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**OBESITY, DIABETES, COFFEE, TEA AND CANNABIS USE ALTER  
RISK FOR ALCOHOL-RELATED CIRRHOSIS IN TWO LARGE  
COHORTS OF HIGH-RISK DRINKERS**

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**Keywords**

Alcohol, cirrhosis, coffee, familial risk, cannabis, diabetes

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### **List of Abbreviations**

|     |                                |
|-----|--------------------------------|
| ALT | Alanine aminotransferase       |
| AST | Aspartate aminotransferase     |
| BMI | Body Mass Index                |
| CI  | Confidence Interval            |
| GGT | Gammaglutamyl transferase      |
| HCC | Hepatocellular Carcinoma       |
| HIV | Human Immunodeficiency Virus   |
| INR | International Normalised Ratio |
| OR  | Odds Ratio                     |
| WHR | Waist/hip ratio                |

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## **Author contributions**

DS, CPD, TRM, PM, PSH, HKS, JBW, BN, LL, FS, TF, AKD and HJC conceived and designed the study. Recruitment and data acquisition was done by SMa, SL, SMu, GPA, FE, DG, AT, FS, MS, AKD, MP, BN, J-MJ, RM, PN, SN, PP, HKS, PM, DS and TRM. JBW and DS led the analyses and writing of the manuscript. All authors read, critically reviewed and approved the final version. DS and TRM are the guarantors.

## **Competing interests**

Authors declare support from the National Institutes of Health (NIH)/NIAAA, during the conduct of the study. Other support outside the submitted work is from Durect Corporation (SL) and INSERM (RM) during the conduct of the study; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; and no other relationships or activities that could appear to have influenced the submitted work.

## **Study Highlights**

What is known:

- Lifetime alcohol exposure reported by patients with alcohol-related cirrhosis varies widely, and only some high-risk drinkers develop cirrhosis

What is new here:

- Susceptibility to cirrhosis among high-risk drinkers is affected by family history of alcohol-related liver disease
- Effects of obesity, diabetes, coffee consumption and beverage preference have been confirmed in data from two independent studies and this information should help in preventing or delaying cirrhosis in patients whose drinking places them at risk.

## **ABSTRACT**

**Background:** Sustained high alcohol intake is necessary but not sufficient to produce alcohol-related cirrhosis. Identification of risk factors, apart from lifetime alcohol exposure, would assist in discovery of mechanisms and prediction of risk.

**Methods:** We conducted a multi-centre case-control study (GenomALC) comparing 1293 cases (with alcohol-related cirrhosis, 75.6% male) and 754 controls (with equivalent alcohol exposure but no evidence of liver disease, 73.6% male). Information confirming or excluding cirrhosis, and on alcohol intake and other potential risk factors, was obtained from clinical records and by interview. Case-control differences in risk factors discovered in the GenomALC participants were validated using similar data from 407 cases and 6573 controls from UK Biobank.

**Results:** The GenomALC case and control groups reported similar lifetime alcohol intake (1374 versus 1412 kg). Cases had a higher prevalence of diabetes (20.5% (262/1288) versus 6.5% (48/734),  $p = 2.27 \times 10^{-18}$ ) and higher pre-morbid BMI ( $26.37 \pm 0.16 \text{ kg/m}^2$ ) than controls ( $24.44 \pm 0.18 \text{ kg/m}^2$ ,  $p = 5.77 \times 10^{-15}$ ). Controls were significantly more likely to have been wine drinkers, coffee drinkers, smokers and cannabis users than cases. Cases reported a higher proportion of parents who died from liver disease than controls (OR 2.25 95% CI 1.55 to 3.26). Data from UK Biobank confirmed these findings for diabetes, BMI, proportion of alcohol as wine and coffee consumption.

**Conclusions:** If these relationships are causal, measures such as weight loss, intensive treatment of diabetes or pre-diabetic states, and coffee consumption should reduce risk of alcohol-related cirrhosis.

Sustained high alcohol intake, often associated with alcohol dependence, can lead to alcohol-related liver diseases including cirrhosis. The usual progression is through fatty liver, frequent in high-risk drinkers but reversible with abstinence, to fibrosis and cirrhosis. Some patients will develop alcoholic hepatitis, and some will develop hepatocellular carcinoma (HCC), generally with cirrhosis as a precursor. Therefore, cirrhosis is not only the end-stage of liver damage, but also increases risk for other life-threatening conditions. Apart from abstinence from alcohol, supportive measures, and liver transplantation in selected abstinent patients, current treatment options for alcohol-related cirrhosis are limited.

The relationship between alcohol intake and cirrhosis has been recognised since the late eighteenth century (1), with subsequent efforts to quantify this association made by Pequinot (2) who noted an increased risk of cirrhosis in people drinking more than 40 grams of alcohol per day. It is known that women are more susceptible to liver damage from alcohol than men (3), and larger studies and meta-analyses (4) have refined the threshold for detectable risk from alcohol intake.

It is notable that only a minority of high-risk drinkers develop cirrhosis. It is difficult to find reliable estimates, but in Denmark 7.7% of patients diagnosed with harmful alcohol use and 8.8% of those diagnosed with alcohol dependence developed cirrhosis over the subsequent 15 years (5). Meta-analysis (6) showed that 7-16% of people in alcohol problem cohorts had cirrhosis after 8-12 years. Variation in susceptibility may be due to genetic variation, and/or presence of other environmental and lifestyle risk factors which increase the probability of liver damage. Apart from alcohol intake and gender, obesity (also associated with non-alcoholic liver disease) has the strongest evidence for increasing risk of alcohol-related cirrhosis. For instance, liver biopsy histology showed more severe abnormalities in patients with alcohol use disorders with greater body weight (7); this was confirmed in a subsequent study (8) which showed that being overweight was a risk factor for steatosis, hepatitis and

cirrhosis in addition to the effects of age, gender and duration of alcohol abuse. Other studies have also found an association between obesity or body mass index (BMI) and liver disease (9, 10), fibrosis (11), alcoholic hepatitis (12) or HCC (13). There is evidence that coffee or tea consumption can reduce risk of liver disease or favourably affect biomarkers associated with liver disease (14-17). Smoking has been associated with increased risk of alcohol-related cirrhosis and of cirrhosis in general, particularly among women (18). A recent report showed that cannabis use protected against liver disease in patients with alcohol use disorders (19), possibly through effects on inflammation mediated by cannabinoid receptors (20).

