between cases and controls in the target sample in the expected direction ( $p = 1.28 \times 10^{-6}$ ). Considering the entire sample as a discovery sample, they also found significant differences when the target samples came from other, previous GWAS.

Among the SNPs included in the calculation of polygenic risk there will be both SNPs with real effects and SNPs unrelated to the disorder, which will generate noise. Thus, there is plenty of room for improvement in the estimate through increasing the size of the discovery sample, which would reduce sampling error in the construction of the polygenic risk model. Another option for improvement would involve

prioritizing the SNPs of the model according to additional, a priori biological criteria, such as prediction of functionality or results of GWAS in related phenotypes. Indeed, using discovery and target samples of different phenotypes, it has been possible to confirm shared genetic vulnerability (Cross-Disorder Group PGC, 2013). This type of analysis will improve our understanding of dual pathology.

Finally, the confirmation that there are persons more vulnerable to becoming drug-dependent from the biological point of view can be relevant both to the reduction of stigma associated with alcoholism and to adolescents' and young people's attitude to alcohol. Various studies have indicated a fair degree of interest in a potential genetic test for individual risk of presenting alcohol dependence, and that the belief in a high individual genetic predisposition constitutes an incentive for changing one's drinking pattern (Dar-Nimrod, Zuckerman, & Duberstein, 2013; Scott et al., 2014).

In sum, GWAS have shown that genetic predisposition to alcoholism is due to multiple genes with a very small individual effect. The study of these genes will have consequences in the medium-term, both increasing our understanding of the biological mechanisms involved in vulnerability to alcoholism, and permitting the stratification of individuals according to their genetic risk of suffering from alcoholism, and this represents an important step in the direction of selective prevention. For example, stratification of adolescents with a use pattern involving risk and/or problematic behaviours (delinquency, violence) based on genetic tests could facilitate more sharply focused interventions. Given the seriousness of the problem, with more than 25,000 people seeking treatment for alcoholism annually in Spain (according to the Spanish Observatory for Drugs and Substance Dependence), any approach that can make a difference should be welcomed. In such a context, work on drug dependence in the coming years should pay attention to the progress made through the use of genomic technologies, given their potential utility. For such potential to be unleashed it will be essential to employ multidisciplinary approaches.

## Conflicts of interest

The author declares that there are no conflicts of interest in relation to this work.

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