Alcohol, ALDH2, and Esophageal Cancer: A Meta-analysis Which Illustrates the Potentials and Limitations of a Mendelian Randomization Approach

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Abstract

Mendelian randomization, the use of common polymorphisms as surrogates for measuring exposure levels in epidemiologic studies, provides one method of assessing the causal nature of some environmental exposures. This can be illustrated by looking at the association between the *ALDH2* polymorphism and esophageal cancer. Alcohol drinking is considered a risk factor for esophageal cancer, and exposure to high levels of acetaldehyde, the principal metabolite of alcohol, may be responsible for the increased cancer risk. The ability to metabolize acetaldehyde is encoded by the *ALDH2* gene, which is polymorphic in some populations. The *ALDH22 allele produces an inactive protein subunit, which is unable to metabolize acetaldehyde. An individual's genotype at this locus may influence their esophageal cancer risk through two mechanisms, first through influencing alcohol intake and second through influencing acetaldehyde levels. We have carried out a meta-analysis of studies looking at the *ALDH2* genotype and esophageal cancer and found that risk was reduced among *2*2 homozygotes [odds ratio (OR), 0.36; 95% confidence interval (95% CI), 0.16-0.80] and increased among heterozygotes (OR, 3.19; 95% CI, 1.86-5.47) relative to **1*1* homozygotes. This provides strong evidence that alcohol intake increases the risk of esophageal cancer and individuals whose genotype results in markedly lower intake, because they have an adverse reaction to alcohol are thus protected. This meta-analysis also provides evidence that acetaldehyde plays a carcinogenic role in esophageal cancer. The two different processes operating as a result of the *ALDH2* genotype have implications for the interpretation of studies using the Mendelian randomization paradigm.** (Cancer Epidemiol Biomarkers Prev 2005;14(8):1967–71)

Introduction

Mendelian Randomization. Associations between modifiable exposures and disease seen in observational epidemiology are sometimes confounded and thus misleading, despite efforts to improve the design and analysis of studies (1). This is especially true of alcohol intake, which tends to be correlated with many other lifestyle factors, including smoking and diet that are risk factors for disease. Reverse causality is also often a problem in observational studies because exposure levels may change when a person becomes ill. In the case of alcohol intake, if anything, individuals are likely to reduce intake which would lead to an underestimate of risk. However, another form of reverse causation could occur through reporting bias, if cases report their exposure history differently to controls because they are searching for a potential reason for their illness. Furthermore, alcohol consumption is likely to be subject to measurement error, which again will lead to an underestimation of risk. Mendelian randomization provides one method for assessing the causal nature of some environmental exposures (1-6). Common polymorphisms exist that influence exposure propensities, or are modifiers of the biological effects of environmental exposure. Therefore, determination of genotype can be used in some incidences as a surrogate for directly measuring the exposure of interest.

This approach is of value when suitable polymorphisms exist, because genotype is determined at conception by the random assortment of maternal and paternal chromosomes and is unchanged by the disease process. Furthermore, the distribution of alleles is largely unrelated to the sorts of confounders that may distort interpretations of findings from observational epidemiologic studies. Hence, the association between a disease and a polymorphism that influences exposure level or mimics the biological link between a proposed exposure and disease is not generally susceptible to reverse causation or confounding that may distort interpretations of conventional observational studies (1). Mendelian randomization provides new opportunities to test causality and explore biological mechanisms and shows how investment in the human genome project may contribute to understanding and preventing the adverse effects on human health of modifiable exposures. The potential and some of the limitations of Mendelian randomization can be illustrated by examining the association between the ALDH2 polymorphism and esophageal cancer as an example.

ALDH2, Alcohol Intake, and Metabolism. Alcohol drinking has been classified as a risk factor for esophageal cancer based on data from epidemiologic studies (7), although ethanol in its pure form does not act as a carcinogen in experimental models (7). Potential reasons why alcohol intake increases risk of esophageal cancer are that alcohol acts as a solvent for tobacco carcinogens or that impurities in alcoholic drinks are the carcinogenic agents (8). Another competing hypothesis is that exposure to high levels of acetaldehyde, the principal metabolite of alcohol, is responsible for the increased cancer risk (9). However, direct evidence that acetaldehyde is a cause of head and neck cancers in humans is hard to obtain.