There is a lack of hard data from twin or family studies on genetic risk for alcohol-related cirrhosis. Alcohol dependence is partially heritable (21) but twin studies on its consequences such as alcohol-related liver disease (22) have been limited by small numbers and lack of adjustment for heritable effects on alcohol exposure (23). Our earlier report (24) suggested that a history of liver disease in a parent with alcohol problems was associated with increased risk of alcohol-related cirrhosis. The known genetic risk loci for cirrhosis in *PNPLA3* and *HSD17B13* (25, 26) are associated with lipid metabolism and potentially with metabolic changes which accompany obesity.

The GenomALC Consortium (24) was initiated to gather data and samples for identification of risk factors for alcohol-related cirrhosis, including a case-control genetic association study. In this paper we focus on comparison of case and control groups for potential clinical and phenotype factors that alter disease risk including beverage preference, other substance use, family history, obesity and diabetes. Where we have identified potential risk-altering factors from our data, we have attempted validation using comparable data from the UK Biobank.

## SUBJECTS AND METHODS

### *GenomALC Study*

Recruitment and data collection were based on our published GenomALC protocol (24). Two groups of patients were recruited between 2012 and 2017 in six countries (Australia, France, Germany, Switzerland, UK and USA).. Cases were recruited through hepatology clinics and controls were recruited from psychiatric clinics or detoxification facilities. All participants gave written informed consent. The study was approved by appropriate Ethics Committees or Institutional Review Board at each site and conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Data and samples were identified by a study-specific code with no personal information.

To be confident that participants either had or were at substantial risk of alcohol-related cirrhosis and to minimise the chance that cirrhosis was caused by factors other than alcohol, we recruited patients with alcohol intake of at least 80 grams per day for men and 50 grams per day for women for 10 years or more. Both cases and controls were required to have negative test results (antibody/antigen/viral load) for hepatitis B and C, and no clinical or serological evidence of human immunodeficiency virus (HIV). Unequivocal evidence of cirrhosis in cases was defined as imaging results (sonography, computed tomography, magnetic resonance imaging) compatible with cirrhosis; together with detectable ascites by imaging or paracentesis, and/or grade 2 or higher spontaneous hepatic encephalopathy, and/or moderate or large oesophageal varices on upper gastrointestinal endoscopy. Histological cirrhosis on biopsy was defined as Metavir fibrosis stage F4 or Ishak fibrosis stage 5 or 6. Liver stiffness (Fibroscan<sup>®</sup>) was accepted as diagnostic for cirrhosis if greater than 22 kPa in the presence of aspartate aminotransferase (AST) less than 100 u/l or  $\geq 30$  kPa if AST between 100-200 IU/L (27, 28) . Other causes of liver disease, including haemochromatosis, Wilson's Disease, and autoimmune liver disease were excluded by laboratory tests or clinical

criteria, and any patient who had received a liver transplant for a condition other than alcohol-related cirrhosis was also excluded. Controls met the alcohol intake criteria but with no evidence or history of liver disease, had normal results for liver function tests (AST, alanine aminotransferase (ALT), bilirubin, albumin, but not necessarily for gammaglutamyl transferase (GGT)), platelet count and International Normalised Ratio (INR), and/or had less than 6 kPa liver stiffness (Fibroscan<sup>®</sup>), while drinking or within seven days of abstinence.

Information was collected on demographics, self-reported ancestry, history of alcohol, tobacco and cannabis use, tea and coffee consumption, clinical symptoms, biopsy results if available, and biochemical and haematological test results. The data collection form (24) is available from the corresponding authors. Data were transferred to a central site, checked for anomalies and if necessary corrected after clarification, and stored in a secure password-protected system.

Analysis of familial transmission of risk for alcohol-related cirrhosis was based on participants' responses to questions about their parents:

- a. *Did your father have problems with alcohol?*
- b. *If YES, did he die of liver disease?*
- c. *Did your mother have problems with alcohol?*
- d. *If YES, did she die of liver disease?*

This analysis was restricted to patients whose fathers or mothers were reported to have had 'problems with alcohol', and assumes that death from liver disease in a parent with alcohol problems is due to alcohol-related liver disease (potentially alcoholic hepatitis or HCC, as well as alcohol-related cirrhosis).

### ***UK Biobank***

Data from the UK Biobank (<https://www.ukbiobank.ac.uk/>, accessed 2018-11-07) on a population cohort of 502,616 participants from the UK were made available under approval

number 18870. Baseline assessment included a demographic, lifestyle and health questionnaire and participants agreed to have their health records accessed for baseline and follow-up outcomes (29). Participants had given informed consent as described at [http://www.ukbiobank.ac.uk/wp-content/uploads/2011/06/Consent\\_form.pdf](http://www.ukbiobank.ac.uk/wp-content/uploads/2011/06/Consent_form.pdf) and ethical approval was given under the UK Biobank Ethics and Governance framework (<https://egcukbiobank.org.uk/>).

For this analysis we extracted information on people who

- (1) reported alcohol intake of  $\geq 80$  grams/day for men or  $\geq 50$  grams/day for women at the time of assessment, with self-reported similar or greater alcohol intake ten years previously, and no reported alcohol-related cirrhosis or other alcohol-related liver disease (controls, N = 6573); or
- (2) had a diagnosis of alcohol-related cirrhosis (ICD10 code K70.3) (cases, N = 407).

Relevant information on these UK Biobank participants included age, sex, calculated BMI, waist/hip ratio (WHR), self-reported current alcohol intake, daily tea and coffee consumption, smoking status (never, former or current smoker), cannabis use (ever), and diabetes status (self-reported in response to the touchscreen question ‘Has a doctor ever told you that you have diabetes?’ and if Yes, confirmed by interview).

