Gene–environment interactions and alcohol use and dependence: current status and future challenges

Carmen S. van der Zwaluw & Rutger C. M. E. Engels
Behavioural Science Institute, Radboud University Nijmegen, the Netherlands

ABSTRACT

Aim To discuss the current status of gene–environment interaction research with regard to alcohol use and dependence. Further, we highlight the difficulties concerning gene–environment studies.


Results Attention to the causative roles of gene–environment interactions in alcohol use and dependence is increasing. Studies with twin designs are beginning to examine gene-shared environment effects, and animal studies have investigated gene–environment interaction effects on alcohol intake in primates. Thirteen studies incorporated gene–environment interactions in examining alcohol use or dependence in humans. These studies held a variety of candidate genes and environmental risk factors and their heterogeneity made it impossible to draw firm general conclusions.

Conclusions Challenges for future gene–environment studies are abundant, and consist of, for example, the development of clear theoretical assumptions about neurobiological mechanisms and the recruitment of large longitudinal samples that already start in childhood. Replication is essential to prevent an overload of false-positive results. Despite the difficulties, it is crucial to include gene–environment interactions in future studies in order to unravel the aetiological factors of human alcohol outcomes.

Keywords Alcohol, dependence, environment, gene, interaction, replication.
affect behaviour; the focus of interest should shift towards the interactions between genes and environment [21]. That is, genetic effects on behaviour occur because they affect an individual’s susceptibility to adverse environments [22]. As such, adverse environments, such as negative or inadequate parental care, may create a risk, depending on genetic susceptibility factors [23,24]. A neo-classical example of a gene–environment interaction (G × E) linked to psychopathology comes from a long-term prospective study by Caspi et al. [25], showing that a functional polymorphism in the monoamine oxidase A (MAOA) gene was associated with later antisocial problems only if children were maltreated by their parents. It should be stressed that research groups who tried to replicate this finding published mixed results [26–30]. From Caspi et al.’s study, it may be concluded that attention should shift from the scientific study of environment or heritability to G × E effects, as has been stressed in the past decade by several scholars (e.g. [31–33]), and that the conditions under which genotypes are expressed in specific drinking behaviours should be examined [22].

With respect to the field of alcohol research, Heath & Nelson [34] pointed to two main reasons for the need to focus on G × E effects in genetic epidemiological research. First, a lack of attention to genetic effects in studies on environmental risk factors may lead to the wrong conclusions about the roles of specific environmental factors for alcohol use and dependence. For instance, if genetic factors are not taken into account, and findings show that peer drinking influences adolescents’ development of alcohol use, this might lead to the erroneous conclusion that adolescents model their peers’ behaviours through various social learning principles. However, it is possible that peer drinking has an effect only if adolescents have a genetic risk for drinking [35,36] and, furthermore, assortative mating with drinking peers may be partly genetically determined [37]. Secondly, studies examining exclusively genetic effects might underestimate the influence of specific genes if these effects are present, or strong and consistent, only under specific environmental circumstances. For instance, a polymorphism in the serotonin transporter gene, 5-HTTLPR, has been linked to alcohol sensitivity, alcohol consumption, frequency of intoxication and alcohol dependence (for a meta-analysis, see [17]). Nilsson et al. [38] showed that the effects of 5-HTTLPR genotypes on alcohol intoxication were particularly strong when adolescents reported poor family relations.

**G × E studies with behavioural genetic designs**

There have been few attempts to examine G × E effects with behavioural genetic designs, for example in twin samples. Heath and colleagues [39] found that alcohol consumption in women was affected by a genotype × marital status interaction; genetic factors were more pronounced in women who were not married. Koopmans et al. [40] found that religious upbringing reduced genetic effects on alcohol use initiation, especially in females. Rose et al. [41] showed that genetic factors had a larger influence on alcohol use when subjects lived in rural areas, compared to urban areas. Harden et al. [42] reported that effects of best friends’ tobacco or alcohol use were highest for adolescents who were genetically vulnerable, indicating a G × E effect. These studies have not yet been replicated. Reviews of G × E effects in twin designs can be found elsewhere [34,43].

**G × E studies in primates**

A renowned line of research deals with G × E effects and alcohol use in non-human primates, such as macaques, baboons and vervet monkeys. Barr and colleagues [44] tested interactions between a polymorphism in the serotonin transporter gene and environmental stressors on voluntary alcohol consumption in macaques. This polymorphism, named rh5-HTTLPR, is orthologous to the functional polymorphism in the serotonin transporter gene promoter in humans. Barr et al. provide evidence for the assumption that monkeys with the rh5-HTTLPR short allele, in particular, experiencing psychosocial stressors (i.e. growing up in parent-deprived, peer-only contexts), consumed high levels of alcohol [45,46]. Due to the high genetic similarity between primates and humans, primates could serve as subjects for research modelling aetiological factors of human alcohol outcomes [45], and in so doing showed G × E effects on alcohol intake.