The major enzyme responsible for the elimination of acetaldehyde is ALDH2 (10). In some populations, *ALDH2* is polymorphic and an individual's genotype at this locus determines blood acetaldehyde concentrations after drinking (11). A single point mutation in *ALDH2* has resulted in the *ALDH2**2 allele. The resultant protein has an amino acid substitution from glutamic acid (glutamate) to lysine at residue 487, an inactive subunit and an inability to metabolize

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acetaldehyde, leading to an accumulation of acetaldehyde after alcohol intake (11). Individuals who are homozygous for the *ALDH2**2 allele have 18 times higher and heterozygotes have five times higher peak blood alcohol levels compared with *1*1 homozygotes (12). *ALDH2**2*2 homozygotes are characterized by a facial flushing response after consumption of alcohol coupled with nausea, drowsiness, headache, and other unpleasant symptoms that prevent them from heavy drinking (12). Heterozygotes have a limited ability to metabolize acetaldehyde but exhibit a less severe reaction than that seen among *ALDH2**2*2 homozygotes. We have carried out meta-analysis of studies looking at the *ALDH2* genotype and esophageal cancer to examine how the Mendelian randomization approach performs in this situation. A priori, we would expect *ALDH2**2 homozygotes to have a reduced esophageal cancer risk due to a lower alcohol intake among these individuals (Fig. 1).

Materials and Methods

Search Strategy and Selection Criteria. Papers published before the end of March 2004 were identified through a search of Medline (http://www.ncbi.nlm.nih.gov) and ISIS web of knowledge (http//:wok.mimas.ac.uk), using the following search terms: ''oesophageal'' or ''esophageal'' and ''ALDH2'' or ''aldehyde dehydrogenase.'' A cited reference search of retrieved articles was carried out and publications were also identified by review of the bibliographies of retrieved articles. Articles reporting on *ALDH2* genotype in cases of esophageal cancer and controls were identified (13-19). Where authors had published more than one article using the same case series, we selected the most recent publication. Our a priori hypothesis was that *ALDH2**2*2 genotype protects against esophageal cancer because individuals with this genotype are unlikely to consume high quantities of alcohol. For this reason, we excluded two studies from the meta-analysis of *2*2 genotype versus **1*1* genotype, which specifically selected cases and/or controls on the basis of alcohol intake (14, 16). We also wanted to test the hypothesis that *ALDH2*1*2* genotype increases esophageal cancer risk relative to **1*1* genotype given a similar level of alcohol intake. We therefore carried out a stratified meta-analysis in which subjects were stratified according to alcohol intake; we excluded two studies that provided no information on alcohol intake by genotype (13, 14).

Statistical Analysis. Alcohol intake was coded as 0 = nondrinkers, 1 = all individuals not classified as nondrinkers or heavy drinkers, and 2 = heavy drinkers. Unadjusted odds ratios (OR) were based on published genotype frequencies. Random effects models were used because the method of case/control ascertainment and the alcohol intake cutoffs both differed between the studies. We quantified the extent of heterogeneity using I^2, the percentage of total variation across studies that is attributable to heterogeneity rather than chance (20). The influence of alcohol intake on the relationship between ALDH2 genotype and esophageal cancer risk was assessed using meta-regression analysis (21). A Monte Carlo permutation procedure was used to determine deviation from Hardy-Weinberg equilibrium among control populations using the HWSIM program provided on the following web site: http://krunch.med.yale.edu/hwsim. All other statistical analysis was carried out in Stata version 8 (Stata Corp., College Station, TX).

Results

Seven studies, (13-19) with a total of 905 cases of esophageal cancer were identified and these are summarized in Table 1. The studies were carried out in Japan, Taiwan, and Thailand. Relative genotype frequencies in all control groups did not deviate from values predicted by Hardy-Weinberg equilibrium. *ALDH2**2 allele frequencies among control populations were lowest in a study in which the controls were alcoholics (16) and were also lower in the Thai study than in the Japanese studies (17). Our meta-analysis gave an overall OR of 0.36 [95% confidence interval (95% CI), 0.16-0.80] for the risk of esophageal cancer among *2*2 homozygotes compared with **1*1* homozygotes (Fig. 2) and an overall OR of 3.19 (95% CI, 1.86-5.47) for heterozygotes compared with **1*1* homozygotes (Fig. 3). Among nondrinkers, there was no strong evidence for an increase in risk among heterozygotes (OR, 1.31; 95% CI, 0.70-2.47) relative to **1*1* individuals. However, among heavy drinkers there was a 7-fold increase in risk (OR, 7.07; 95% CI, 3.67-13.6). Among all others with an intermediate alcohol intake the risk among heterozygotes versus **1*1* homozygotes was 2.49 (95% CI, 1.29-4.79). Meta-regression analysis showed evidence that alcohol intake influenced the effect of the **1*2* genotype on esophageal cancer risk (P = 0.008) and that the larger the amount of alcohol consumption (i.e., the greater the OR of **1*2* versus **1*1* genotypes).