### ***Data analysis***

Data analyses used SPSS Version 22 (IBM Corp., 590 Madison Avenue New York, NY 10022). Alpha (p-value)  $< 0.05$  and Odds Ratio (OR) when 95% Confidence Interval (CI) excluded 1.00 were considered significant. Statistical tests for differences between case and control groups were based on contingency tests for categorical variables, and ANOVA for quantitative variables. Logistic regression analysis to evaluate independent predictors was based on stepwise entry until all significant ( $p < 0.05$ ) variables had been entered. For

evaluation of the effects of family history, the possibility of differential transmission of effects to male and female patients was taken into account using patient sex for stratification, testing for heterogeneity of OR across strata with the Breslow-Day test and, if no heterogeneity was found, estimating the common OR. Similarly, for testing whether case-control differences were consistent across country of recruitment, countries were treated as the strata and heterogeneity and common odds ratios were evaluated.

## RESULTS

### *GenomALC participants – case-control comparisons*

1293 cases and 754 controls were recruited between 2012 and 2017. There were 978 male and 315 female cases, and 555 male and 199 female controls. Clinical features of the cases are summarised in Supplementary Table 1. Most participants reported only ‘European’ ancestry, with the highest proportion in Germany (99%) and lowest in the US (88%).

Cases drank significantly less alcohol per day than controls, but had been drinking for significantly longer. Total lifetime alcohol intake did not differ significantly between male cases and controls, and in female cases was slightly lower than for controls (Table 1). A breakdown by country of recruitment is given in Supplementary Table 2, with comparisons of lifetime alcohol intake in cases and controls by country in Supplementary Figure 1. Controls reported taking a significantly higher proportion of their total alcohol in the form of wine (Table 2), but were less likely to report usually drinking with (rather than between) meals.

Forty eight percent of cases but only 28% of controls were currently living with a spouse or partner. There was no significant difference in years of education. Controls were more likely than cases to have been coffee drinkers during the time they were drinking alcohol heavily, and to have drunk more coffee per day, but there was no significant difference for tea consumption (Table 2). A slightly higher but statistically significant proportion of controls reported drinking green tea (7% of cases and 9% of controls). Most people in both groups were or had been smokers, but the proportion was significantly higher in controls (83%) than cases (72%). Regular cannabis use was about three times more common among the controls (27%) than cases (9%) (Table 2) but the proportion decreased with age (in both cases and controls) and the case-control difference was non-significant in patients aged over 60 years (Figure 1(a)).

Mean BMI was higher among the cases than the controls (Table 2). Because this difference might be secondary to the disease, e.g. through fluid retention in the cases or through inadequate diet in the controls, we also compared patients' pre-morbid BMI. This was estimated from participants' reports on their weight at age 40 (for those over 40) or else at age 20, with the intention of avoiding effects of the disease on BMI. Again, there was a highly significant difference with the cases having a higher mean for this measure of obesity. A larger proportion of cases, 262 out of 1280, but only 48 out of 734 controls were reported to be diabetic (Odds Ratio 3.68, 95% CI 2.66 to 5.08) (Table 2). Information about whether reported diabetes was Type 1 or Type 2 was not available. As expected, the prevalence of diabetes increased with age (Figure 1(b)), and diabetes was significantly associated with cirrhosis risk only in patients aged over 40 years.

We also tested whether the differences between cases and controls showed variation between countries, with results shown in Supplementary Table 4.

When all the risk factors were tested together, using multiple logistic regression to identify independent effects on risk of alcohol-related cirrhosis (Table 3), the most significant effects were from cannabis use (protective), coffee and possibly tea consumption (each decreasing risk to a similar extent). Diabetes and pre-morbid BMI, but not current BMI, were associated with increased risk.

### ***GenomALC participants – family history***

Among those whose fathers had a reported alcohol problem, 21.5% of cases versus 9.4% of controls reported that their fathers died of liver disease (OR 2.64, 95% CI 1.68 to 4.14).

Among those whose mothers had a reported alcohol problem, 17.9% of cases versus 12.5% of controls reported that their mothers died of liver disease (OR 1.53, 95% CI 0.79 to 2.97).

We also tested for differential effects by sex of the participants, analysing effects on sons and daughters (male and female patients) separately (Figure 2). Risk of cirrhosis was significantly increased in both male and female patients if the Father was reported as excessive alcohol user and to have died from liver disease. There were trends towards increased risk in both sexes if the Mother was affected, but these did not reach statistical significance. Combining data from all four groups gave an odds ratio of 2.25 (95% CI 1.55 – 3.26).

### ***UK Biobank – case-control comparisons***

Means and distributions of alcohol-related characteristics for cases and controls from UK Biobank are shown in Supplementary Table 3. Ages were similar, but reported alcohol intake differed substantially, largely because of the minimum current drinking level required for controls but not cases, but perhaps also from reduction or cessation of alcohol intake by cases with poor health.

There were significant differences (Table 4) between cases and controls for prevalence of diabetes, obesity, coffee consumption, and smoking but not for cannabis use. Beverage preferences also differed significantly, with controls taking a higher proportion of their alcohol as wine (32%, against 26% for cases) and cases taking a higher proportion as spirits (15%, against 8% for controls).

To test all potential risk factors simultaneously and attempt to identify independent effects, multivariate logistic regression was performed with results shown in Table 5. Cannabis use was excluded from the multivariate analyses because it was only available for a subset of the UK Biobank participants and its inclusion in an analysis involving listwise deletion greatly reduced the available numbers. Coffee and tea consumption, measures of obesity and prevalence of diabetes were independently significant. When both BMI and WHR were included, their effects were in opposite directions, with higher WHR associated with higher

risk and higher BMI with lower risk. In this analysis, the proportion of alcohol taken as sprits was independently significant but the proportion as wine was not.

## **DISCUSSION**

We have a number of important findings about factors associated with alcohol-related cirrhosis in high-risk drinkers. The novelty of the study lies in the fact that we used high-risk drinkers as controls, and well-defined selection of cases and controls allowed evaluation of the factors specifically altering risk for alcohol-related cirrhosis. Importantly, validation in an independent cohort enhances confidence in our results. Unlike previous studies that reported association with individual risk factors for alcohol-related cirrhosis, our study has simultaneously evaluated multiple potential aspects of risk in well characterised large cohorts of high-risk drinkers.

