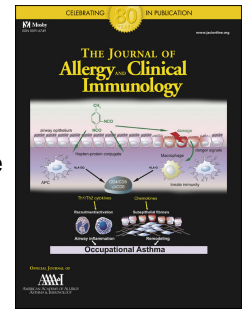


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High alcohol consumption causes high IgE levels, but not high risk of allergic disease

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Abstract

Background:

High alcohol consumption is associated with high IgE levels in observational studies; however, whether high alcohol consumption leads to high IgE levels and allergic disease is unclear.

Objective:

We tested the hypothesis that high alcohol consumption is associated with high IgE levels and with allergic disease, observationally and genetically using a Mendelian randomization design free of reverse causation and largely free of confounding.

Methods:

Among 111,408 individuals aged 20-100 from the general population, 50,019 had plasma IgE measurements and 102,270 were genotyped for alcohol metabolizing enzymes *ADH-1B*(rs1229984) and *ADH-1C*(rs698). Observationally we investigated the associations between IgE levels and allergic disease (allergic asthma, rhinitis, and eczema) and between alcohol consumption and IgE levels and allergic disease. Genetically, we explored potential causal relationships between alcohol consumption and IgE levels and allergic disease.

Results:

The multivariable adjusted odds ratio for IgE levels above versus below 150 kU/L and compared to individuals without allergic disease was 2.3 (95% CI:2.2-2.5) for 1 allergic disease, 3.9 (3.5-4.4) for 2 allergic diseases, and 7.5 (6.2-9.0) for 3 allergic diseases. High alcohol consumption was associated with high IgE levels, but not with high risk of allergic disease. The odds ratio for high versus low IgE levels per 1 alcoholic drink/week higher consumption was 1.12 (95% CI:1.02-1.23) genetically and 1.01 (1.01-1.02) observationally; for allergic disease the corresponding odds ratios were 0.95 (0.92-0.99) genetically and 1.00 (1.00-1.00) observationally.

Conclusion:

High alcohol consumption is associated observationally and genetically with high IgE levels, but not with high risk of allergic disease.

Key words:

Ethanol; Asthma; Rhinitis; Eczema; Immunoglobulin E; Alcohol Dehydrogenase; Mendelian Randomization; General Population

Key messages:

High alcohol consumption does not lead to allergic disease

High alcohol consumption may explain high IgE levels, in individuals with or without allergic disease.

Abbreviations used:

IgE: Immunoglobulin E

ADH-1B Alcohol dehydrogenase 1B

ADH-1C Alcohol dehydrogenase 1C

TH1 T helper cell 1

TH2 T helper cell 2

69 **Capsule Summary:**

70 In this study of the general population including 111,408 individuals, we found that high alcohol
71 consumption is associated observationally and genetically with high IgE levels, but not with high
72 risk of allergic disease.

Introduction

Immunoglobulin E (IgE) specific to allergens is thought to play an important role in the pathogenesis of allergic diseases such as allergic asthma, allergic rhinitis, and allergic eczema(1). Thus, high total IgE levels in plasma might be a useful marker of allergic disease in individuals in the general population, considering that total IgE levels often are elevated in patients with versus without allergic disease(2) and as higher levels of specific IgE are associated with more severe allergic disease(3;4). Interestingly, moderately high alcohol consumption as well as alcoholism possibly are associated with high levels of IgE(5-12). Taken together, these observations illustrate that high alcohol consumption may lead to high IgE levels as well as to allergic disease. This is a very important question to address, given the high consumption of alcohol in many countries coupled with the current increase in allergic diseases worldwide(13). However, whether high alcohol consumption directly leads to high IgE levels and to allergic disease is unclear, particularly as the observational association referred to above could be explained by confounding and/or reverse causation.

To better address these important questions, large-scale, well-adjusted observational studies can be coupled with Mendelian randomization studies(14;15). In the latter, naturally occurring genetic variation in human populations can be used as proxies for adulthood high and low alcohol consumption, largely free of confounding and certainly free of reverse causation (Figure 1). This is because genotypes are distributed at random at conception, irrespective of the lifestyle adopted and diseases developed later in life by an individual. Specifically, Mendelian randomization can be used to investigate possible causal relationships of high alcohol consumption with high IgE levels and with risk of allergic diseases, using genetic variants in two alcohol dehydrogenase genes (*ADH-1B*, *ADH-1C*) known to be causally associated with high alcohol consumption(14-16). *ADH-1B.2* and *ADH-1C.1* encode proteins with fast alcohol degradation,

while *ADH-1B.1* and *ADH-1C.2* encode proteins with slow alcohol degradation. Mechanistically then, people with fast alcohol degradation have higher levels of acetaldehyde within cells and people with such genetic predisposition in consequence tend to feel more discomfort when consuming alcohol, and therefore consume less alcohol than people with slow alcohol degradation(17). A Mendelian randomization study can be compared to a randomized controlled trial. In a randomized controlled trial (Figure 1), study participants are randomized to either control or active treatment (low or high alcohol consumption), independent on other factors, securing that the two study groups have similar distributions of confounders. Using this study design, reverse causation is not possible since the outcome cannot affect randomization. Similar to that, the genes used in Mendelian randomization studies are randomly distributed at birth and therefore not affected by lifestyle adopted and diseases developed later in life. Like the randomized controlled trial, Mendelian randomization studies are not at risk of reverse causation (Figure 1). Unlike the two types of studies mentioned, in observational studies it is crucial to adjust for all possible confounders and even if all confounders are adjusted for, it is still possible that the results are caused by confounding and/or reverse causation. In summary, Mendelian randomization studies have several strengths compared to classic observational studies: first, it is not subject to confounding and reverse causation and, second, it is based on genes associated life-long with risk factors. Therefore it is possible to study causality using the Mendelian randomization study design.

We tested the hypothesis that high alcohol consumption is associated with high IgE levels and with allergic diseases in the general population, observationally and genetically. For this purpose, we studied 111,408 individuals from the Copenhagen General Population Study and the Copenhagen City Heart Study, using a Mendelian randomization design including both observational and genetic associations. Genetic association using *ADH-1B* and *ADH-1C* variants as a proxy for adulthood high alcohol consumption can address potential causal associations as

121 genotypes generally are unrelated to classical confounding factors, and as reverse causation is not
122 an issue, that is, high IgE levels and allergic disease cannot change a person's genotype(18).
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Methods

Study population

We used the Copenhagen General Population Study and the Copenhagen City Heart Study(19-22). In both studies individuals aged 20-100 years were recruited from the counties in and around Copenhagen by random selection from the Danish Civil Registration System, to represent the Danish general population. There was no overlap of participants in the two studies. Information was obtained from self-administered questionnaires reviewed with an investigator on the day of attendance, physical examinations, and from blood drawn for biochemical analysis and genotyping.

A total of 111,408 Whites of Danish descent, 99,137 from the Copenhagen General Population Study and 12,271 from the Copenhagen City Heart Study, were included in the study. Of these, 106,664 had information on alcohol consumption, 50,019 had measurements of plasma IgE, and 102,270 were genotyped for *ADH-1B* (rs1229984) and *ADH-1C* (rs698).

All participants gave written informed consent. The study was approved by Herlev and Gentofte Hospital and by Danish ethical committees.

Alcohol consumption

Information on weekly alcohol consumption was obtained from questionnaires, where participants reported average weekly consumption of bottles of beer and standard glasses of wine and spirits, assuming that each drink was the size of a standard drink containing 12 g of pure alcohol. Alcohol consumption was then calculated in drinks/week; we asked participants about weekly alcohol consumption rather than daily consumption as alcohol consumption may differ between weekends and during the week.. Information on lifetime abstinence was not available, and participants reporting no weekly consumption would be expected to be a mixture of lifetime abstainers and those giving up alcohol.

Allergic disease

Information on allergic disease, that is, allergic asthma, allergic rhinitis, and allergic eczema, was obtained through the questionnaire. In the Copenhagen General Population Study participants were asked the following question: “Does food, medicine, grass, flowers, animal hair or other things trigger asthma (yes/no), rhinitis (yes/no), or eczema (yes/no)?” Such information was not available for participants in the Copenhagen City Heart Study.

Covariates

Through the questionnaire, smoking behaviour was reported as smoking status (current, former, never) and years of smoking, and smoking amount was reported as average number of cigarettes, cheroots, cigars and packages of pipe tobacco consumed per day. Based on this information amount of daily smoking was calculated as number of cigarettes or equivalent smoked per day, and total amount ever smoked was calculated as pack-years, where one pack-year is 20 cigarettes or equivalent smoked daily for one year.

Information on educational level and civil status was obtained through the questionnaire, while body mass index (BMI) was calculated from measured weight (kg) divided by the square of measured height (m^2).

Information on physical activity was obtained from questionnaire. Physical activity at work was reported in five categories: “mainly sitting”, “sitting, standing, walking”, “walking and lifting”, “heavy workload”, and “not working”. Physical activity during leisure time was reported in four categories: “< 2 hours/week”, “2-4 hours/week”, “> 4 hours light training per week”, and “> 4 hours heavy training per week”.

IgE measurements

Total plasma IgE was measured from either frozen or fresh plasma samples, as described previously(23). In the Copenhagen City Heart Study, 10,251 samples were frozen at -20°C immediately after blood draw in 1981-1983 and stored until measurements were conducted in 2009-2011. They were analysed using the ADVIA Centaur® Total IgE quantitative assay. In the Copenhagen General Population Study, 8,102 samples frozen at -80°C immediately after blood draw in 2003-2004 were analysed in 2011-2012. Finally, 32,062 measurements from the Copenhagen General Population Study were made on fresh samples. All samples from the Copenhagen General Population Study were analysed using the Diasys Immunoglobulin E FS assay. Due to the differences in the assay methods and storage conditions used, results obtained from the two set of frozen samples were adjusted slightly according to the distribution of results from the fresh samples to make the measurements comparable. However, if statistical analyses were conducted on raw IgE measurements, the results were similar to those presented. Importantly, the selection of individuals for IgE measurement was completely random and was only determined by when it was possible to measure this large number of individuals; thus, no systematic bias was introduced in the selection of individuals for IgE measurements.

Genotyping

DNA was isolated from whole blood. The *ADH-1B* and *ADH-1C* genotypes were identified using TaqMan assays(24) in the Copenhagen General Population Study and using Nanogen technology(16;25) in the Copenhagen City Heart Study. The minor allele frequency was 2% for *ADH-1B* and 42% for *ADH-1C* with similar distributions in the two studies. The linkage disequilibrium coefficient D' was 0.87 and r^2 was 0.01 between the two genotypes. The genotype distributions did not differ from Hardy Weinberg equilibrium in either study (all $p > 0.05$).

Individuals were further divided into four groups of genotype combinations based on alcohol dehydrogenase activity: fast metabolizers, intermediate-fast, intermediate-slow and slow metabolizers, as done previously(26) (Supplementary Table 1).

Statistical analyses

We used Stata version 13.1.

Observational analyses

Allergic disease and IgE

To examine the association between allergic disease and IgE levels, individuals were given an allergic disease score according to self-reported prevalence of allergic asthma, allergic rhinitis, and/or allergic eczema (no allergic disease, 1 allergic disease out of 3, 2 out of 3, or all 3 allergic diseases); however, we also examined each of the three allergic diseases separately. Total IgE levels were assessed both as a continuous variable and as a dichotomous variable; however, previous studies have been inconsistent regarding the optimal cut-off value for total IgE. Thus, we calculated odds ratios for allergic disease with different cut-off values of IgE levels (Supplementary Figure 1), showing levelling off of odds ratios at 120 kU/L and at 170 kU/L. Based on this data, we chose the cut-off value of 150 kU/L being largely midway between these two values and as sensitivity would be lost at higher cut-off values and specificity would be lost at lower cut-off values. Logistic regressions calculated multivariable adjusted odds ratios with 95% confidence intervals for high versus low IgE levels with individuals with no allergic disease as the reference group. Multivariable adjustment was for sex, age, BMI, smoking status, civil status, and educational level, because these variables have previously been associated with allergic disease and/or alcohol consumption. Since IgE levels were skewed toward higher levels, log-transformed IgE levels were used to calculate the

geometric mean and corresponding confidence interval after back transformation for each allergic disease score and for individual allergic diseases with a 95% confidence interval.

Alcohol consumption and IgE

To examine the association between alcohol consumption and IgE levels, individuals were divided into 6 categories according to self-reported weekly alcohol consumption: 0, 1-6, 7-13, 14-20, 21-27, and ≥ 28 drinks/week. Logistic regressions were performed to calculate multivariable adjusted (as above) odds ratios for high versus low IgE levels with 95% confidence interval; as individuals reporting no alcohol consumption would be comprised of true abstainers and those giving up alcohol, individuals drinking 1-6 drinks/week was used as the reference group. Similarly to the analysis of allergic disease and IgE levels, geometric means were calculated for each of the alcohol consumption groups. Test for trend was by logistic regression on a continuous scale.