There was no evidence of between study heterogeneity in the analysis of *2*2 versus **1*1* genotype (χ^2 = 2.14, P = 0.71, I^2 = 0.0%), but there was evidence of heterogeneity in the analysis of **1*2* versus **1*1* genotype (χ^2 = 53.5, P < 0.001,

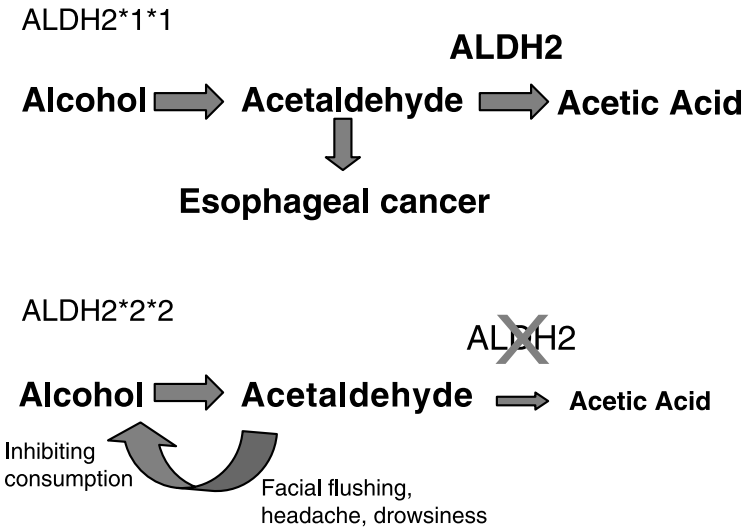


Figure 1. Schematic diagram of the postulated relationships among ALDH2, alcohol consumption, acetaldehyde levels, and esophageal cancer.

Table 1. Studies of the ALDH2 polymorphism and esophageal cancer risk

Reference	Population	No. cases	No. controls	*2 Allele frequency in controls
Hori et al. (13)	Japan	93	70 healthy individuals	0.33
Chao et al. (14)	Taiwan	88 (59 alcoholics)	105 nonalcoholics	0.29
Matsuo et al. (15)	Japan	102	241 hospital outpatients	0.28
Yokoyama et al. (16)	Japan	112* alcoholics	526 alcoholics	0.05
Boonyaphiphat et al. (17)	Thailand	202	261 hospital inpatients	0.10
Itoga et al. (18)	Japan	74	241 healthy individuals	0.22
Yokoyama et al. (19)	Japan	234	634 population	0.27

*Figures for genotype frequencies by case-control status obtained by personal correspondence.

I^2 = 81.3%). This heterogeneity was slightly reduced within strata after stratification for alcohol intake and was confined to alcohol drinkers, both heavy drinkers (χ^2 = 9.01, P = 0.03, I^2 = 66.7%) and others (χ^2 = 11.6, P = 0.009, I^2 = 74.0%), and probably reflects differences in crude estimates of alcohol intake between studies, particularly as there was no between study heterogeneity among nondrinkers (χ^2 = 0.35, P = 0.84, I^2 = 0.0%). The overall test of heterogeneity among the three effect estimates for the pooled strata above gave the following results: χ^2 =32.6 and P < 0.001.

In the studies that provided alcohol use by genotype in the control group, heavy drinking was more common among individuals with the **1*1* genotype compared with the **1*2* genotype and there were no heavy drinkers among individuals with the *2*2 genotype (Table 2).

An Egger test (22) provided no evidence that effect estimates were related to study size (P = 0.61 for *2*2 analysis and P = 0.11 for **1*2* analysis), providing some reassurance that small study bias, such as publication bias has not distorted the findings.