### ***Alcohol use***

Aspects of alcohol use, other than quantity, differed significantly between cases and controls and may affect risk of developing cirrhosis. In the GenomALC data, a higher proportion of total alcohol intake as wine was observed in the control group. When considered in the logistic regression model, a higher proportion of alcohol as wine was significantly associated with lower risk of cirrhosis but drinking with or between meals had no significant effect. The differential effect of wine, compared to other alcoholic beverages, is consistent with results of several previous studies (30-32) but we cannot distinguish between direct effects from some components of wine and confounding by other characteristics of drinkers who prefer wine. Nor can we be sure that we are seeing a protective effect of wine rather than a harmful effect associated with a preference for other beverages, because the UK Biobank data suggest that a higher proportion of alcohol taken as spirits is associated with higher risk of cirrhosis. It would be inappropriate, and potentially harmful, to infer that wine consumption is beneficial.

### ***Tea and coffee***

We found replicated evidence for a protective effect of coffee consumption. In the GenomALC case-control comparison (Table 2) controls were more likely to have been a coffee drinker during the period of excessive drinking and to have drunk more coffee per day. In the UK Biobank data the number of cups of coffee per day was higher among the controls than cases (Table 4). These results are consistent with the reported protective effects of coffee on liver disease (14, 33), on liver function test abnormality (14, 34, 35), and (at least in moderate amounts) on overall mortality (36). This is the first study to demonstrate an independent association of coffee in subjects with well-characterised alcohol use and cirrhosis directly assessed for this analysis. However, there is still uncertainty about which components of coffee confer protection and whether it is protective after liver damage is already present.

The GenomALC case-control comparison showed marginally significant protective effects of tea consumption when both tea and coffee were included in the multivariate analysis (Table 3). At least among the cases, tea and coffee tended to be alternative beverages; tea drinkers were less likely to drink coffee and *vice versa*. There were not many users of green tea (<10%) in our cohort, and there was only marginally significant protective effect (Table 2). In similar UK Biobank comparisons, coffee and tea were each significantly associated with lower risk and had comparable effect sizes (Table 5).

### ***Other substance use***

Smoking was more common among controls than cases in the GenomALC participants (Table 2), and the UK Biobank data confirmed this (Table 4) with current smoking being more frequent and never smoking being less frequent in the controls. One interpretation could be that smoking is protective against cirrhosis, but this is contrary to its effects on most diseases and cannot be accepted without other evidence. It is possible that cases had more contact with the healthcare system than controls and had received more intensive and

effective counselling about the risks of smoking, but this would not have affected the proportions who had never smoked. Even if smoking were protective against cirrhosis, its adverse impact on cardiovascular and respiratory diseases and cancers would outweigh any benefits.

There has been uncertainty about whether cannabis use is protective or harmful. However, a recent study of over 300,000 people with a past or current history of abusive alcohol use showed that cannabis use was associated with lower ORs for all stages of alcohol-related liver disease (19). Our GenomALC data showing cannabis use was more common among the controls confirms this (Table 2). In addition, multivariate regression in the GenomALC cohort corroborated the association of cannabis as an independent protective factor for cirrhosis (37-39). Nevertheless many of the controls were recruited from addiction clinics and may have had other substance use disorders (including for cannabis) that could confound these results. In the UK Biobank, cannabis use had no significant effect but the proportion of participants with information on cannabis use was small. We observed that among GenomALC participants, younger patients were more likely to have used cannabis (Figure 1(a)) but the ORs associated with reported cannabis use were consistent across age groups. There is independent evidence for a biological link between liver damage and cannabinoids and/or cannabinoid receptors (37-39), and for the therapeutic potential of several components of the cannabinoid system against liver cirrhosis (40).

### ***Obesity, diabetes and metabolic risk***

Our expectation, based on previous reports, was that obesity would be a risk factor for cirrhosis. This was confirmed in the GenomALC case-control comparison (Table 2), and when the effects of obesity and diabetes were considered together (Table 3) both were independently significant. Distinction between type 1 and type 2 diabetes was not specifically recorded in our data, but over 90% would be expected to be type 2 given the age range of our

study participants (41). Results in the UK Biobank were similar (Tables 4, 5) but waist/hip ratio showed a stronger association than BMI. Prevalence of diabetes increased with age, as expected (Figure 1(b)), and high-risk drinkers who have diabetes in middle age are particularly likely to progress to cirrhosis. The association between obesity and/or diabetes and risk of cirrhosis, including alcohol-related cirrhosis, has been described in community based cohort studies (42-44) and may reflect a similarity with non-alcoholic liver disease, which is related to metabolic syndrome and dysregulation of carbohydrate and lipid metabolism.

### ***Family History***

Our data show that risk of alcohol-related liver disease is transmitted in families, as we previously reported for a subset of our patients (24). Familial/genetic risk is well-established for excessive alcohol intake or alcohol dependence (21), but not for the medical complications of alcohol use such as cirrhosis. The transmission from fathers to offspring was statistically significant, with a trend for similar risk transmission from mothers (Figure 2). This apparent difference in risk transmission from fathers and from mothers is likely due to chance, to lower incidence of cirrhosis in mothers (i.e. insufficient power) and/or recall bias by the study participants. Transmission of risk from parents to offspring is likely to be genetic, given the discovery in recent years of loci associated with alcohol-related cirrhosis (25, 26, 45). If differential transmission of risk from fathers and mothers is a real phenomenon, it may be mediated through genetic/epigenetic imprinting or other mechanisms of selective transmission from father versus mother; multigenerational epigenetic adaptation to hepatic wound healing response has been elucidated in animal models (46). Confirming or refuting such differential transmission will require replication in other studies with family data, or molecular studies on epigenetic changes in candidate genes (47).