Alcohol consumption and allergic disease

To examine the association between alcohol consumption and allergic disease, individuals were divided into those with and without allergic disease to obtain maximal statistical power; these analyses were also stratified for sex, median age, and smoking status to examine robustness of the finding. Multivariable adjusted (as above) odds ratios for allergic disease were calculated for each of the alcohol consumption groups compared to those drinking 1-6 drinks/week. Test for trend was by logistic regression on a continuous scale.

Genetic analyses

To investigate a potential causal relationship between alcohol consumption and IgE levels and allergic disease, we conducted a study in steps according to the Mendelian randomization studies approach(27). We first examined whether alcohol consumption was observationally associated with high levels of IgE and with allergic disease, as described above.

Genotype and alcohol consumption

We then examined whether the *ADH* genotypes were associated with differences in alcohol consumption. The association between alcohol consumption and the individual genotypes were investigated as well as the association between alcohol consumption and the four genotype combination groups.

Genotype and IgE

Next, we examined whether the genotype combination groups were associated with high levels of IgE. Age and sex adjusted logistic regressions calculated odds ratios with 95% confidence intervals with fast metabolizers as the reference group; because genotype is unrelated to measured potential confounders, we adjusted only for age and sex.

Genotype and allergic disease

Likewise, we examined whether the genotype combination groups were associated with high risk of allergic disease. Age and sex adjusted logistic regressions calculated odds ratios with 95% confidence intervals with fast metabolizers as the reference group.

Genetic versus observational estimates

Finally, we performed an instrumental variable analysis to explore the magnitude of the relationship between alcohol consumption and high IgE levels as well as with allergic disease. In a two-stage regression model we estimated the causal effect of an increase of 1 alcoholic drink/week. The first stage was a linear regression of genotype combination on levels of alcohol consumption. F-statistics greater than 10 indicates sufficient statistical strength to carry out an instrumental variable analysis and R-square indicates the effect of the genotype combination on outcome(18); for genotype combination groups we observed $F=12.5$ and $R^2=0.0009$; although this is a relative low R^2 we still had sufficient power to demonstrate that alcohol consumption influences IgE levels (see Results) In

267 the second stage, we performed a logistic regression to calculate odds ratios with 95% confidence
268 intervals for high versus low IgE levels and for risk of allergic disease, for each genetically
269 determined increase in 1 alcoholic drink/week, and compared this estimate with the corresponding
270 observational estimate.

271

Results

Baseline characteristics in the combined study population of 111,480 individuals as well as of those included in the observational analyses and in the genetic analyses were largely similar (Table I, Figure 2).

Observational analyses

Allergic disease and IgE

The multivariable adjusted odds ratio for IgE levels above versus below 150 kU/L and compared with individuals without allergic disease was 2.3 (95% CI: 2.2-2.5) for those with 1 allergic disease, 3.9 (3.5-4.4) for 2 allergic diseases, and 7.5 (6.2-9.0) for those with 3 allergic diseases (Figure 3). The geometric mean levels for IgE was 18 (16-20) for those without allergic disease, 32 (30-34) for those with 1 allergic disease, 43 (41-45) for those with 2 allergic diseases, and 74 (72-76) for those with 3 allergic diseases. Baseline characteristics of the study population used in these analyses are shown in Supplementary Tables 2 and 3.

Alcohol consumption and IgE

High alcohol consumption was associated with high levels of IgE, overall as well as separately in those with and without allergic disease (Figure 4). For the dichotomous analysis of high versus low IgE levels and compared to those drinking 1-6 drinks/week, the odds ratios increased from 0.90 (95% CI: 0.81-1.01) for those not drinking to 1.70 (1.51-1.91) for those drinking ≥ 28 drinks/week (p-value for trend across 6 groups of < 0.001). Also, the mean IgE levels increased with increasing amount of alcohol consumption, overall as well as separately for those with and without allergic

disease. Baseline characteristics of the study population included in these analyses are shown in Supplementary Table 4.

Alcohol consumption and allergic disease

High alcohol consumption was not associated with high risk of allergic disease (Figure 5); if anything, there was a trend toward lower risk (p for trend of 0.03). Baseline characteristics of the study population used in these analyses are shown in Supplementary Table 5. Results observed overall were similar when stratified by sex, median age, and smoking status (compare Supplementary Figures 3, 4 and 5 with Figure 5)

Genetic analyses

Genotype and alcohol consumption

Compared to homozygotes for the *ADH-1B.2* genotype (fast metabolizers), homozygotes for the *ADH-1B.1* genotype (slow metabolizers) were on average drinking 3.4 drinks more per week (47% more) (p-value for trend across 3 genotypes of < 0.001) (Supplementary Table 1). For the other genotype, homozygotes for the *ADH-1C.2* genotype (slow metabolizers) were on average drinking 0.4 drinks more per week (3% more) compared to homozygotes for the *ADH-1C.1* genotype (fast metabolizers) (p-value for trend of < 0.001). In genotype combination groups, the slow metabolizers (group 4) were on average drinking 1.9 drinks more per week (21% more) compared to the fast metabolizers (group 1) (p-value for trend across 4 groups of < 0.001). Baseline characteristics of the study population used in these analyses are shown in Supplementary Table 6.

Genotype and IgE

Compared with fast alcohol metabolizers, the odds ratio for high versus low IgE levels was 1.17 (95% CI: 0.98-1.40) for intermediate-fast metabolizers with 18% higher alcohol consumption, 1.26 (1.06-1.50) for intermediate-slow metabolizers with 19% higher alcohol consumption, and 1.23 (1.02-1.48) for slow metabolizers with 21% higher alcohol consumption (Figure 6). Baseline characteristics of the study population used in this analysis are shown in Supplementary Table 7.

Genotype and allergic disease

Compared with fast alcohol metabolizers, the odds ratio for allergic disease was 0.93 (95% CI: 0.86-1.01) for intermediate-fast metabolizers, 0.92 (0.85-0.99) for intermediate-slow metabolizers, and 0.93 (0.86-1.00) for slow metabolizers (Supplementary Figure 2). Baseline characteristics of the study population used in this analysis are shown in Supplementary Table 8.

Genetic versus observational estimates

The odds ratio per 1 alcoholic drink/week higher consumption for high versus low IgE levels was 1.12 (95% CI: 1.02-1.23) genetically and 1.01 (1.01-1.02) observationally (Figure 7), meaning that an increase in consumption of 1 alcoholic drink/week resulted in an odds ratio of 1.12 for high versus low IgE levels genetically and 1.01 observationally. For allergic disease the corresponding odds ratios were 0.95 (0.92-0.99) genetically and 1.00 (1.00-1.00) observationally. Taken together, this means that high alcohol consumption leads to high IgE levels, but not to allergic disease.

Discussion

Examined in 111,408 individuals from the general population, we observed that high alcohol consumption was associated observationally and genetically with high IgE levels, but not with high risk of allergic disease. The genetic data thus indicate that alcohol consumption leads to high IgE levels without an influence on allergic disease, the latter a reassuring finding. To the best of our knowledge, genetic Mendelian randomization studies like the present have never before been conducted to evaluate a potential causal relationship between alcohol consumption and IgE levels and allergic disease.

Mechanistically, it may seem puzzling that high alcohol consumption leads to high IgE levels, but not to allergic disease. The effects of alcohol on IgE production is not fully understood; however, it has been suggested that alcohol changes the balance of Th1 and Th2 responses towards a Th2 response of the immune system, resulting in increased levels of IgE(8;28); however, some studies involving humans have found increased levels of IgE in alcoholics despite a Th1 pattern of cytokine production, suggesting that the increase in IgE is by another mechanism. It has, thus, been suggested that either alcohol directly affect B-cells favoring an IgE switch or the increase of IgE is caused by an alcohol induced increased uptake of endotoxins from the gut(6;29).

Previous observational studies on moderately high alcohol consumption and alcoholism support that high alcohol consumption is associated with high plasma levels of IgE(5-12). The present data suggest that this is a causal relationship, which mechanistically may be because alcohol consumption could influence immune modulation, with cytokine production changing towards a Th2-dominated response in favor of IgE production(30). However, the exact mechanism from high alcohol consumption to high levels of IgE in plasma is largely unknown.

It is interesting that despite the apparent causal relationship between alcohol consumption and IgE levels, we found no association observationally or genetically between

alcohol intake and risk of allergic disease. Our observational results contradict those of a recent study on 34,525 adults, where ever drinking more than 12 drinks annually compared with lifetime abstaining was positively associated with eczema(31). Unlike our study, that study did however not include unconfounded genetic data free of reverse causation, and it investigated the risk of any type of eczema whereas our study only included allergic eczema.

A strength of the present study is the very large study population, resulting in sufficient statistical power even for the genetic Mendelian randomization studies. Also, the study population was selected randomly from the white Danish general population, securing a genetically homogenous study population and thus excluding population admixture as a problem in the present studies. Thus, because of the relative ethnic/racial homogeneity of the study population the number of variables is reduced, which reduces the possible confounding but decreases potential generalizability of the finding to other ethnic groups. However, we are not aware of data to suggest that the present findings should not apply to most ethnic groups.

Our study used self-reported alcohol consumption, which represents a potential limitation. Thus, even though the validity of self-reported alcohol consumption has been compared to alternative measurements and the reliability has been demonstrated to be reasonable (32), we cannot exclude a reporting bias since the questionnaire is based on standard drinks and thus expecting participants to know the size of standard drinks for each type of beverage. Furthermore, individuals with large alcohol consumption might have considered a standard drink to be larger than individuals with small alcohol consumption, resulting in the true difference in alcohol consumption between high and low consumers to be larger than that reported. However, for observational analyses this would only bias the results toward the null hypothesis and thus cannot explain our positive results. Theoretically, alcohol consumption could also influence our genetic results through

alcohol dehydrogenase gene expression; however, unlike for the microsomal ethanol oxidizing system, high alcohol consumption does not have a major influence on expression of ADH(33).

Further, as our studies was based on a self-reported questionnaire, it is unclear if individuals in the higher alcohol group was not bothered by allergy because some individuals in that group had had a lot of alcohol, if they had poor memory because of the high alcohol consumption, or sensory deficits associated from higher alcohol consumption. To clarify this, it would have been ideal to assess responses with standardized questionnaires looking at symptom scores, such as MiniRQLQ, SCORAD or ACQ, FEV1 comparisons, standardized memory levels, as alcohol can affect memory, B vitamin levels and liver function tests, and evaluation for neuropathy. However even in individuals with moderate alcohol consumption up to 20 drinks per week, a level of alcohol consumption unlikely to lead to poor memory or sensory deficit(34-37), we found no indication of increased risk of allergic disease. Another limitation is that the high alcohol group was more skewed towards consisting of males and smokers. Indeed, several studies suggest that males may have less allergy after puberty than females(38-42). Furthermore, other studies have suggested that the prevalence of allergic disease decreases with increasing age(39;43). Hence, it was possible that our findings were more a finding associated with sex, smoking variation, or age than true correlation to alcohol, but in analyses stratified for sex, age, and smoking status, high alcohol consumption was still not associated with high risk of allergic disease. Results from stratification should, however, always be interpreted carefully as stratification introduces the risk of collider stratification bias(44). Also, as birth order as well as sibship size may influence risk for allergic disease(45), it is a limitation of our study that such information was not available for adjustment. Further, compared to our observational estimate the genetic, causal estimate of an odds ratio for high versus low IgE levels for an increase of 1 alcoholic drink/week was much higher, a difference that should be interpreted cautiously. This is because the genetic calculation using instrumental

variable analyses depends on self-reported alcohol consumption, which may differ by genotype. In other words, people with the genotypes leading to high alcohol consumption may consume larger servings of beer, wine and spirit and may prefer the versions of these beverages with the highest alcohol content, compared to people with genotypes leading to low alcohol consumption. If this was the case, then the genetic instrumental variable estimate would be too high. Importantly, however, such a reporter bias on alcohol consumption cannot explain that we observed alcohol consumption to be likely causally associated with high IgE levels, it can only influence the magnitude of the effect. Alternatively, it is also possible that the higher genetic, causal estimate compared with the observational estimate is because genetics typically monitor an adulthood lifelong effect of elevated alcohol consumption, while the observational estimate just represents a single point estimate of the association between alcohol consumption and IgE levels. It is also worth considering if the alcohol dehydrogenase genes get expressed differently in relation to alcohol exposure. In other words, is there any compensatory mechanism, such that if there is higher exposure to alcohol chronically, more protein expression occurs to compensate for the chronic exposure, which may differ by genotype. The alcohol dehydrogenase genes have very complex regulatory mechanisms, and animal studies investigating the effect of alcohol on gene expression have indeed suggested an increased expression with alcohol exposure.(46;47)

Information on allergic disease was also based on questionnaires and was not reevaluated by physical examination or biochemical analyses, representing another potential limitation as we cannot be sure that all individuals have been properly diagnosed. Furthermore, as we could not define whether the reported eczema in the individual person represents atopic dermatitis or allergic contact eczema, we simply decided to use the term allergic eczema. However, study participants reporting allergic disease are probably suffering from allergic symptoms, and due

to the extensive and free Danish health care system, most individuals will likely have been diagnosed by a physician.