Discussion

This meta-analysis shows strong evidence that the *ALDH2*2*2* genotype reduces esophageal cancer risk by ~3-fold, and this is likely to be due to markedly lower levels of alcohol consumption in *2*2 versus **1*1* homozygotes. *ALDH2*2*2* homozygotes are intolerant to alcohol and can exhibit a severe reaction following intake of a small amount of alcohol; hence, this genotype protects against esophageal cancer because it influences propensity to drink alcohol (Table 2). This provides support to the existing evidence from epidemiologic studies that the association between alcohol intake and esophageal cancer is causal in nature. As *ALDH2* genotype is determined at birth, the findings with respect to genotype are not subject to reverse causation - ill-health influencing the exposure measure - unlike the findings with respect to directly measured alcohol intake. Furthermore, it is often difficult to separate the effects of heavy alcohol intake and smoking in observational studies, because the two are highly correlated. In a large Japanese cohort study, smoking was shown to be strongly associated with heavy alcohol intake (ref. 23; Table 3), however, smoking was not associated with *ALDH2* genotype (refs. 15, 24; Table 3). Hence measuring *ALDH2* genotype as a surrogate for alcohol intake is not subject to confounding, and can be used to verify the findings of epidemiologic studies. Finally, a tendency to inaccurately report alcohol intake, most probably underreporting, may be related to other risk factors for disease, leading to bias in the association between reported alcohol intake and disease. This inaccurate reporting will not be an issue if genotype is used as a proxy for alcohol intake.

A meta-analysis of observational studies of alcohol intake and esophageal cancer risk found relative risks of 1.8, 2.38, and 4.36 for risk among light, moderate, and heavy drinkers, respectively, compared with nondrinkers (25). In the control population in the study by Yokoyama et al. (19), 9.4% of **1*1* individuals were nondrinkers (never plus ex drinkers) and 28.2%, 39.6%, and 22.9% were light, moderate, and heavy drinkers, respectively. Whilst the cutoff between light and moderate drinkers was different in the two studies (~29 g/d in the study by Yokoyama et al. and 39.99 g/d in the meta-analysis of observational studies); other cutoff points were similar. We therefore used the relative risks associated with different levels of alcohol consumption in the meta-analysis of

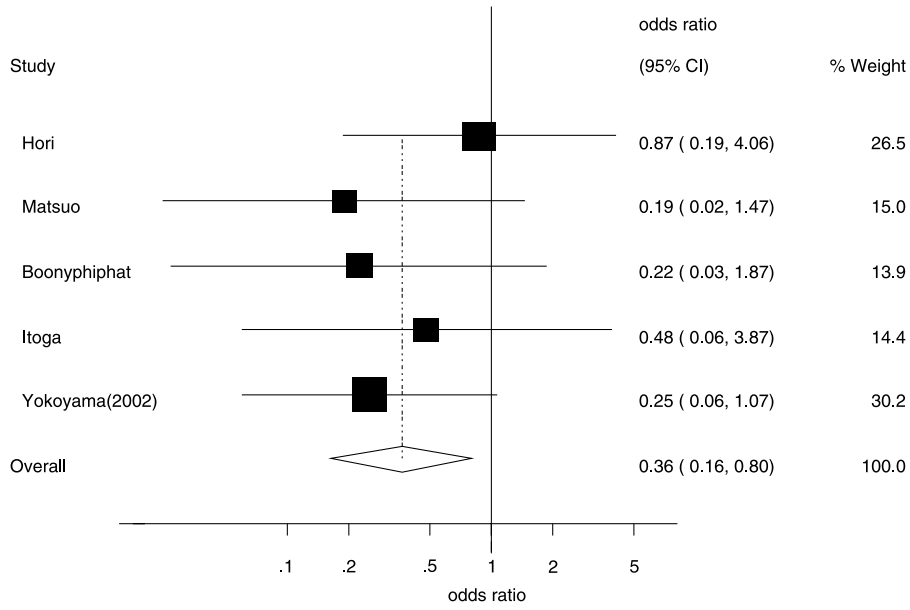
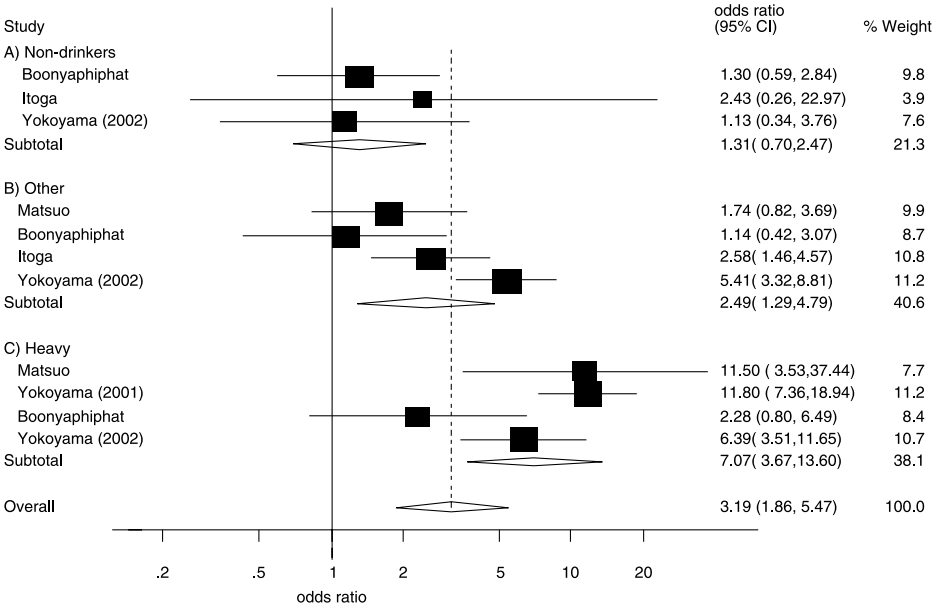