### ***Strengths and limitations***

Our study design has both strengths and weaknesses. One of the issues to be addressed in planning a case-control study is the choice of appropriate criteria for the two groups. For the GenomALC cases, we restricted our recruitment to patients with alcohol-related cirrhosis and definition of criteria for this did not present any significant difficulty. The choice of controls was more complex; it is necessary to have a control group with alcohol intake which puts them at risk of cirrhosis and with similar lifetime alcohol exposure to the cases. In practice we recruited controls from clinics for treatment of substance use disorders and from detoxification facilities, accepting the risk that these controls might have different pattern of psychiatric comorbidities from the cases. In the data analysis, we sought to overcome the problem of non-causative differences between the GenomALC cases and controls by checking for consistency with results from a population-based second source of data, the UK Biobank.

The recruitment of GenomALC participants in six countries is a source of strength in that it provides diversity and allows comparison of results (see Supplementary Table 4). In general the results do not differ significantly across countries, except for cannabis use and possibly smoking status where heterogeneity is driven by stronger effects in France. The GenomALC participants were mostly of European descent and the extent to which our results can be generalised to other populations remains to be determined.

From the UK Biobank data, diagnoses of alcohol-related cirrhosis or alcohol-related liver disease were based on hospital discharge diagnoses or death certification. For the control group from UK Biobank, we cannot exclude liver disease and if it was present in a substantial proportion of these controls then power to detect effects on risk would be reduced. However, any such reduction in power may be mitigated by the much larger number of controls in the UK Biobank dataset. Reduction in power would lead to a failure to find a true difference

between cases and controls (false negative result) rather than producing a significant but false difference (false positive).

The GenomALC study was not prospective as patients were assessed after diagnosis, however the research questions were planned and the data collected were for the purposes of these analyses. The lack of prospective design is not a problem for assessment of genetic risk for which these patients were primarily recruited, but recall may be biased by patients' knowledge of their diagnosis, and some of the postulated risk factors such as BMI may change as a consequence of disease. Case-control differences may be causative but could also be due to modes of recruitment (particularly for other drug use, including smoking). Methods using instrumental variables such as Mendelian Randomisation can address causation, but they depend on genetic association results being available for the postulated causative factors. Study design included definition of data and samples to be collected, but it is inevitable that questions will arise, often due to other research published during the course of a study, that were not envisaged at the outset. Although we have identified multiple risk factors for development of cirrhosis among high-risk drinkers, there are other factors such as variation in the microbiome (48), perhaps in turn associated with obesity, or infection with hepatotropic viruses other than B or C (49), about which we have no data.

A further limitation, which applies to many epidemiological studies, is that associations with risk may not reflect cause-and-effect relationships. For all risk factors, but particularly for the apparent effects of smoking, cannabis use and beverage preference (wine versus spirits) unmeasured confounders could produce the observed associations and we caution against changes in these areas without further evidence.

## ***Conclusions***

We identified significant associations between family history of liver disease; diabetes and obesity; tea, coffee, wine and cannabis consumption, and risk of cirrhosis. Our findings may have public health consequences if the causal relationships can be confirmed; measures such as weight loss, intensive treatment of diabetes or pre-diabetic states, and encouragement of coffee consumption may be useful lifestyle interventions to reduce the risk of alcohol-related cirrhosis.

## **ACKNOWLEDGEMENTS**

We gratefully acknowledge the participation of patients as cases or controls in the GenomALC study, and the work of staff in Australia, France, Germany, Switzerland, UK and USA in screening, recruitment, data and sample collection and sample processing.

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**Table 1.** Comparison of alcohol consumption in cases and controls from the GenomALC study. Results are shown as means  $\pm$  SEM. For the log-transformed alcohol measures, grams of alcohol per day and lifetime alcohol consumption, the means converted back to grams or kilograms (geometric means) are shown in italics.

|                                  | Age (for cases, at diagnosis) | Log Alcohol grams/day   | Years of excessive drinking | Log lifetime alcohol, kg |
|----------------------------------|-------------------------------|-------------------------|-----------------------------|--------------------------|
| Male (978 cases, 555 controls)   |                               |                         |                             |                          |
| Controls                         | 49.8 $\pm$ 0.41               | 2.333 $\pm$ 0.0010      | 21.9 $\pm$ 0.40             | 3.192 $\pm$ 0.013        |
|                                  |                               | <i>215 grams</i>        |                             | <i>1556 kg</i>           |
| Cases                            | 52.4 $\pm$ 0.28               | 2.282 $\pm$ 0.0095      | 25.1 $\pm$ 0.36             | 3.195 $\pm$ 0.011        |
|                                  |                               | <i>191 grams</i>        |                             | <i>1566 kg</i>           |
| p-values                         | 5.06 x 10 <sup>-8</sup>       | 5.26 x 10 <sup>-4</sup> | 1.06 x 10 <sup>-8</sup>     | 0.882                    |
| Female (315 cases, 199 controls) |                               |                         |                             |                          |
| Controls                         | 50.7 $\pm$ 0.72               | 2.239 $\pm$ 0.0167      | 18.57 $\pm$ 0.53            | 3.034 $\pm$ 0.020        |
|                                  |                               | <i>173 grams</i>        |                             | <i>1082 kg</i>           |
| Cases                            | 50.3 $\pm$ 0.52               | 2.160 $\pm$ 0.0016      | 19.4 $\pm$ 0.53             | 2.960 $\pm$ 0.020        |
|                                  |                               | <i>144 grams</i>        |                             | <i>912 kg</i>            |
| p-values                         | 0.603                         | 0.0013                  | 0.288                       | 0.013                    |
| All (1293 Cases, 754 Controls)   |                               |                         |                             |                          |
| Controls                         | 50.0 $\pm$ 0.36               | 2.308 $\pm$ 0.0085      | 21.0 $\pm$ 0.33             | 3.150 $\pm$ 0.011        |
|                                  |                               | <i>203 grams</i>        |                             | <i>1412 kg</i>           |
| Cases                            | 51.9 $\pm$ 0.25               | 2.252 $\pm$ 0.0084      | 23.7 $\pm$ 0.31             | 3.138 $\pm$ 0.010        |
|                                  |                               | <i>179 grams</i>        |                             | <i>1374 kg</i>           |
| p-values                         | 1.10 x 10 <sup>-5</sup>       | 1.30 x 10 <sup>-5</sup> | 1.23 x 10 <sup>-8</sup>     | 0.426                    |