The clinical importance of our findings is worth considering. First, as high alcohol consumption did not lead to allergic disease, we see no reason to advise against alcohol consumption to prevent allergic disease. Second, in some individuals and patients with or without allergic disease, but with high IgE levels, high IgE levels may be explained by high alcohol consumption, rather than by allergic disease.

In conclusion, high alcohol consumption was associated observationally and genetically with high IgE levels, but not with high risk of allergic disease. Thus, high alcohol consumption seems to cause high IgE levels in plasma, but this IgE increase does not seem to lead to high risk of allergic disease like allergic asthma, allergic rhinitis or allergic eczema, the latter a reassuring finding.

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Contributors

Contribution: Frederikke K Lomholt, Sune F Nielsen, and Børge G Nordestgaard designed the study together. Frederikke K Lomholt and Sune F Nielsen analyzed and interpreted the data. Børge G Nordestgaard oversaw all analyses and contributed to the data interpretation. Frederikke K

456 Lomholt wrote the first draft of the paper. Sune F Nielsen and Børge G Nordestgaard edited the
457 paper. All authors approved the paper in its final form.

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Reference List

- (1) Platts-Mills TA. The role of immunoglobulin E in allergy and asthma. *Am J Respir Crit Care Med* 2001;164:S1-S5.
- (2) Carosso A, Bugiani M, Migliore E, Anto JM, DeMarco R. Reference values of total serum IgE and their significance in the diagnosis of allergy in young European adults. *Int Arch Allergy Immunol* 2007;142:230-8.
- (3) Pastorello EA, Incorvaia C, Ortolani C, Bonini S, Canonica GW, Romagnani S, et al. Studies on the relationship between the level of specific IgE antibodies and the clinical expression of allergy: I. Definition of levels distinguishing patients with symptomatic from patients with asymptomatic allergy to common aeroallergens. *J Allergy Clin Immunol* 1995;96:580-7.
- (4) Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001;107:891-6.
- (5) Barbee RA, Halonen M, Lebowitz M, Burrows B. Distribution of IgE in a community population sample: correlations with age, sex, and allergen skin test reactivity. *J Allergy Clin Immunol* 1981;68:106-11.
- (6) Dominguez-Santalla MJ, Vidal C, Vinuela J, Perez LF, Gonzalez-Quintela A. Increased serum IgE in alcoholics: relationship with Th1/Th2 cytokine production by stimulated blood mononuclear cells. *Alcohol Clin Exp Res* 2001;25:1198-205.
- (7) Gonzalez-Quintela A, Vidal C, Gude F, Tome S, Lojo S, Lorenzo MJ, et al. Increased serum IgE in alcohol abusers. *Clin Exp Allergy* 1995;25:756-64.
- (8) Gonzalez-Quintela A, Vidal C, Lojo S, Perez LF, Otero-Anton E, Gude F, et al. Serum cytokines and increased total serum IgE in alcoholics. *Ann Allergy Asthma Immunol* 1999;83:61-7.
- (9) Linneberg A, Nielsen NH, Madsen F, Frolund L, Dirksen A, Jorgensen T. Factors related to allergic sensitization to aeroallergens in a cross-sectional study in adults: The Copenhagen Allergy Study. *Clin Exp Allergy* 2001;31:1409-17.
- (10) Omenaas E, Bakke P, Elsayed S, Hanao R, Gulsvik A. Total and specific serum IgE levels in adults: relationship to sex, age and environmental factors. *Clin Exp Allergy* 1994;24:530-9.
- (11) Vidal C, Quintela AG, Millan I, Gude F, Cuervas-Mons V. Serum IgE levels in liver cirrhosis. Contrasting results in alcoholic and non-alcoholic patients. *Clin Exp Allergy* 1994;24:540-8.
- (12) Wuthrich B, Schindler C, Medici TC, Zellweger JP, Leuenberger P. IgE levels, atopy markers and hay fever in relation to age, sex and smoking status in a normal adult Swiss

population. SAPALDIA (Swiss Study on Air Pollution and Lung Diseases in Adults) Team. *Int Arch Allergy Immunol* 1996;111:396-402.

- (13) Pawankar R, Canonica GW, Holgate ST, Lockey RF, Blaiss M. The WAO White Book on Allergy (Update. 2013). 2013.
- (14) Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 2003;32:1-22.
- (15) Smith GD, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. *Int J Epidemiol* 2004;33:30-42.
- (16) Tolstrup JS, Nordestgaard BG, Rasmussen S, Tybjaerg-Hansen A, Gronbaek M. Alcoholism and alcohol drinking habits predicted from alcohol dehydrogenase genes. *Pharmacogenomics J* 2008;8:220-7.
- (17) Hurley TD, Edenberg HJ. Genes encoding enzymes involved in ethanol metabolism. *Alcohol Res* 2012;34:339-44.
- (18) Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey SG. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med* 2008;27:1133-63.
- (19) Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjaerg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *N Engl J Med* 2014;371:32-41.
- (20) Nielsen SF, Bojesen SE, Schnohr P, Nordestgaard BG. Elevated rheumatoid factor and long term risk of rheumatoid arthritis: a prospective cohort study. *BMJ* 2012;345:e5244.
- (21) Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007;298:299-308.
- (22) Thomsen M, Ingebrigtsen TS, Marott JL, Dahl M, Lange P, Vestbo J, et al. Inflammatory biomarkers and exacerbations in chronic obstructive pulmonary disease. *JAMA* 2013;309:2353-61.
- (23) Helby J, Bojesen SE, Nielsen SF, Nordestgaard BG. IgE and risk of cancer in 37 747 individuals from the general population. *Ann Oncol* 2015.
- (24) Lawlor DA, Nordestgaard BG, Benn M, Zuccolo L, Tybjaerg-Hansen A, Davey SG. Exploring causal associations between alcohol and coronary heart disease risk factors: findings from a Mendelian randomization study in the Copenhagen General Population Study. *Eur Heart J* 2013;34:2519-28.
- (25) Sethi AA, Tybjaerg-Hansen A, Andersen RV, Nordestgaard BG. Nanogen microelectronic chip for large-scale genotyping. *Clin Chem* 2004;50:443-6.

- (26) Wium-Andersen MK, Orsted DD, Tolstrup JS, Nordestgaard BG. Increased alcohol consumption as a cause of alcoholism, without similar evidence for depression: a Mendelian randomization study. *Int J Epidemiol* 2015;44:526-39.
- (27) Nordestgaard BG, Palmer TM, Benn M, Zacho J, Tybjaerg-Hansen A, Davey SG, et al. The effect of elevated body mass index on ischemic heart disease risk: causal estimates from a Mendelian randomisation approach. *PLoS Med* 2012;9:e1001212.
- (28) Starkenburg S, Munroe ME, Waltenbaugh C. Early alteration in leukocyte populations and Th1/Th2 function in ethanol-consuming mice. *Alcohol Clin Exp Res* 2001;25:1221-30.
- (29) Gonzalez-Quintela A, Vidal C, Gude F. Alcohol, IgE and allergy. *Addict Biol* 2004;9:195-204.
- (30) Linneberg A, Roursgaard M, Hersoug LG, Larsen ST. Effects of alcohol consumption on the allergen-specific immune response in mice. *Alcohol Clin Exp Res* 2008;32:553-6.
- (31) Silverberg JI, Greenland P. Eczema and cardiovascular risk factors in 2 US adult population studies. *J Allergy Clin Immunol* 2015;135:721-8.
- (32) Del Boca FK, Darkes J. The validity of self-reports of alcohol consumption: state of the science and challenges for research. *Addiction* 2003;98 Suppl 2:1-12.
- (33) Lieber CS. Metabolism of alcohol. *Clin Liver Dis* 2005;9:1-35.
- (34) Britton A, Singh-Manoux A, Marmot M. Alcohol consumption and cognitive function in the Whitehall II Study. *Am J Epidemiol* 2004;160:240-7.
- (35) Kalmijn S, van Boxtel MP, Verschuren MW, Jolles J, Launer LJ. Cigarette smoking and alcohol consumption in relation to cognitive performance in middle age. *Am J Epidemiol* 2002;156:936-44.
- (36) Panza F, Capurso C, D'Introno A, Colacicco AM, Frisardi V, Lorusso M, et al. Alcohol drinking, cognitive functions in older age, predementia, and dementia syndromes. *J Alzheimers Dis* 2009;17:7-31.
- (37) Zuccala G, Onder G, Pedone C, Cesari M, Landi F, Bernabei R, et al. Dose-related impact of alcohol consumption on cognitive function in advanced age: results of a multicenter survey. *Alcohol Clin Exp Res* 2001;25:1743-8.
- (38) Almqvist C, Worm M, Leynaert B. Impact of gender on asthma in childhood and adolescence: a GA2LEN review. *Allergy* 2008;63:47-57.
- (39) Osman M, Hansell AL, Simpson CR, Hollowell J, Helms PJ. Gender-specific presentations for asthma, allergic rhinitis and eczema in primary care. *Prim Care Respir J* 2007;16:28-35.
- (40) Simpson BM, Custovic A, Simpson A, Hallam CL, Walsh D, Marolia H, et al. NAC Manchester Asthma and Allergy Study (NACMAAS): risk factors for asthma and allergic disorders in adults. *Clin Exp Allergy* 2001;31:391-9.

- (41) Siroux V, Curt F, Oryszczyn MP, Maccario J, Kauffmann F. Role of gender and hormone-related events on IgE, atopy, and eosinophils in the Epidemiological Study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy. *J Allergy Clin Immunol* 2004;114:491-8.
- (42) Vink NM, Postma DS, Schouten JP, Rosmalen JG, Boezen HM. Gender differences in asthma development and remission during transition through puberty: the TRacking Adolescents' Individual Lives Survey (TRAILS) study. *J Allergy Clin Immunol* 2010;126:498-504.
- (43) Wolkewitz M, Rothenbacher D, Low M, Stegmaier C, Ziegler H, Radulescu M, et al. Lifetime prevalence of self-reported atopic diseases in a population-based sample of elderly subjects: results of the ESTHER study. *Br J Dermatol* 2007;156:693-7.
- (44) Cole SR, Platt RW, Schisterman EF, Chu H, Westreich D, Richardson D, et al. Illustrating bias due to conditioning on a collider. *Int J Epidemiol* 2010;39:417-20.
- (45) Strachan DP, Ait-Khaled N, Foliaki S, Mallol J, Odhiambo J, Pearce N, et al. Siblings, asthma, rhinoconjunctivitis and eczema: a worldwide perspective from the International Study of Asthma and Allergies in Childhood. *Clin Exp Allergy* 2015;45:126-36.
- (46) Crabb DW, Matsumoto M, Chang D, You M. Overview of the role of alcohol dehydrogenase and aldehyde dehydrogenase and their variants in the genesis of alcohol-related pathology. *Proc Nutr Soc* 2004;63:49-63.
- (47) He L, Ronis MJ, Badger TM. Ethanol induction of class I alcohol dehydrogenase expression in the rat occurs through alterations in CCAAT/enhancer binding proteins beta and gamma. *J Biol Chem* 2002;277:43572-7.

Figure legends

Figure 1.

Comparison of the study design from observational studies, Mendelian randomization studies, and randomized controlled trials as applied to high and low alcohol consumption on IgE levels and risk of allergic disease.

Figure 2.

Number individuals entering into the different analyses.

Figure 3.

Associations between allergic disease and IgE levels examined in 38,802 individuals from the Copenhagen General Population Study. The top graph shows the association between allergic disease score and IgE levels, while the bottom graph shows the association between individual allergic diseases and IgE levels. Left: multivariable adjusted odds ratios. Right: geometric means. OR= odds ratio. CI= confidence interval.

Figure 4.

Association between self-reported alcohol consumption and allergic disease and IgE levels, examined in 47,336 individuals from the Copenhagen General Population Study and Copenhagen City Heart Study combined. The association was studied both overall and stratified for allergic disease status. Left: multivariable adjusted odds ratios. Right: crude geometric mean. OR= odds ratio. CI= confidence interval.

Figure 5.

Association between self-reported alcohol consumption and IgE levels, examined in 102,843 individuals from the Copenhagen General Population Study and Copenhagen City Heart Study combined. OR= odds ratio. CI= confidence interval.

Figure 6.

Associations between ADH-genotype combinations and IgE levels examined in 44,848 individuals from the Copenhagen General Population Study and Copenhagen City Heart Study combined. OR= odds ratio. CI= confidence interval.

Figure 7.

Observational and genetic estimates for increase in IgE levels with an increase in alcohol consumption of 1 drink/week. 102,270 individuals with information on genotype from the Copenhagen General Population Study and Copenhagen City Heart Study were included in the analyses. The analysis was performed based on the entire study group and stratified for gender. OR= odds ratio. CI= confidence interval.