**G × E studies in humans**

A PubMed search revealed a small number of studies (n = 13) that tested G × E effects on alcohol outcomes in humans. The keywords used were ‘gene’, ‘interaction’ and ‘alcohol’. Reference sections of the identified articles were used to find additional relevant studies. A detailed table of G × E studies in humans can be obtained from the first author. Bau et al. [47] and Madrid et al. [48] examined the TaqI A1 allele of the dopamine receptor D2 gene (DRD2) in relation to alcohol dependence. Both studies showed that individuals with the DRD2 A1 allele who also experienced high levels of stress reported higher levels of alcohol dependence, providing support for interaction effects between the DRD2 A1 allele and stress on alcohol dependence.

Several studies examined a polymorphism (5-HTTLPR) in the 5’ regulatory region of the serotonin transporter gene, and its interaction with a variety of environmental factors in relation to alcohol use, with contradictory results. Interactions were found between

---

© 2009 The Authors. Journal compilation © 2009 Society for the Study of Addiction

Addiction, 104, 907–914
5-HTTLPR s/s genotype and quality of family relations [37] and maltreatment [49] on (adolescent) alcohol use. Covault et al. [50] found the 5-HTTLPR s/s genotype to increase the risk for drinking in college students if they had experienced multiple negative life events, while Olssen et al. [51] found the 5-HTTLPR s/s genotype to protect against binge drinking if subjects showed a secure attachment style. In contrast, Dick and coworkers [52] and Gacek et al. [53] did not find an interaction between 5-HTTLPR genotype and stressful life events on alcohol dependence and binge drinking, respectively.

Three studies found interactions between a polymorphism in the promoter region of the monoamine oxidase A gene (MAOA-LPR) and alcohol use or dependence. Nilsson et al. [54] showed that maltreatment and quality of family relations interacted with the MAOA-LPR 3-repeat allele in predicting alcohol-related problem behaviour in males. This relationship was also present for females, although for only the MAOA-LPR 4-repeat allele [55]. Ducci and coworkers [56] found that an interaction between the MAOA-LPR 3-repeat allele and sexual abuse during childhood was associated with antisocial alcoholism, but not with alcoholism in general. Interactions between MAO-B haplotypes and sexual abuse were not significantly related to (antisocial) alcoholism.

Dick et al. [57] found an interaction between a single nucleotide polymorphism (SNP) in the GABRA2 gene (rs 279871) and marital status on alcohol dependence; married individuals with the GABRA2 A/A genotype were affected more often by alcohol dependence than were married individuals without the GABRA2 risk allele.

Finally, Blomeyer et al. [58] found that adolescents homozygous for the C allele of a SNP in the CRHR1 gene (rs 1876831) consumed more alcohol and showed more heavy drinking if they experienced more than three negative life events than adolescents carrying the T allele.

Current status of G × E studies in alcohol use and dependence

Although the above-mentioned studies provide new and exciting insights into the interplay between specific genotypes and environmental stressors on alcohol outcomes, their heterogeneity makes it difficult to compare them. Variations in terms of genes, environmental risk factors, phenotypes (alcohol use, alcohol-related problems and dependence measures), sample sizes and characteristics and study designs make it impossible to draw conclusions about patterns of findings across studies. Flint & Munafò [59] warn of the possibility that all significant G × E reports may be false positives, due mainly to the small sample sizes of most G × E studies. Although this is perhaps a little too pessimistic (for a more positive view, see [60]), it is clear that testing for G × E interactions brings with it many difficulties that, in turn, complicate independent replication of the above-stated findings, which is necessary to reduce the possibility of reporting false positives [61].