**Table 2.** Putative risk factors compared (one at a time) in the GenomALC cases and controls. N for the tested risk factors varied from 1070 to 1293 for cases and from 609 to 754 for controls. Means  $\pm$  SE, or proportions.

|  | Controls                         | Cases                              | p-value                  |
|--|----------------------------------|------------------------------------|--------------------------|
| Beer, percent of total alcohol*                    | 44.7 $\pm$ 1.58                  | 41.7 $\pm$ 1.22                    | 0.130                    |
| Wine, percent of total alcohol*                    | 37.7 $\pm$ 1.66                  | 30.1 $\pm$ 1.18                    | 1.76 x 10 <sup>-4</sup>  |
| Spirits, percent of total alcohol*                 | 35.1 $\pm$ 1.53                  | 38.2 $\pm$ 1.22                    | 0.124                    |
| Other, percent of total alcohol*                   | 5.5 $\pm$ 0.93                   | 7.6 $\pm$ 0.78                     | 0.101                    |
| Usually drink with meals /<br>between meals / both | 56/225/463<br>(7.5%/30.2%/62.2%) | 139/333/818<br>(10.8%/25.8%/63.4%) | 0.013                    |
| BMI, kg/m <sup>2</sup>                             | 25.51 $\pm$ 0.19                 | 27.47 $\pm$ 0.16                   | 3.55 x 10 <sup>-14</sup> |
| Premorbid BMI, kg/m <sup>2</sup>                   | 24.44 $\pm$ 0.18                 | 26.37 $\pm$ 0.15                   | 5.77 x 10 <sup>-15</sup> |
| Diabetes present                                   | 48 out of 734 (6.5%)             | 262 out of 1280 (20.5%)            | 2.27 x 10 <sup>-18</sup> |
| Tea drinker  | 185 out of 751 (24.6%)           | 273 out of 1288 (21.2%)            | 0.078                    |
| Tea, cups per day†                                 | 3.10 $\pm$ 0.23                  | 3.07 $\pm$ 0.19                    | 0.933                    |
| Green Tea drinker                                  | 70 out of 749 (9.3%)             | 85 out of 1283 (6.6%)              | 0.030                    |
| Green Tea, cups per day†                           | 2.41 $\pm$ 0.22                  | 2.29 $\pm$ 0.19                    | 0.664                    |
| Coffee drinker                                     | 502 out of 753 (66.7%)           | 685 out of 1290 (53.1%)            | 1.91 x 10 <sup>-9</sup>  |
| Coffee, cups per day†                              | 4.06 $\pm$ 0.19                  | 3.46 $\pm$ 0.12                    | 0.0053                   |
| Smoking, Ever                                      | 624 out of 754 (82.8%)           | 929 out of 1293 (71.8%)            | 1.72 x 10 <sup>-8</sup>  |
| Cannabis user > 5 years                            | 200 out of 747 (26.8%)           | 121 out of 1287 (9.4%)             | 4.22 x 10 <sup>-24</sup> |

\* Note that although the percentages of alcohol as beer, wines, spirits or other for each person sum to 100%, the mean percentages for cases or controls do not. When the comparison was repeated using the non-parametric Mann-Whitney U-test the results were similar; percentage of alcohol as wine differed significantly ( $p < 0.001$ ) between cases and controls but percentages as beer, spirits or other alcoholic beverages did not ( $p > 0.05$ ).

† In participants who reported drinking tea/green tea/coffee, as appropriate.

**Table 3.** Putative risk factors compared in the GenomALC cases and controls, using multivariate logistic regression to identify independent effects. Sex, age, daily alcohol intake and duration of excessive drinking are included to adjust for any deviation from case-control matching. N = 1362 with data on all of the listed predictors. OR, odds ratio per unit change in predictor variable; 95% CI, 95% confidence intervals.

|                                       | OR    | 95% CI        | p-value                  |
|---------------------------------------|-------|---------------|--------------------------|
| Variables in the equation:            |       |               |                          |
| Cannabis user (0=No, 1=Yes)           | 0.331 | 0.237 – 0.464 | 1.18 x 10 <sup>-10</sup> |
| Diabetic (0=No, 1=Yes)                | 3.086 | 2.020 – 4.715 | 1.85 x 10 <sup>-7</sup>  |
| Premorbid BMI, kg/m <sup>2</sup>      | 1.057 | 1.031– 1.0884 | 1.12 x 10 <sup>-5</sup>  |
| Coffee drinker (0=No, 1=Yes)          | 0.643 | 0.498 – 0.830 | 6.87 x 10 <sup>-4</sup>  |
| Ever smoker (0=No, 1=Yes)             | 0.619 | 0.450 – 0.853 | 0.0033                   |
| Tea drinker (0=No, 1=Yes)             | 0.701 | 0.517 – 0.952 | 0.023                    |
| Spirits, percent of total             | 1.004 | 1.001 – 1.007 | 0.019                    |
| Duration of excessive drinking, years | 1.024 | 1.012 – 1.036 | 1.39 x 10 <sup>-4</sup>  |
| Variables not in the equation:        |       |               |                          |
| Sex                                   |       |               | 0.438                    |
| Age, years                            |       |               | 0.944                    |
| Alcohol intake, grams/day             |       |               | 0.223                    |
| BMI, kg/m <sup>2</sup>                |       |               | 0.122                    |
| Wine, percent of total                |       |               | 0.549                    |
| Drink with meals?                     |       |               | 0.413                    |
| Green tea drinker (0=No, 1=Yes)       |       |               | 0.897                    |
| Beer, percent of total                |       |               | 0.447                    |

**Table 4.** Putative risk factors compared (one at a time) in the UK Biobank participants.Means  $\pm$  SE and N, or proportions.