Figure 2. Risk of esophageal cancer in individuals with the ALDH2*2*2 versus ALDH2*1*1 genotype.



Non-drinkers = non-drinkers (Boonyaphiphat et al and Itoga et al), and never and ex-drinkers (Yokoyama et al, 2002).
Others = All those who were not heavy drinkers (Matsuo et al, 2001), drinkers ≤60g/day (Boonyaphiphat et al), habitual drinkers (Itoga et al, 2002) and 1-17.9 units/week; where 1 unit=22g ethanol (Yokoyama et al, 2002).
Heavy drinkers = 75ml ethanol per day ≥5 days/week (Matsuo et al, 2001), alcoholics (Yokoyama et al, 2001), >60g ethanol per day (Boonyaphiphat et al) and ≥18 units/week; where 1 unit=22g ethanol (Yokoyama et al, 2002).

Figure 3. Risk of esophageal cancer in individuals with the *ALDH2*1*2* versus *ALDH2*1*1* genotype.

observational studies (25) to calculate an approximate overall probability of disease given alcohol intake as reported by **1*1* individuals in the study by Yokoyama et al. (19). To calculate this probability, we used the following equation: $RR = \Sigma RRi \times P_i$, where i denotes the drinking category (non, light, moderate, and heavy), RR_i is the relative risk in the ith drinking category estimated by a meta-analysis (25), and P_i is the assumed proportion of ith drinking category among controls (19). We estimated an overall relative risk for this group of around 2.54 compared with nondrinkers (equivalent to **2*2* individuals as virtually all of these individuals are nondrinkers). The OR of esophageal cancer for **1*1* homozygotes versus **2*2* homozygotes in this meta-analysis was 2.77 (95% CI, 1.25-6.12). Risk ratios and ORs are virtually equivalent for esophageal cancer as the disease is very rare. Hence the greater risk seen among **1*1* homozygotes is what would be expected given their drinking behavior and findings from observational epidemiologic studies relating drinking behavior to esophageal cancer risk.