Table I. Baseline characteristics of 111,408 individuals from the general population

	Total population	Observational analyses			Genetic analyses		
	n = 111,480	Allergic disease and IgE n = 38,802	Alcohol consumption and IgE n = 47,336	Alcohol consumption and allergic disease n = 102,843	Genotype and alcohol consumption n = 97,687	Genotype and IgE n = 44,848	Genotype and allergic disease n = 98,457
Sex							
Men	45	46	46	46	46	45	55
Women	55	54	54	54	54	56	45
Age	57 (48-67)	59 (49-68)	58 (49-67)	57 (48-67)	57 (48-66)	58 (48-67)	57 (47-66)
BMI	26 (23-28)	26 (23-28)	26 (23-28)	26.1 (23-28)	26.1 (23-28)	26.0 (23-28)	26.1 (23-28)
Education							
Elementary (1 - 9 years)	30	24	33	30	28	29	29
High school (10 - 12 years)	53	57	51	53	54	54	54
Academic (> 12 years)	17	19	16	17	17	17	17
Civil status							
Married/cohabiting	74	76	74	75	75	75	75
Unmarried	8	6	8	8	8	7	8
Separated/divorced	10	9	10	10	10	10	10
Widow/widower	8	8	8	8	8	8	8
Smoking status							
Never smoker	39	43	38	39	40	40	40
Former smoker	38	42	38	39	40	40	39
Current smoker	22	15	24	22	20	20	21
Pack-years	22 (7-31)	21 (6-30)	23 (8-32)	22 (7-32)	22 (7-30)	22 (7-30)	22 (7-30)
Cigarettes/day	15 (10-20)	15 (9-20)	16 (10-20)	15 (10-20)	15 (10-20)	15(10-20)	15 (10-20)
Physical activity during work							
Mainly sitting	30	31	31	31	30	30	30
Sitting, standing, walking	25	23	26	25	25	25	25
Walking and lifting	15	12	14	14	15	14	15
Heavy workload	3	3	3	3	3	3	3
Not working	27	32	26	27	28	28	28
Physical activity during							

leisure

< 2 hours/week	8	7	8	7	7	7	7
2-4 hours/week	43	41	43	43	43	43	43
> 4 hours/week light	43	46	43	43	44	44	44
> 4 hours/week heavy	6	7	6	6	6	6	6

Values are shown as percent or mean (interquartile range).

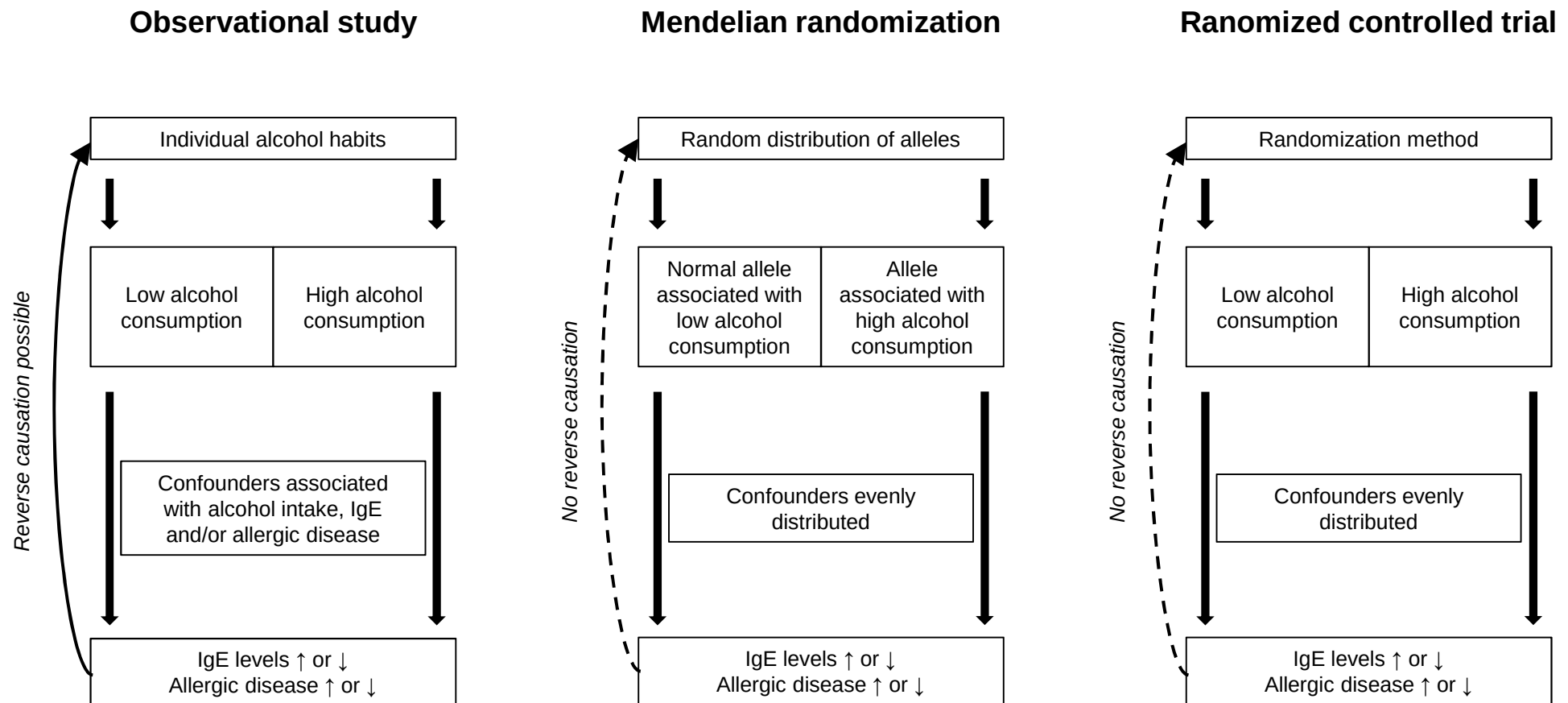


Figure 1

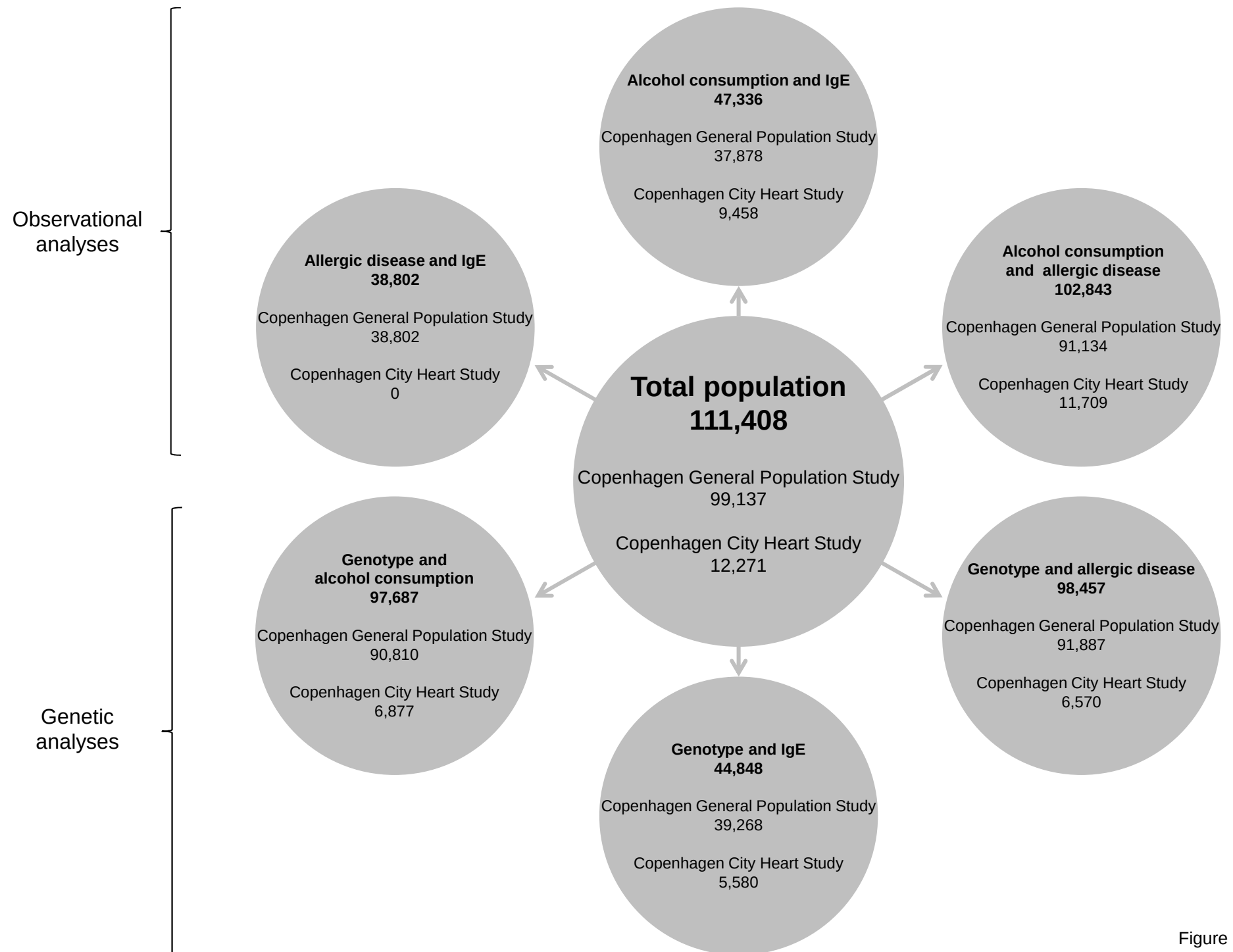


Figure 2

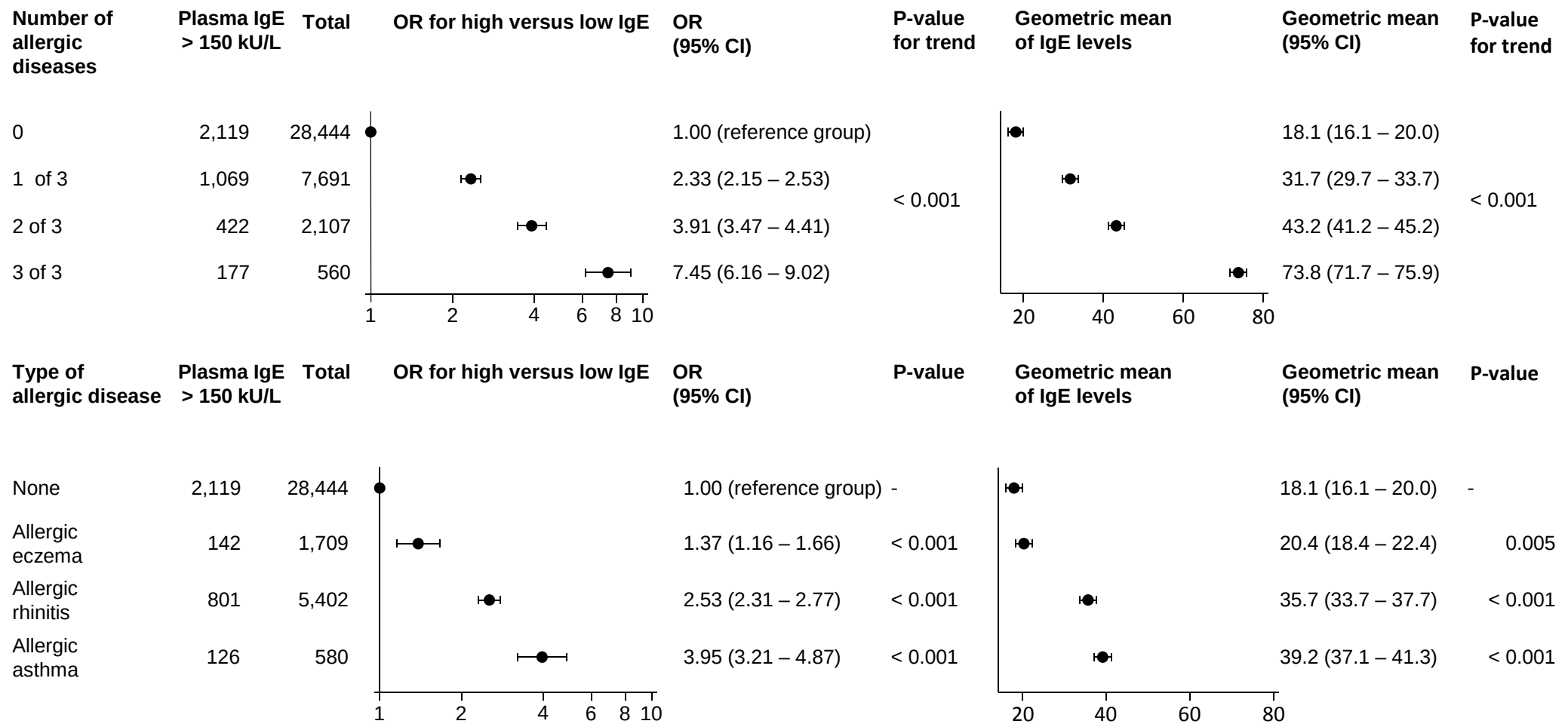
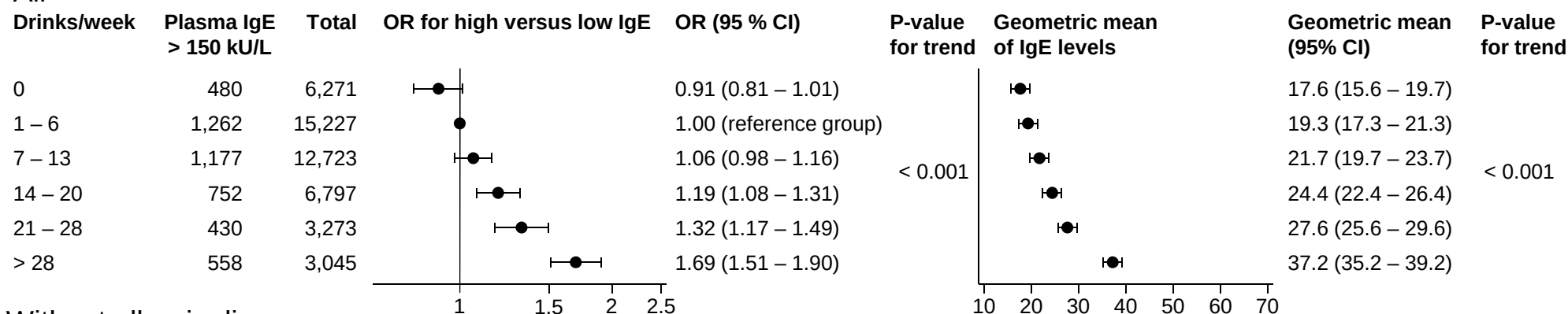
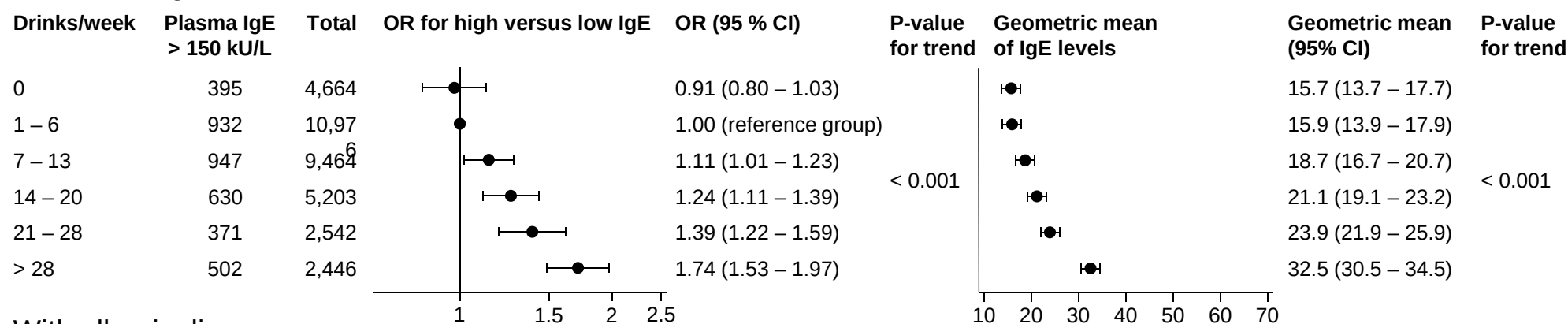