|   | Controls                 | Cases                   | p-value                  |
|---|--------------------------|-------------------------|--------------------------|
| BMI, kg/m <sup>2</sup>                            | 28.05 $\pm$ 0.06 (6534)  | 28.87 $\pm$ 0.27 (401)  | 6.51 x 10 <sup>-4</sup>  |
| Waist/Hip Ratio                                   | 0.931 $\pm$ 0.001 (6550) | 0.969 $\pm$ 0.004 (403) | 7.67 x 10 <sup>-20</sup> |
| Diabetes present                                  | 353 out of 6573 (5.4%)   | 122 out of 407 (30.0%)  | 4.05 x 10 <sup>-50</sup> |
| Red or white wine, percent of total alcohol       | 32.3 $\pm$ 0.44% (6515)  | 25.9 $\pm$ 2.12% (251)  | 0.0049                   |
| Beer or cider, percent of total alcohol           | 58.4 $\pm$ 0.47% (6525)  | 57.4 $\pm$ 2.53% (253)  | 0.679                    |
| Spirits, percent of total alcohol                 | 8.4 $\pm$ 0.23% (6526)   | 15.4 $\pm$ 1.72% (254)  | 9.65 x 10 <sup>-9</sup>  |
| Tea, cups per day                                 | 3.24 $\pm$ 0.04 (6298)   | 3.13 $\pm$ 0.18 (382)   | 0.512                    |
| Coffee, cups per day                              | 2.18 $\pm$ 0.03 (6064)   | 1.91 $\pm$ 0.12 (366)   | 0.026                    |
| Smoking, Ever                                     | 4895 out of 6557 (74.7%) | 283 out of 404 (70.0%)  | 0.046                    |
| Cannabis (ordinal measure of number of occasions) | 0.930 $\pm$ 0.034 (1518) | 0.871 $\pm$ 0.211 (31)  | 0.805                    |

**Table 5.** Putative risk factors compared in the UK Biobank cases and controls. Multivariate logistic regression with stepwise inclusion of potential predictors of case-control status, to identify independent and significant effects. Sex and age are included to adjust for any deviation from case-control matching. OR, odds ratio per unit change in predictor variable; 95% CI, 95% confidence intervals. Note that cannabis information is not included as a potential predictor because it would reduce the available numbers too greatly.

|   | OR    | 95% CI        | p-value                  |
|---|-------|---------------|--------------------------|
| Variables in the equation:                  |       |               |                          |
| Diabetic (0=No, 1=Yes)                      | 6.25  | 4.41 – 8.86   | 6.21 x 10 <sup>-25</sup> |
| Waist/Hip Ratio (WHR x 100)                 | 1.062 | 1.040 – 1.084 | 2.28 x 10 <sup>-8</sup>  |
| Spirits, percent of total alcohol           | 1.011 | 1.006 - 1.017 | 8.51 x 10 <sup>-5</sup>  |
| Body mass index, kg/m <sup>2</sup>          | 0.940 | 0.908 – 0.974 | 5.90 x 10 <sup>-4</sup>  |
| Tea (cups per day)                          | 0.928 | 0.877 - 0.981 | 0.0088                   |
| Coffee (cups per day)                       | 0.915 | 0.851 - 0.984 | 0.017                    |
| Variables not in the equation:              |       |               |                          |
| Sex   |       |               | 0.088                    |
| Smoking (Ever)                              |       |               | 0.156                    |
| Age, years                                  |       |               | 0.589                    |
| Red or white wine, percent of total alcohol |       |               | 0.681                    |
| Beer or cider, percent of total alcohol     |       |               | 0.834                    |

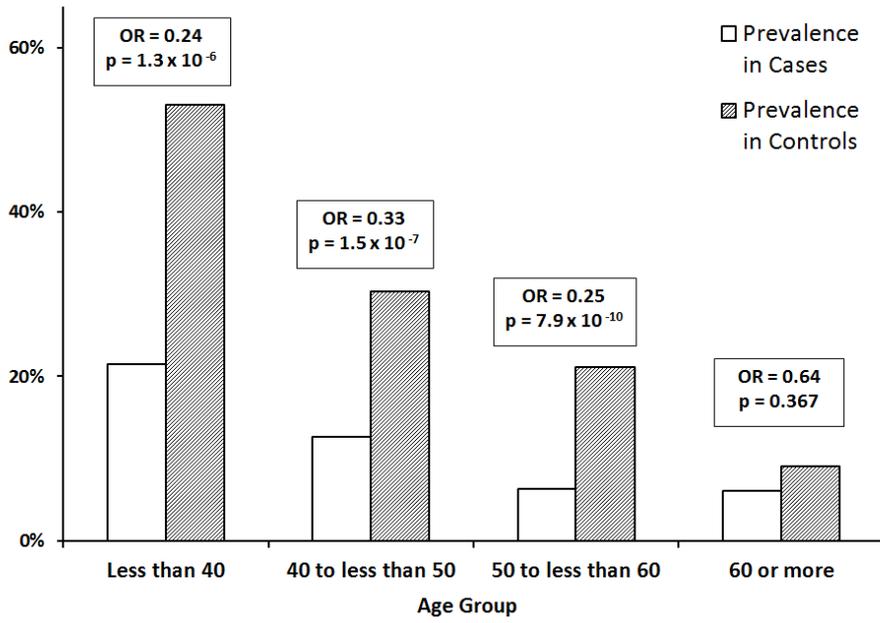
## LEGENDS TO FIGURES

**Figure 1.** Prevalence and Odds Ratios (ORs) by age group for (a) reported cannabis use, (b) diabetes, in GenomALC cases and controls. For cannabis use, Odds Ratios did not show significant heterogeneity between age groups ( $p = 0.200$ ) but for diabetes Odds Ratios showed significant heterogeneity between age groups ( $p = 0.0044$ ).

**Figure 2.** Odds ratios for alcoholic cirrhosis in male and female GenomALC participants, by reported parental death from liver disease (if the parent was reported to have had alcohol problems).