There are markedly increased acetaldehyde levels among heterozygotes who drink alcohol, and heterozygosity is associated with an ~3-fold overall increase in risk for esophageal cancer (Fig. 3). This suggests that acetaldehyde may be the mechanism through which drinking alcohol increases the risk of esophageal cancer. This meta-analysis and the results of the meta-regression show that the

Table 2. Drinking status by ALDH2 genotype among controls

Reference	Drinking categories	**1*1*, *n* (%)	**1*2*, *n* (%)	**2*2*, *n* (%)
Matsuo et al. (15)	Others	104 (82.5)	92 (95.8)	19 (100)
	Heavy drinkers	22 (17.5)	4 (4.2)	0 (0)
Yokoyama et al. (16)	Alcoholics	476 (100)	50 (100)	0 (0)
Boonyaphiphat et al. (17)	Nondrinker	104 (48.4)	24 (60.0)	Excluded due to small numbers
	≤60g/d	66 (30.7)	11 (27.5)	
	>60g/d	45 (20.9)	5 (12.5)	
Itoga et al. (18)	Nondrinkers	8 (5.6)	23 (26.1)	10 (100)
	Drinkers	135 (94.4)	65 (73.9)	0 (0)
Yokoyama et al. (19)	Never/rare	21 (6.2)	80 (32.0)	41 (95.3)
	Light	96 (28.2)	103 (41.2)	2 (4.7)
	Moderate	135 (39.6)	35 (14.0)	0 (0)
	Heavy	78 (22.9)	27 (10.8)	0 (0)
	Ex drinker	11 (3.2)	5 (2.0)	0 (0)

Table 3. Smoking status by heavy alcohol intake and by ALDH2 genotype

Smoking status	Alcohol intake (%)		
	Moderate (1-149 g/wk)	Heavy (≥450 g/wk)	
Inoue and Tsugene (22), (men, *n* = 35,007)			
Never	30.2	15.5	
Former	22.7	20.1	
Current	47.1	64.4	
	ALDH2 genotype		
	**2*2*	**1*2*	**1*1*
Takagi et al. (23), (men, *n* = 1919)			
Current smokers	35.9	39.5	39.7
Matsuo et al, (15), (controls, *n* = 241)	**2*2* and **1*2*		
Never	62.6		54.0
Former	15.7	22.0	
Current	21.7	23.8	

association between ALDH2 heterozygote genotype and esophageal cancer is dependent on alcohol consumption. Among nondrinkers, there is no strong evidence of an increased risk. Whereas among heavy drinkers, a substantial elevated risk is seen. This suggests that possession of an *ALDH2*2* allele does not increase risk of esophageal cancer unless alcohol is consumed.

Limitations. In this meta-analysis, we did not have access to individual level data and were not able to reclassify individuals by alcohol intake; instead, we were forced to use the cutoffs used by the different studies as approximate measures of nondrinking, heavy drinking, and other. This is likely to have been a source of heterogeneity in the meta-analysis. Similarly, we did not have access to individual data on smoking and thus were unable to assess whether alcohol intake, as determined by ALDH2 genotype, influences the number of cigarettes smoked. However, we suggest that cigarette smoking is unlikely to be a confounder given the apparent independence of ALDH2 genotype and smoking status (Table 3).

In this example, it is easy to observe the two different processes operating as a result of the ALDH2 genotype because this polymorphism in *ALDH2* is well characterized with reliable differences in phenotype by genotype. However, whereas this polymorphism can be viewed as having two related effects with opposing influences on disease risk, there are very few examples of well-characterized polymorphisms where this can be understood. Finally, canalization, the developmental buffering against the effect of a polymorphism during fetal development, is also often a potential problem in studies that apply the Mendelian randomization concept (1). It is unlikely to be a factor in this example, however, as alcohol consumption is only adopted in adolescence or adulthood.

In summary, this polymorphism influences the propensity for exposure to alcohol and modifies exposure to acetaldehyde among alcohol drinkers and can therefore be used to characterize both alcohol intake and acetaldehyde as components of a causal chain increasing the risk of esophageal cancer. In using *ADLH2*2*2* as a surrogate for measuring alcohol intake, this study shows that alcohol drinking is related to elevated risk of esophageal cancer thus illustrating the potential of the Mendelian randomization concept. However, this study also shows that *ALDH2*1*2* is related to both lower alcohol consumption and elevated risk of esophageal cancer when the amount of alcohol consumed is identical; thus, analyzing the relationship between *ALDH2*1*2* and risk of esophageal cancer without considering the amount of alcohol consumption is misleading. The result of *ALDH2*1*2* illustrates a potential limitation of a Mendelian randomization approach in that in less well characterized situations, similar gene-environment interactions may be occurring which are not recognized, leading to spurious conclusions being drawn from looking at the main effects.

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