Figure 3

All



Without allergic disease



With allergic disease

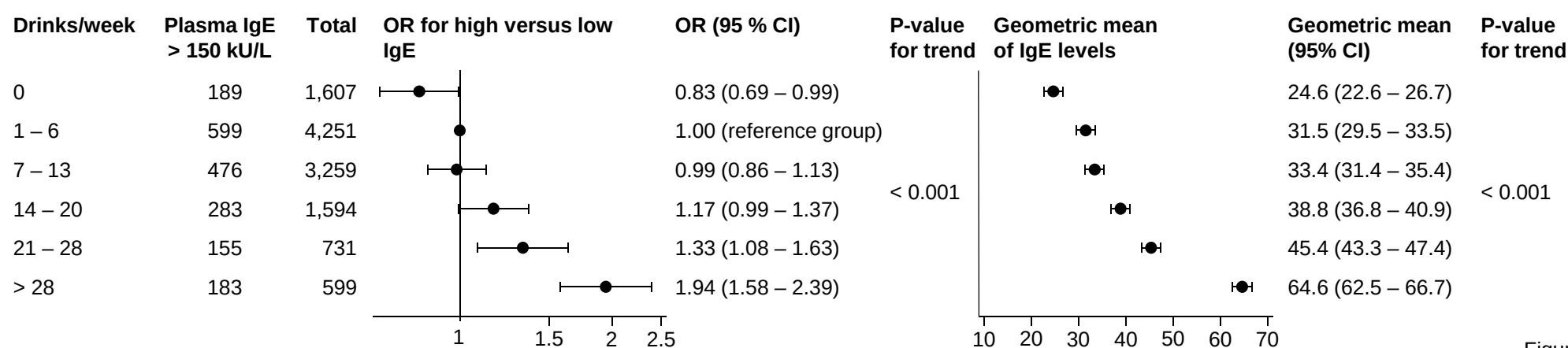


Figure 4

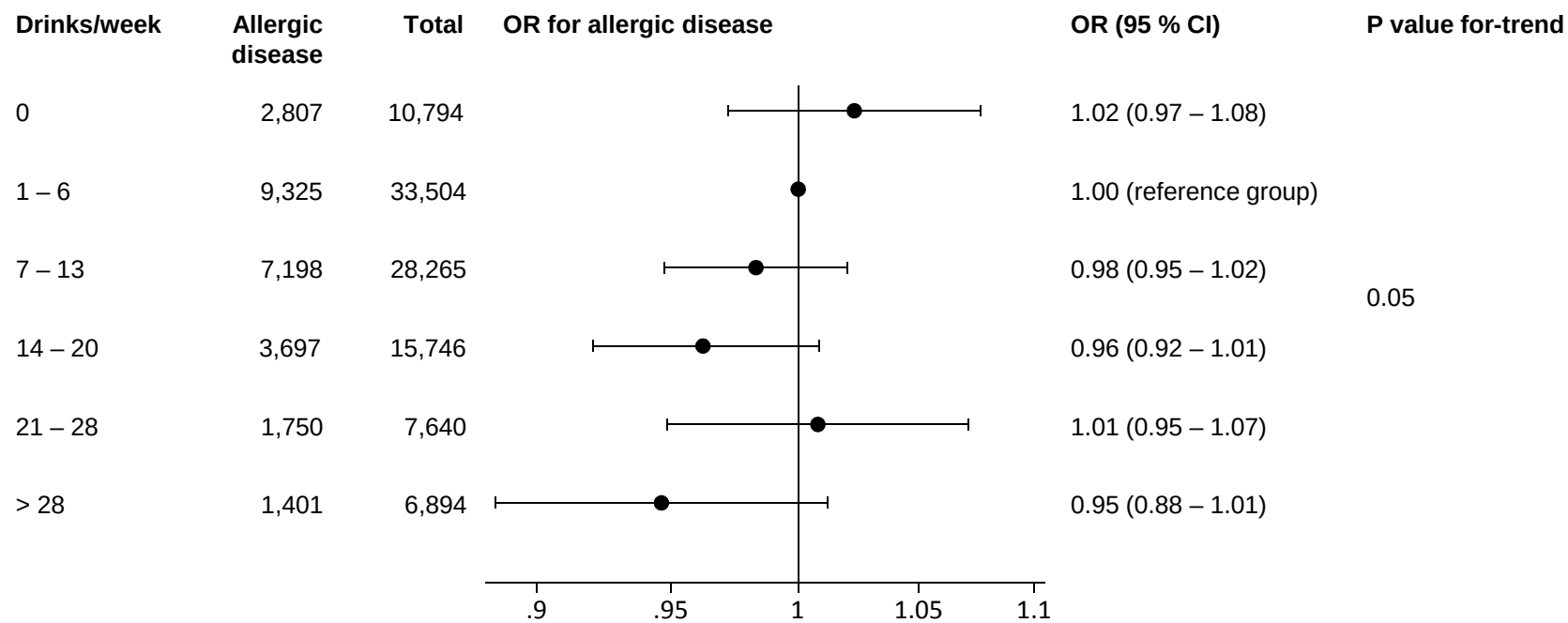


Figure 5

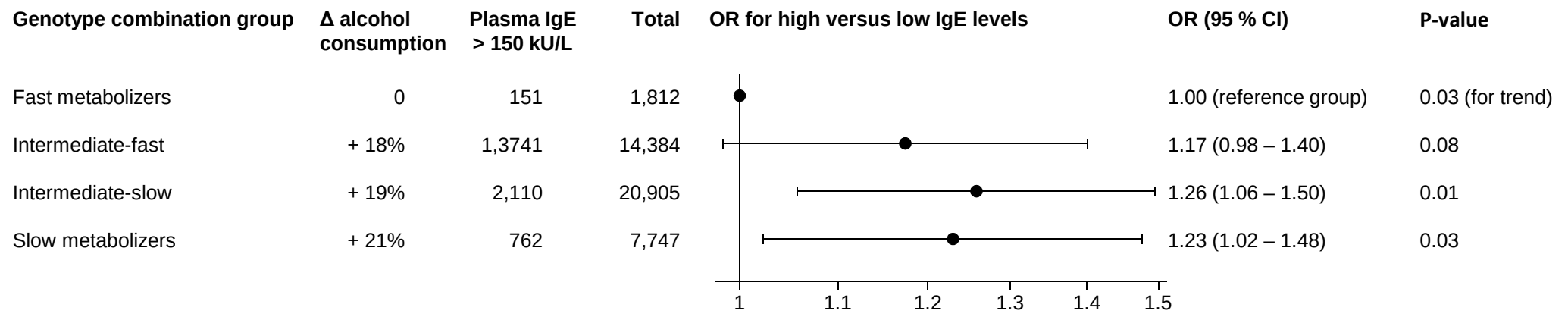


Figure 6

High versus low IgE

Type of analysis	IgE > 150 kU/L	Total		OR (95 % CI)	P-value
Observational	4,876	48,961		1.013 (1.011 – 1.016)	< 0.001
Genetic	4,394	44,848		1.118 (1.018 – 1.228)	0.02

Allergic disease versus no allergic disease

Type of analysis	Allergic disease	Total		OR (95 % CI)	P-value
Observational	26,178	102,843		0.999 (0.997 – 1.000)	0.12
Genetic	25,283	98,457		0.959 (0.923 – 0.996)	0.03

OR (95% CI) per 1 alcoholic drink/week

Table E1. Association between genotype and alcohol consumption**Genotype**

ADH-1B		n	Mean (drinks/week)	SE (mean)	% difference	P	R ²	F
ADH-1B.2/2	fast	39	7.4	1.26	0	6·10 ⁻²⁵	0.0011	53
ADH-1B.2/1	fast	3,905	9.1	0.14	+ 23 %			
ADH-1B.1/1	slow	93,743	10.8	0.03	+ 47 %			

ADH-1C		n	Mean (drinks/week)	SE (mean)	% difference	P	R ²	F
ADH-1C.1/1	Fast	33,177	10.6	0.06	0	0.0002	0.0004	8
ADH-1C.1/2	Intermediate	47,514	10.7	0.05	+ 0.3 %			
ADH-1C.2/2	slow	16,996	11.0	0.08	+ 3.0 %			

Genotype combination groups

Genotype combination group	n	Mean	SE (mean)	% difference	P	R ²	F
(1) Fast metabolizers	3,944	9.1	0.14	0	9·10 ⁻¹³	0.0012	38
(2) Intermediate-fast	31,023	10.7	0.06	+ 18 %			
(3) Intermediate-slow	45,818	10.8	0.05	+ 19 %			
(4) Slow metabolizers	16,902	11.0	0.08	+ 21 %			

Genotype combination group	ADH-1B	ADH-1C	n	Mean	Combined
(1) Fast metabolizers	Fast (2/2)	Fast (1/1)	32	6.6	9.1
(1) Fast metabolizers	Fast (2/2)	Intermediate (1/2)	7	10.9	
(1) Fast metabolizers	Fast (2/1)	Fast (1/1)	2,122	9.1	
(1) Fast metabolizers	Fast (2/1)	Intermediate (1/2)	1,689	9.1	
(1) Fast metabolizers	Fast (2/2)	Slow (2/2)	94	9.1	
(2) Intermediate-fast	Slow (1/1)	Fast (1/1)	31,023	10.7	10.7
(3) Intermediate-slow	Slow (1/1)	Intermediate (1/2)	45,818	10.8	10.8
(4) Slow metabolizers	Slow (1/1)	Slow (2/2)	16,902	11	11

Table E2. Baseline characteristics of 38,802 individuals with information on number of allergic diseases and IgE measurement