Challenges in G × E studies

First, specific genes and polymorphisms are often selected based on previous linkage studies in which the gene is related directly to the disorder. However, these studies frequently fail to find consistent associations between gene and phenotype (see e.g. [19,20]), making the choice for such a gene or polymorphism seem somewhat random. Genome-wide association studies may provide additional information and background on chromosomal areas related to alcohol dependence (e.g. [62,63]). Also, providing a plausible biological mechanism by which the (functional) polymorphism may affect behaviour and/or environment makes the choice for a particular gene more well founded [64] (for an example, see [25]). However, even if the selection of a particular gene seems justified by building upon previous research, biological mechanisms and genome-wide initiatives, there is another difficulty that applies specifically to G × E studies: the fact that we expect a G × E effect implies that a gene is—in some cases—related exclusively to alcohol use or dependence when certain environmental factors are present. As such, those genes that did not appear to be linked directly to alcohol use or dependence in linkage or genome-wide association studies might be ignored erroneously [32]. An additional problem that also plays a role in ‘plain’ genotype–phenotype association studies is the selection of one marker on one gene, implying that the marker itself is involved in predisposition to the disease, or that it is in linkage disequilibrium with markers involved in the disease. Testing several markers on one gene or creating haplotypes are ways to (partly) resolve this selection problem but these methods may, in turn, create difficulties of their own, such as multiple testing (e.g. [65,66]).

Secondly, according to Moffitt, Caspi & Rutter [32], it is essential to assess environmental risk factors precisely and reliably by using: (i) proximal measures of environmental pathogens, preferably not retrospectively; (ii) multi-informant data; (iii) developmental-specific assessments; and (iv) by noticing cumulative effects of environmental influences. Furthermore, it is important to realize that variations in exposure to environmental risk factors are, in many cases, influenced genetically (e.g. [31]). In the context of family influences, passive gene–environment correlations [67] refer to the fact that parental genes (that are passed on to a child) also affect the child’s environment. For example, it is likely that an adverse environmental factor for the child, such as bad parenting, is influenced by genetic variations in the
parents via, for example, personality traits [68] or psychopathology [33,69]. In addition, evocative (or active) gene–environment correlations refer to the case that the links between parental behaviours and child (or adult) outcome behaviours are caused (evoked) by the child’s genotype (see e.g. [70]). As such, shaping environment and selection of possible environmental risk factors is influenced by parental characteristics, as well as by the child’s characteristics. As Riley [71] states, it is imperative in G × E studies to exclude gene–environment correlations as the main source of the G × E effect (as was performed in, e.g. [72]). Another difficulty includes the different (quality of) measurements of environmental factors between studies. Using questionnaires that have proved to be highly reliable and valid may partly tackle this problem. In addition, some scholars (e.g. [73]) have argued that interactions may be artificial because of their scale dependence: transforming gene or behaviour variables can eliminate or create an interaction [59]. Moffitt et al. [32], for example, tested for scaling artefacts in the MAOA-maltreatment study [25] in several ways, and showed that it was unlikely that the interactions were due to artefacts of scaling. Naturally, researchers are encouraged to carry out similar tests in their future studies.

Thirdly, and theoretically, examining the combined effect of genes and environment increases statistical power if a subgroup of individuals with a particular (known) adverse environment and genotype can be investigated (see e.g. [74]). However, environmental and genetic involvements are often unknown, requiring more testing, thus causing a decrease in power. In addition, samples of the G × E studies on alcohol outcomes in humans were often small, which also reduces power. The appropriate sample size in G × E studies depends upon several factors, such as study design (case–control, case–sibling, case–parent), disease prevalence, exposure to the environmental factor, prevalence of the genetic risk allele, inheritance (dominant or recessive) and requested power. Gauderman [75] provides several examples, tables and a website to allow researchers to determine their required sample size following their specific design parameters. Ideally, to reduce the odds of finding false positives, scholars should compute the appropriate sample sizes for their intended study before starting it. However, one of the complicating factors includes the presumed knowledge of certain parameters, such as G × E effect size, which is often precisely the parameter that researchers are planning to study. Nevertheless, it is clear that large samples are needed to tackle power problems and to carry out reliable (replication) studies [76]. In addition, meta-analyses and systematic reviews can be applied to synthesize data [60].

Except for two reports [52,53], all the above-described G × E studies on alcohol use and dependence in humans reported significant interaction effects. This points to a publication bias in which journals generally have a tendency to publish significant, and therefore probably more interesting, findings. The magnitude of this so-called file drawer effect can hardly be established [77]. As a result of the tendency of many journals to publish only significant findings, it is probable that researchers will search for the best (significant) results in their data. As Flint & Munafo [59] point out, this ‘data dredging’, as they term it, risks flooding the literature with false positive findings that are hard to replicate and provide no additional insights. They also reject the fact that in some studies the failure to find an overall G × E effect is solved by creating subgroups in which the effect appears to operate. However, while pursuing significant findings without clear theoretical and biological assumptions is objectionable, it is also very likely that certain genetic factors affect only specific subgroups or subtypes of behaviour. The exclusion of important moderating factors may thus lead erroneously to the conclusion that there is no genotype–phenotype or G × E–phenotype association when there is in fact an association, although only when moderating variables are taken into account [78].