	Number of allergic diseases				P-value for trend
	0	1 of 3	2 of 3	3 of 3	
Sex					< 0.001
Men	13,619 (48)	3,298 (43)	766 (36)	179 (32)	
Women	14,825 (52)	4,393 (57)	1,341 (64)	381 (68)	
Age	60 (50-69)	57 (47-65)	55 (46-63)	54 (46-62)	< 0.001
BMI	26 (23-28)	26 (23-28)	26 (23-28)	27 (23-29)	0.65
Education					< 0.001
Elementary (1 – 9 years)	7,491 (26)	1,415 (18)	345 (16)	89 (16)	
High school (10 – 12 years)	15,903 (56)	4,579 (60)	1,248 (59)	335 (60)	
Academic (> 12 years)	5,050 (18)	1,697 (22)	514 (24)	136 (24)	
Civil status					0.11
Married/cohabiting	21,717 (76)	5,903 (77)	1,623 (77)	417 (75)	
Unmarried	1,745 (6)	536 (7)	167 (8)	44 (8)	
Separated/divorced	2,524 (9)	769 (10)	218 (10)	72 (13)	
Widow/widower	2,458 (9)	483 (6)	99 (5)	27 (5)	
Smoking status					< 0.001
Never smoker	11,661 (41)	3,614 (47)	949 (45)	250 (45)	
Former smoker	12,106 (43)	3,154 (41)	908 (43)	226 (40)	
Current smoker	4,677 (16)	923 (12)	250 (12)	84 (15)	
Pack-years	22 (7-31)	19 (5-26)	17.0 (5-24.0)	18 (6-28)	< 0.001
Cigarettes/day	15 (9-20)	15 (9-20)	14 (7-20)	15 (10-20)	0.22
Alcohol consumption (drinks/week)					< 0.001
0	2,525 (9)	685 (9)	229 (11)	72 (13)	
1 – 6	8,958 (32)	2,694 (35)	819 (39)	208 (37)	
7 – 13	8,194 (29)	2,233 (29)	586 (28)	153 (27)	
14 – 20	4,599 (16)	1,138 (15)	267 (13)	67 (12)	
21 – 27	2,277 (8)	529 (7)	124 (6)	42 (8)	
≥ 28	1,891 (7)	412 (5)	82 (4)	18 (3)	
Physical activity during work					< 0.001
Mainly sitting	8,245 (29)	2,671 (35)	767 (36)	186 (33)	
Sitting, standing, walking	6,246 (22)	1,877 (24)	562 (27)	159 (28)	
Walking and lifting	3,413 (12)	1,032 (13)	290 (14)	72 (13)	
Heavy workload	709 (2)	136 (2)	33 (2)	8 (1)	

Not working	9,831 (35)	1,975 (26)	455 (22)	135 (24)	
Physical activity during leisure					0.17
< 2 hours/week	1,718 (6)	416 (5)	136 (6)	42 (8)	
2-4 hours/week	11,763 (41)	3,185 (41)	864 (41)	249 (44)	
> 4 hours/week light	13,011 (46)	3,562 (46)	949 (45)	235 (42)	
> 4 hours/week heavy	1,954 (7)	528 (7)	158 (8)	34 (6)	

Values are shown as number (percent) or mean (interquartile range).

Table E3. Baseline characteristics of 38,802 individuals with information on allergic disease and IgE measurement

	Type of allergic disease				P-value versus no allergic disease		
	None	Allergic eczema	Allergic rhinitis	Allergic asthma	Allergic eczema	Allergic rhinitis	Allergic asthma
Sex					< 0.001	0.45	< 0.001
Men	13,619 (48)	453 (27)	2,617 (49)	228 (39)			
Women	14,825 (52)	1,256 (74)	2,785 (52)	352 (61)			
Age	60 (50-69)	57 (47-66)	56 (47-65)	60 (50-69)	< 0.001	< 0.001	0.65
BMI	26 (23-28)	26 (23-28)	26 (23-28)	27 (24-29)	0.002	< 0.001	< 0.001
Education					< 0.001	< 0.001	0.50
Elementary (1 – 9 years)	7,491 (26)	349 (20)	912 (17)	154 (27)			
High school (10 – 12 years)	15,903 (56)	983 (58)	3,286 (61)	310 (54)			
Academic (> 12 years)	5,050 (18)	377 (22)	1,204 (22)	116 (20)			
Civil status					0.002	< 0.001	0.15
Married/cohabiting	21,717 (76)	1,240 (73)	4,236 (78)	427 (74)			
Unmarried	1,745 (6)	137 (6)	360 (7)	39 (7)			
Separated/divorced	2,524 (9)	197 (12)	510 (9)	62 (11)			
Widow/widower	2,458 (9)	135 (8)	296 (6)	52 (9)			
Smoking status					0.90	< 0.001	0.82
Never smoker	11,661 (41)	693 (41)	2,677 (50)	244 (42)			
Former smoker	12,106 (43)	725 (42)	2,171 (40)	258 (45)			
Current smoker	4,677 (16)	291 (17)	554 (10)	78 (14)			
Pack-years	22 (7-31)	21 (6-29)	17 (5-25)	22 (6-32)	0.02	< 0.001	0.73
Cigarettes/day	15 (9-20)	16 (10-20)	14 (7-20)	17 (10-20)	0.78	0.07	0.20
Alcohol consumption (drinks/week)					< 0.001	< 0.001	< 0.001
0	2,525 (9)	186 (11)	432 (9)	67 (12)			
1 – 6	8,958 (32)	632 (37)	1,853 (34)	209 (36)			
7 – 13	8,194 (29)	464 (27)	1,609 (30)	160 (28)			
14 – 20	4,599 (16)	244 (14)	819 (15)	75 (13)			
21 – 27	2,277 (8)	109 (6)	385 (7)	35 (6)			
≥ 28	1,891 (7)	74 (4)	304 (6)	34 (6)			
Physical activity during work					< 0.001	< 0.001	< 0.001
Mainly sitting	8,245 (29)	1,113 (32)	2,920 (37)	730 (34)			
Sitting, standing, walking	6,246 (22)	923 (27)	2,028 (26)	527 (24)			
Walking and lifting	3,413 (12)	510 (15)	1,035 (13)	283 (13)			
Heavy workload	709 (2)	53 (2)	133 (2)	40 (2)			

Not working	9,831 (35)	870 (25)	1,830 (23)	590 (27)			
Physical activity during leisure					0.03	0.16	0.06
< 2 hours/week	1,718 (6)	234 (7)	437 (6)	143 (7)			
2-4 hours/week	11,763 (41)	1,485 (42)	3,246 (41)	929 (43)			
> 4 hours/week light	13,011 (46)	1,542 (44)	2,691 (46)	932 (43)			
> 4 hours/week heavy	1,952 (7)	208 (6)	572 (7)	166 (8)			

Values are shown as number (percent) or mean (interquartile range).

Table E4. Baseline characteristics of 47,336 individuals with information on alcohol consumption and IgE measurements

	Alcohol consumption (drinks/week)						P-value for trend
	0	1 – 6	7 – 13	14 – 20	21 – 27	≥ 28	
Sex							< 0.001
Men	1,615 (26)	5,337(35)	6,058 (48)	4,006 (59)	2,324 (71)	2,574 (85)	
Women	4,656 (74)	9,890 (65)	6,665 (52)	2,791 (41)	949 (29)	471 (16)	
Age	58 (49-67)	56 (46-65)	59 (50-68)	61 (52-69)	62 (54-69)	60 (53-68)	< 0.001
BMI	26 (23-29)	26 (23-28)	26 (23-28)	26 (23-28)	26 (24-28)	27 (24-29)	< 0.001
Education							< 0.001
Elementary (1 – 9 years)	3,415 (55)	4,440 (29)	3,542 (28)	1,972 (29)	947 (29)	1,164 (38)	
High school (10 – 12 years)	2,133 (34)	7,875 (52)	6,956 (55)	3,806 (56)	1,871 (57)	1,511 (50)	
Academic (> 12 years)	723 (12)	2,912 (19)	2,225 (18)	1,019 (15)	455 (14)	370 (12)	
Civil status							< 0.001
Married/cohabiting	3,763 (60)	11,239 (74)	9,926 (78)	5,389 (79)	2,614 (80)	2,279 (75)	
Unmarried	780 (12)	1,251 (8)	720 (6)	379 (6)	171 (5)	248 (8)	
Separated/divorced	862 (14)	1,541 (10)	1,050 (8)	541 (8)	269 (8)	338 (11)	
Widow/widower	866 (14)	1,196 (8)	1,027 (8)	488 (7)	219 (7)	180 (6)	
Smoking status							< 0.001
Never smoker	2,247 (36)	6,932 (46)	5,168 (41)	2,211 (33)	904 (28)	563 (19)	
Former smoker	1,783 (29)	5,242 (34)	5,147 (41)	3,079 (45)	1,536 (47)	1,313 (43)	
Current smoker	2,241 (36)	3,053 (20)	2,408 (19)	1,507 (22)	833 (26)	1,169 (38)	
Pack-years	24 (10-34)	20(56-28)	21 (6-30)	23 (8-33)	27 (11.40)	35 (16-46)	< 0.001
Cigarettes/day	15 (10-20)	14 (9-20)	15 (9-20)	15 (10-20)	17 (10-20)	21 (14-27)	< 0.001
Allergic disease							< 0.001
No allergic disease	4,664 (74)	10,976 (72)	9,464 (74)	5,203 (77)	2,542 (78)	2,446 (80)	
Allergic disease	1,607 (26)	4,251 (28)	3,259 (26)	1,594 (24)	731 (22)	599 (20)	
Physical activity during work							< 0.001
Mainly sitting	1,739 (28)	5,125 (34)	4,162 (33)	2,064 (30)	977 (30)	782 (26)	
Sitting, standing, walking	2,030 (32)	4,120 (27)	3,159 (25)	1,619 (24)	671 (21)	608 (20)	
Walking and lifting	1,103 (18)	2,426 (16)	1,489 (12)	764 (11)	366 (11)	438 (14)	
Heavy workload	117 (2)	282 (2)	258 (2)	190 (3)	114 (3)	221 (7)	
Not working	1,282 (20)	3,274 (22)	3,655 (29)	2,160 (32)	1,145 (35)	996 (33)	
Physical activity during leisure							< 0.001
< 2 hours/week	979 (16)	1,137 (7)	718 (6)	421 (6)	209 (6)	341 (11)	
2-4 hours/week	2,957 (47)	6,641 (44)	5,230 (41)	2,775 (41)	1,344 (41)	1,247 (41)	

> 4 hours/week light	2,062 (33)	6,599 (43)	5,918 (47)	3,159 (47)	1,489 (45)	1,268 (42)
> 4 hours/week heavy	273 (4)	850 (6)	857 (7)	442 (7)	231 (7)	189 (6)

Values are shown as number (percent) or mean (interquartile range).

Table E5. Baseline characteristics of 102,843 individuals with information on alcohol consumption and allergic disease

	Alcohol consumption (drinks/week)						P-value for trend
	0	1 – 6	7 – 13	14 – 20	21 – 28	> 28	
Sex							< 0.001
Men	2,764 (26)	11,364 (34)	13,102 (46)	8,986 (57)	5,386 (71)	5,750 (83)	
Women	8,030 (74)	22,140 (66)	15,163 (54)	6,760 (43)	2,254 (30)	1,144 (17)	
Age	57 (47-67)	55 (45-64)	58 (48-67)	60 (51-68)	61 (53-69)	60 (53-68)	< 0.001
BMI	27 (23-29)	26 (22-28)	26 (23-28)	26 (23-28)	26 (24-28)	27 (24-29)	< 0.001
Education							0.002
Elementary (1 – 9 years)	5,251 (49)	8,979 (27)	7,678 (27)	4,416 (28)	2,156 (28)	2,447 (36)	
High school (10 – 12 years)	4,119 (39)	17,824 (53)	15,660 (55)	8,903 (57)	4,387 (57)	3,605 (52)	
Academic (> 12 years)	1,424 (13)	6,701 (20)	4,927 (17)	2,427 (15)	1,097 (14)	842 (12)	
Civil status							< 0.001
Married/cohabiting	6,414 (60)	24,511 (73)	22,007 (78)	12,468 (79)	6,145 (80)	5,214 (76)	
Unmarried	1,426 (13)	2,902 (9)	1,762 (6)	859 (6)	434 (6)	574 (8)	
Separated/divorced	1,541 (14)	3,469 (10)	2,378 (8)	1,291 (8)	561 (7)	709 (10)	
Widow/widower	1,413 (13)	2,622 (8)	2,118 (8)	1,128 (7)	500 (7)	397 (6)	
Smoking status							< 0.001
Never smoker	4,118 (38)	15,611 (47)	11,722 (42)	5,293 (34)	2,116 (28)	1,373 (20)	
Former smoker	3,138 (29)	11,590 (36)	11,440 (41)	7,048 (45)	3,634 (48)	3,012 (44)	
Current smoker	3,538 (33)	6,303 (16)	5,103 (18)	3,405 (22)	1,890 (25)	2,509 (37)	
Pack-years	24 (10-34)	18.5 (5-27)	20 (6-30)	23 (8-32)	26 (10-37)	34 (15-45)	< 0.001
Cigarettes/day	15 (10-20)	14 (8 -20)	14 (8-20)	15 (10-20)	17 (10-20)	21 (14-25)	< 0.001
Physical activity during work							< 0.001
Mainly sitting	2,830 (26)	10,953 (33)	9,030 (32)	4,724 (30)	2,287 (30)	1,799 (26)	
Sitting, standing, walking	3,173 (29)	8,927 (27)	7,122 (25)	3,736 (25)	1,630 (21)	1,351 (20)	
Walking and lifting	1,994 (18)	5,545 (17)	3,576 (13)	1,896 (12)	873 (11)	943 (14)	
Heavy workload	217 (2)	681 (2)	635 (2)	446 (3)	252 (3)	461 (7)	
Not working	2,580 (24)	7,398 (22)	7,902 (28)	4,944 (31)	2,598 (34)	2,340 (34)	
Physical activity during leisure							< 0.001
< 2 hours/week	1,606 (15)	2,421 (7)	1,572 (6)	880 (6)	444 (6)	676 (10)	
2-4 hours/week	5,127 (48)	14,843 (44)	11,733 (42)	6,553 (42)	3,150 (41)	2,853 (41)	
> 4 hours/week light	3,554 (33)	14,283 (43)	13,111 (46)	7,311 (56)	3,504 (46)	2,905 (42)	
> 4 hours/week heavy	507 (5)	1,957 (6)	1,849 (7)	1,002 (6)	542 (7)	460 (7)	

Values are shown as number (percent) or mean (interquartile range).