Fourthly, study samples differ strongly across studies, due to variations in inclusion criteria, sample procedures and sample characteristics. Some scholars over-sample children who are risk-prone, leading to variations in environmental risks (e.g. levels of abuse or domestic violence, divorce rates or proportion of children in deprived families) between samples. In addition, patients included in many case–control studies (in both genetic association studies and G × E studies) are often recruited from addiction medical clinics, hospitals or psychiatric wards. As the majority of alcohol-dependent individuals will not go into treatment centres or hospitals, this is likely to be a selective sample of alcohol-dependent individuals in the population. Therefore, it is possible that a sample selection bias occurs, making it difficult to generalize findings to the population level. For designs that avoid social selection effects or allocation biases, see Rutter [79]. Another sampling issue concerns the fact that allele frequencies differ between ethnic populations, which may cause population stratification [80]. This applies only to case–control designs, as family-controlled studies have an inherent control for population stratification [81], and as such are one of the solutions to handling population stratification. Another solution is to include ethnicity as a confounder in the analyses.

Fifthly, there is a need to adopt a developmental perspective. None of the above-described studies had a longitudinal design. As such, it is impossible to test whether G × E effects are causative factors for alcohol consumption or alcohol dependence, or to examine specific transitions in use or in transient stages of subclinical
symptoms. Developmental psychologists have stressed the significance of testing age-specific theoretical models [82], as explanatory factors may differ in magnitude across the life-span [83]. For example, the impact of family stress in childhood is likely to differ for the predictions of alcohol consumption in 16-year-olds compared to 66-year-olds. How expressions of genes in relation to alcohol use differ across the life-course, and whether specific G × E effects vary in influence at various stages, are essential issues to examine. Further, most molecular genetic studies on alcohol outcomes focus upon adult populations, and disregard early precursors of alcohol use and dependence. In his review, Rose [84] argued for behavioural childhood measures such as impulsivity, self-control, conduct problems and aggression in genetic studies on alcohol use, as they are early predictors of heavy alcohol use, alcohol-related problems and dependence later in life (see also empirical studies [85–87]). These behavioural problems are assumed to be strong mediators of genetic effects on alcohol dependence [88,89]. This underlines the call for longitudinal designs to test G × E effects across stages of use and age groups. In particular, prospective multi-informant family studies are needed in which several children within a nuclear family and their parents are followed from childhood to adulthood. As alcohol dependence symptoms and problem drinking emerge in early adulthood, studies need to cover a span of at least 15 years (cf. [34]).

Finally, classification of alcohol dependence is based regularly on DSM-IV categorization, but may be very variable in its clinical presentation. Moreover, for alcohol outcomes such as ‘heavy drinking’ there are no internationally applied standards, which increases heterogeneity between studies, and in turn diminishes replicability and generalizability. One way to avoid this is to focus upon biological, neurological and cognitive levels that are likely to mediate genetic effects on phenotypes. The concept of endophenotypes was applied first to psychiatric disorders by Gottesman & Shields [90], and a recent review provided an excellent overview of the rationale to use endophenotypes in gene identification efforts in psychiatry [91]. With regard to alcohol use and dependence a broad range of endophenotypes has been examined, such as alcohol metabolism [92], hormonal changes after consumption [93], psychomotor responses [94], craving [95,96], sensitivity to the effects of alcohol [15,97], electroencephalography (EEG) or event-related potential’s (ERP) [91,98,99], expectations of alcohol consumption [100,101] and learned responses to alcohol stimuli [102–104].

In conclusion, it has become clear that studies examining G × E effects on alcohol outcomes are only beginning to emerge. Although the difficulties that accompany G × E studies are abundant (see above), both environmental and genetic factors and their interplay need to be included in future studies to unravel aetiological factors of human behaviour reliably. Essential in so doing are clear theoretical assumptions about the neurobiological mechanism between genotype and outcome, and between genotype and environmental features. Very large longitudinal studies that already start in childhood are required to capture various G × E effects across the life-span. Clearly, replication is crucial (but until now almost non-existent) to prevent an overload of false-positive published G × E studies. The road to the implication of G × E studies in (clinical) practice is still long but, to quote Kaufman & Gelernter [105], ‘we are not discouraged by the failure to consistently replicate gene–environment interactions, rather extremely excited by the potential of new investigative techniques to study risk and resiliency across species’ (p. 545).

Declarations of interest
None.

Acknowledgement
This research was supported by the Dutch Organization for Scientific Research (no. 400-05-051).

References