Table E6. Baseline characteristics of 97,687 individuals with information on genotype and alcohol consumption

	Genotype combination group				P-value for trend
	Fast metabolizers	Intermediate-fast	Intermediate-slow	Slow metabolizers	
Sex					< 0.001
Men	1,873 (48)	14,324 (46)	20,872 (46)	7,534 (45)	
Women	2,071 (53)	16,699 (54)	24,946 (55)	9,368 (55)	
Age	58 (48-67)	57 (47-66)	57 (48-66)	57 (48-66)	0.79
BMI	26 (23-28)	26 (23-28)	26 (23-28)	26 (23-28)	0.06
Education					0.05
Elementary (1 – 9 years)	1,053 (27)	8,644 (28)	12,984 (28)	4,795 (28)	
High school (10 – 12 years)	2,188 (56)	16,914 (55)	24,932 (54)	9,145 (54)	
Academic (> 12 years)	703 (18)	5,465 (18)	7,902 (17)	2,962 (18)	
Civil status					0.07
Married/cohabiting	2,947 (75)	23,309 (75)	34,439 (75)	12,839 (76)	
Unmarried	308 (8)	2,404 (8)	3,444 (8)	1,264 (8)	
Separated/divorced	384 (10)	2,968 (10)	4,375 (10)	1,559 (9)	
Widow/widower	305 (8)	2,342 (8)	3,560 (8)	1,240 (7)	
Smoking status					0.26
Never smoker	1,520 (39)	12,480 (40)	18,377 (40)	6,785 (40)	
Former smoker	1,613 (41)	12,313 (40)	18,077 (40)	6,669 (40)	
Current smoker	811 (21)	6,230 (20)	9,364 (20)	3,448 (20)	
Pack-years	22 (7-32)	22 (7-30)	22 (7-30)	22 (7-30)	0.60
Cigarettes/day	15 (9-20)	15 (10-20)	15 (10-20)	15 (10-20)	0.94
Physical activity during work					0.72
Mainly sitting	1,223 (31)	9,374 (30)	13,733 (30)	5,167 (31)	
Sitting, standing, walking	955 (24)	7,751 (25)	11,329 (25)	4,191 (25)	
Walking and lifting	554 (14)	4,547 (15)	6,738 (15)	2,401 (14)	
Heavy workload	108 (3)	809 (3)	1,221 (3)	428 (3)	
Not working	1,104 (28)	8,542 (28)	12,797 (28)	4,715 (28)	
Physical activity during leisure					0.52
< 2 hours/week	249 (6)	2,087 (7)	3,011 (7)	1,144 (7)	
2-4 hours/week	1,661 (42)	13,296 (43)	19,805 (43)	7,328 (43)	
> 4 hours/week light	1,761 (45)	13,670 (44)	20,165 (44)	7,397 (44)	
> 4 hours/week heavy	273 (7)	1,970 (6)	2,837 (6)	1,033 (6)	

Values are shown as number (percent) or mean (interquartile range).

Table E7. Baseline characteristics of 44,848 individuals with information on genotype and IgE measurements

	Genotype combination group				P-value for trend
	Fast metabolizers	Intermediate-fast	Intermediate-slow	Slow metabolizers	
Sex					0.003
Men	862 (47.6)	6,619 (46)	9,465 (45)	3,434 (44)	
Women	950 (52.4)	7,765 (54)	11,440 (55)	4,313 (56)	
Age	59 (49-68)	58 (48-67)	58 (48-67)	58 (49-67)	0.97
BMI	26 (23-28)	26 (23-28)	26 (23-28)	26 (23-28)	0.045
Education					0.008
Elementary (1 – 9 years)	507 (28)	4,121 (29)	6,146 (29)	2,310 (30)	
High school (10 – 12 years)	994 (55)	7,690 (54)	11,147 (53)	4,146 (54)	
Academic (> 12 years)	311 (17)	2,573 (18)	3,612 (17)	1,291 (17)	
Civil status					0.68
Married/cohabiting	1,352 (75)	10,789 (75)	15,767 (75)	5,816 (75)	
Unmarried	133 (7)	1,070 (7)	1,512 (7)	567 (7)	
Separated/divorced	192 (11)	1,387 (10)	1,987 (10)	734 (10)	
Widow/widower	135 (8)	1,138 (8)	1,639 (8)	630 (8)	
Smoking status					0.86
Never smoker	714 (40)	5,815 (40)	8,317 (40)	3,107 (41)	
Former smoker	734 (41)	5,681 (40)	8,253 (40)	3,079 (40)	
Current smoker	364 (20)	2,888 (20)	4,335 (21)	1,561 (20)	
Pack-years	23 (7-32)	22 (7-30)	22 (7-30)	22 (7-30)	0.87
Cigarettes/day	16 (10-20)	15 (10-20)	16 (10 – 20)	15 (10-20)	0.59
Physical activity during work					0.67
Mainly sitting	566 (31)	4,412 (31)	6,300 (30)	2,375 (31)	
Sitting, standing, walking	420 (23)	3,623 (25)	5,202 (25)	1,889 (24)	
Walking and lifting	263 (15)	1,986 (14)	2,975 (14)	1,057 (14)	
Heavy workload	48 (3)	342 (2)	529 (3)	195 (3)	
Not working	515 (28)	4,021 (28)	5,899 (28)	2,231 (29)	
Physical activity during leisure					0.24
< 2 hours/week	110 (6)	992 (7)	1,410 (7)	550 (7)	
2-4 hours/week	758 (42)	6,089 (42)	8,914 (43)	3,335 (43)	
> 4 hours/week light	818 (45)	6,361 (44)	9,322 (45)	3,358 (43)	
> 4 hours/week heavy	126 (7)	942 (7)	1,259 (6)	504 (7)	

Values are shown as number (percent) or mean (interquartile range).

Table E8. Baseline characteristics of 98,457 individuals with information on genotype and allergic disease

	Genotype combination group				P-value for trend
	Fast metabolizers	Intermediate-fast	Intermediate-slow	Slow metabolizers	
Sex					0.0004
Men	1,874 (47)	14,292 (46)	20,786 (45)	7,557 (44)	
Women	2,137 (53)	16,979 (54)	25,333 (55)	9,499 (56)	
Age	58 (48-67)	57 (47-66)	57 (47-66)	57 (47-66)	0.84
BMI	26 (23-28)	26 (23-28)	26 (23-28)	26 (23-28)	0.04
Education					0.07
Elementary (1 – 9 years)	1,095 (27)	8,821 (28)	13,203 (29)	4,902 (29)	
High school (10 – 12 years)	2,205 (55)	16,954 (54)	24,979 (54)	9,173 (54)	
Academic (> 12 years)	711 (18)	5,496 (18)	7,937 (17)	2,981 (18)	
Civil status					0.11
Married/cohabiting	2,969 (74)	23,344 (75)	34,362 (75)	12,858 (75)	
Unmarried	321 (8)	2,481 (8)	3,611 (8)	1,308 (8)	
Separated/divorced	403 (10)	3,051 (10)	4,487 (10)	1,587 (9)	
Widow/widower	318 (8)	2,395 (7)	3,659 (8)	1,303 (8)	
Smoking status					0.34
Never smoker	1,565 (39)	12,585 (40)	18,452 (40)	6,846 (40)	
Former smoker	1,626 (41)	12,311 (39)	18,104 (39)	6,656 (39)	
Current smoker	820 (21)	6,375 (20)	9,563 (21)	3,554 (21)	
Pack-years	22 (7-32)	22.0 (7-30)	22 (7-30)	22 (6-30)	0.48
Cigarettes/day	15 (10-20)	15 (10-20)	15 (10-20)	15 (10-20)	0.68
Physical activity during work					0.60
Mainly sitting	1,239 (31)	9,362 (30)	13,701 (30)	5,160 (30)	
Sitting, standing, walking	968 (24)	7,730 (25)	11,292 (24)	4,210 (25)	
Walking and lifting	568 (14)	4,625 (15)	6,900 (15)	2,436 (14)	
Heavy workload	111 (3)	806 (3)	1,222 (3)	428 (3)	
Not working	1,125 (28)	8,748 (28)	13,004 (28)	4,822 (28)	
Physical activity during leisure					0.78
< 2 hours/week	274 (7)	2,165 (7)	3,130 (7)	1,179 (7)	
2-4 hours/week	1,695 (42)	13,493 (43)	20,011 (43)	7,429 (44)	
> 4 hours/week light	1,770 (44)	13,633 (44)	20,136 (44)	7,398 (43)	
> 4 hours/week heavy	272 (7)	1,980 (6)	2,842 (6)	1,050 (6)	

Values are shown as number (percent) or mean (interquartile range).

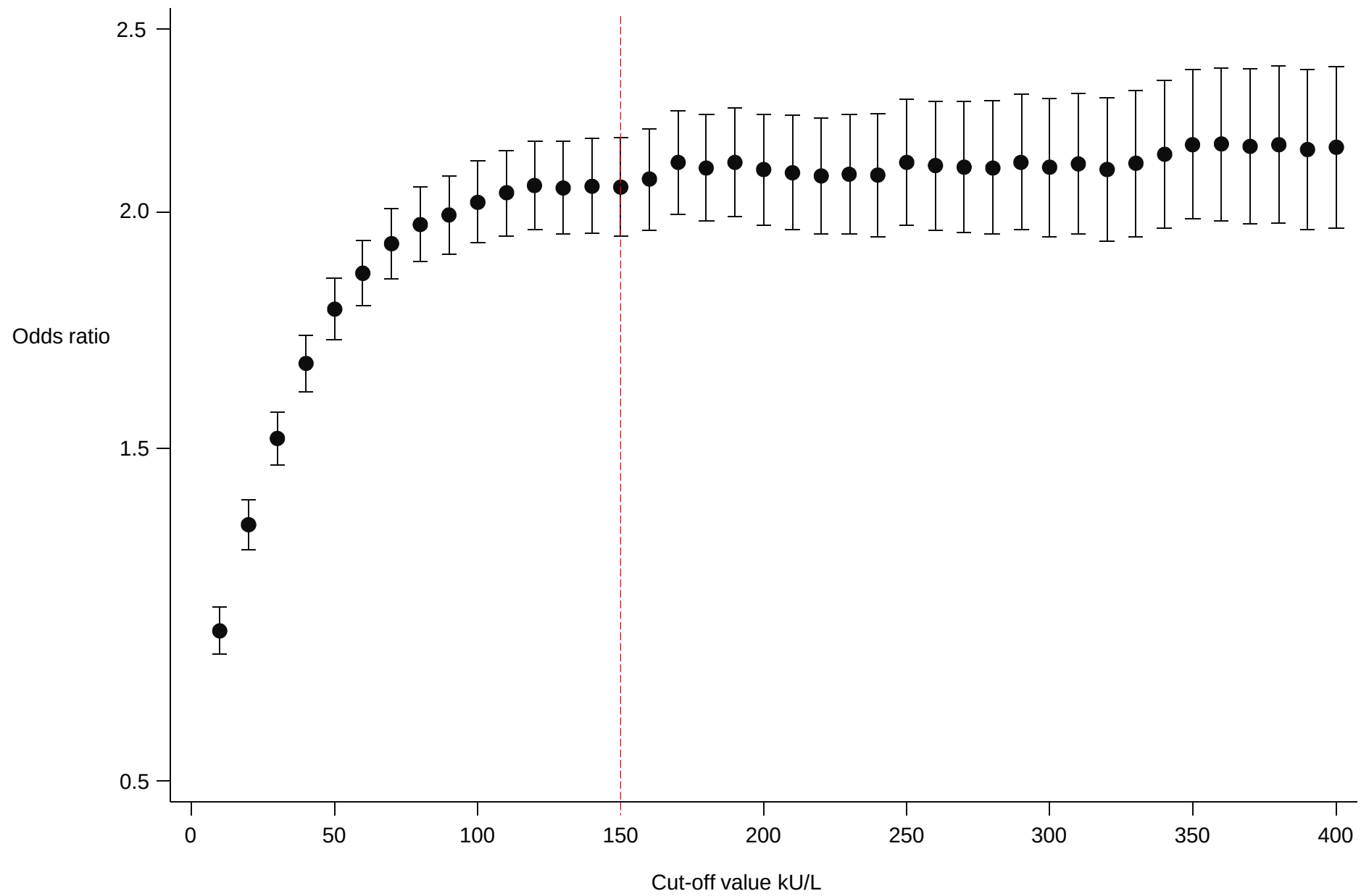


Figure E1

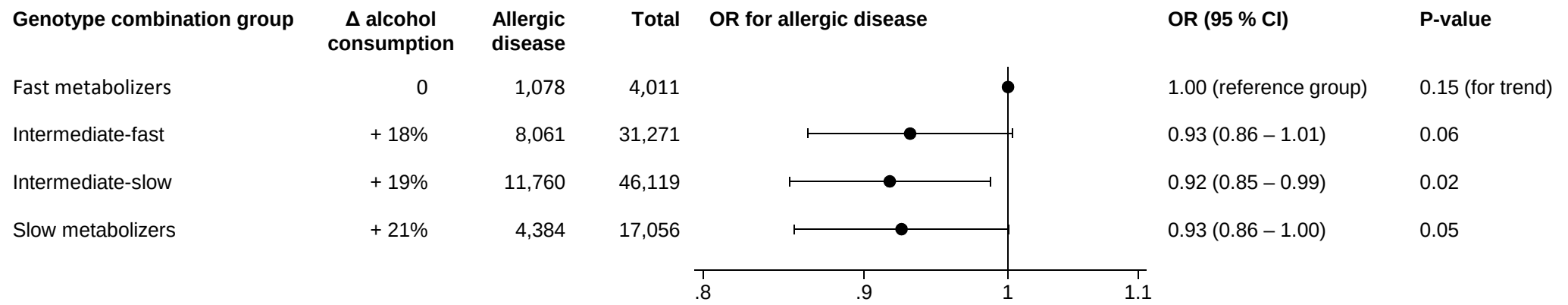


Figure E2

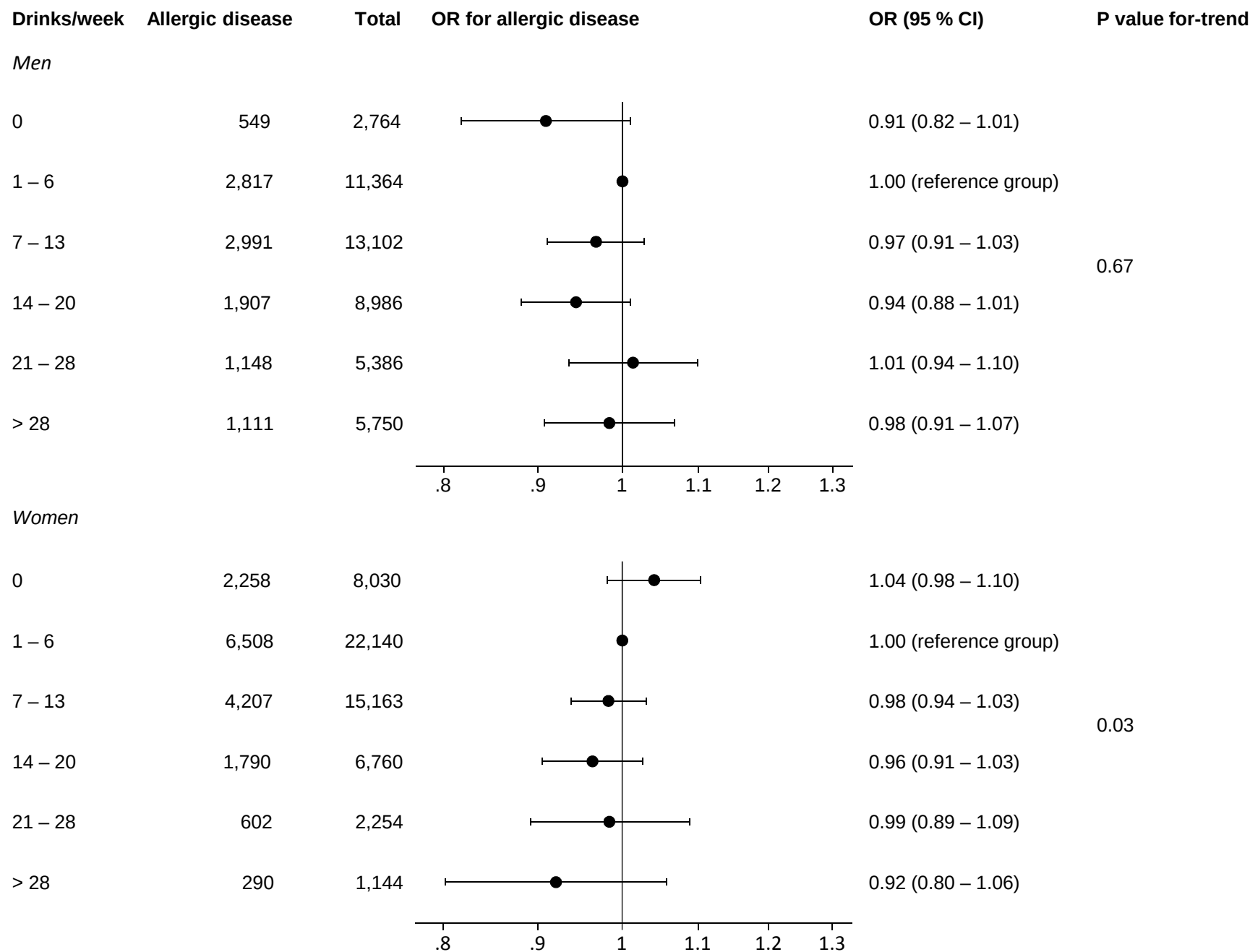


Figure E3

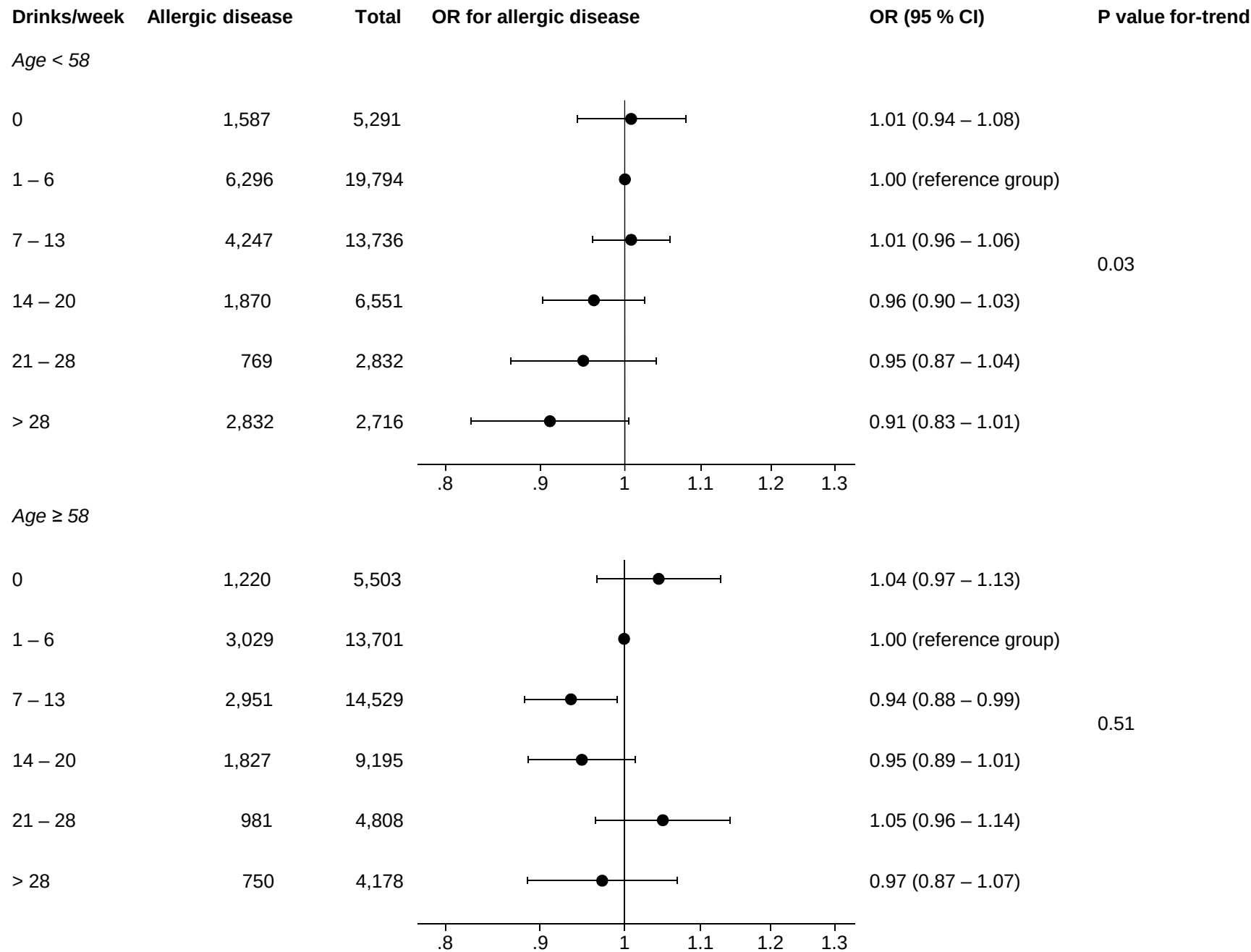


Figure E4

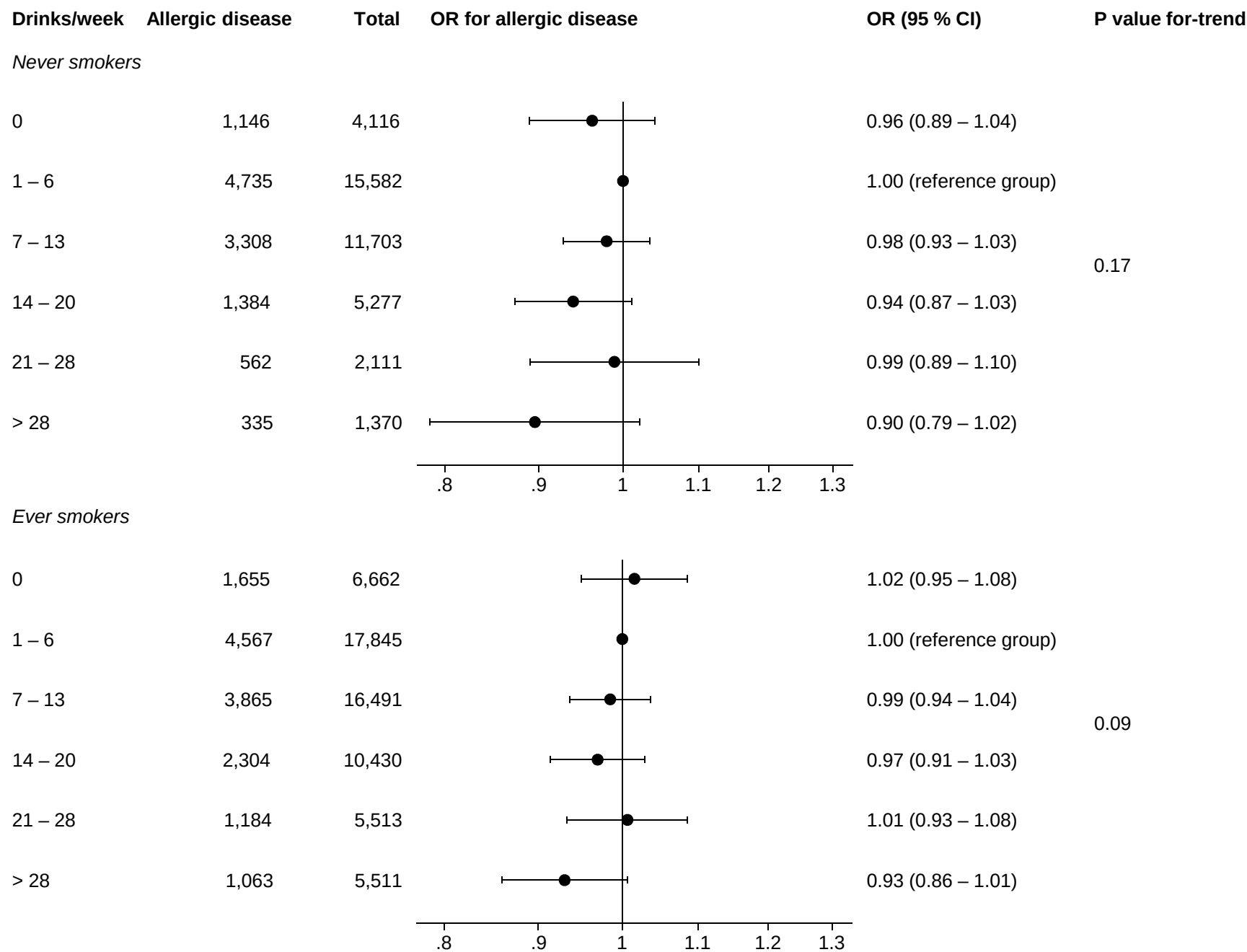


Figure E5