Figure 1.

a)



b)

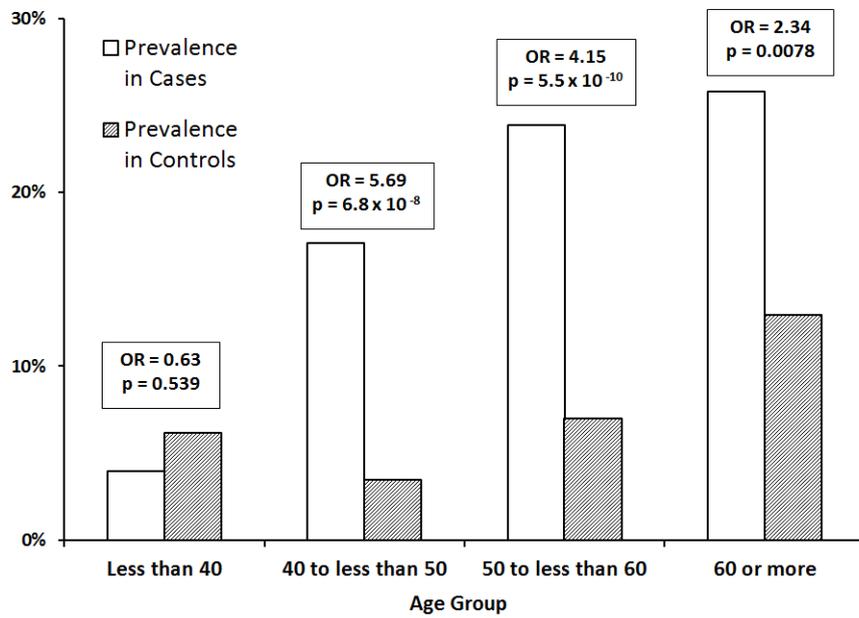
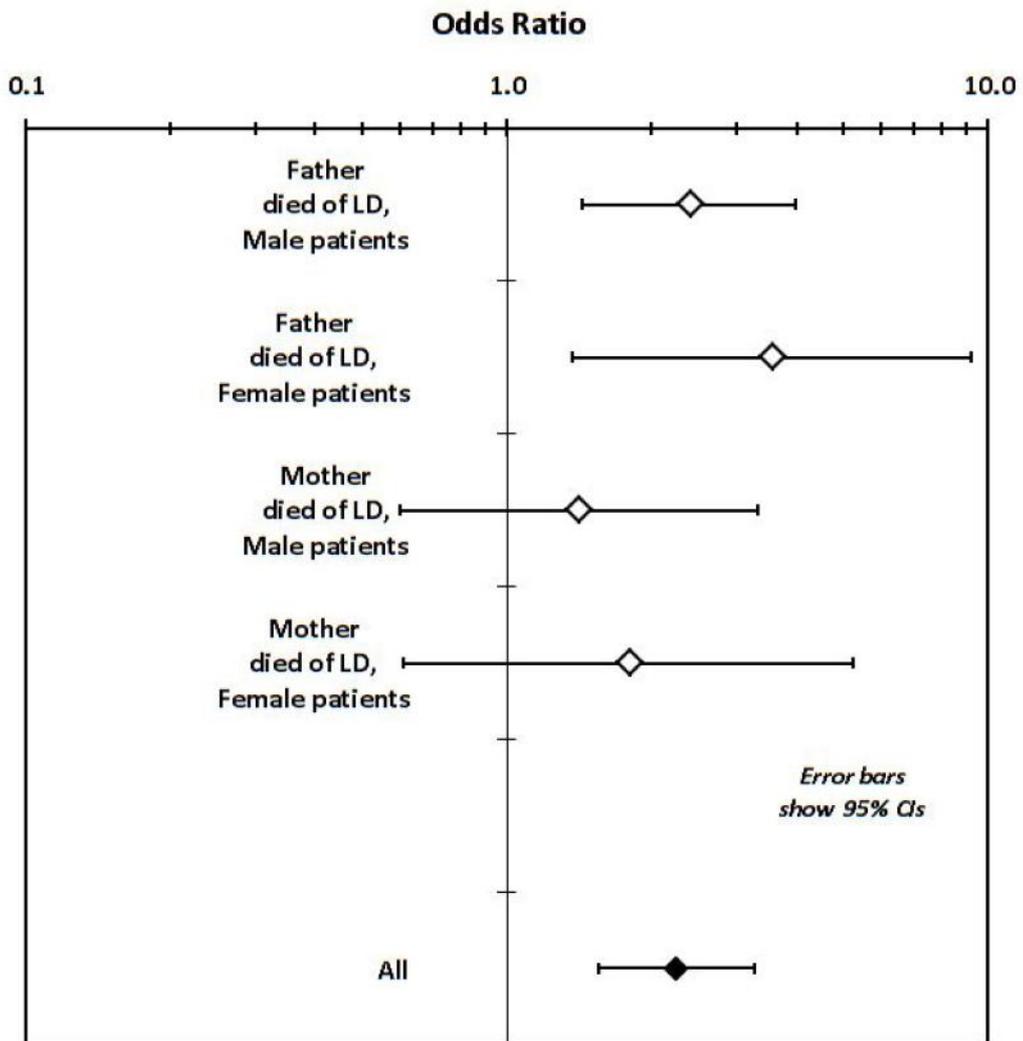


Figure 2.



**STROBE Statement**—Checklist of items that should be included in reports of *case-control studies*

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|                              | <b>Item No</b> | <b>Recommendation</b>  | <b>Section/Page</b>   |
|------------------------------|----------------|--|---|
| <b>Title and abstract</b>    | 1              | (a) Indicate the study's design with a commonly used term in the title or the abstract   | Abstract/pg 6   |
|                              |                | (b) Provide in the abstract an informative and balanced summary of what was done and what was found  | Abstract/pg 6   |
| <b>Introduction</b>          |                |  |   |
| Background/rationale         | 2              | Explain the scientific background and rationale for the investigation being reported   | Pg 7-8  |
| Objectives                   | 3              | State specific objectives, including any prespecified hypotheses   | Pg 8  |
| <b>Methods</b>               |                |  |   |
| Study design                 | 4              | Present key elements of study design early in the paper  | Pg 9-11   |
| Setting                      | 5              | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection  | Pg 9-11   |
| Participants                 | 6              | (a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls                  | Pg 9-11   |
|                              |                | (b) For matched studies, give matching criteria and the number of controls per case  | Pg 9-11, 13, 15   |
| Variables                    | 7              | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable   | Pg 9-11   |
| Data sources/<br>measurement | 8*             | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | Pg 9-11   |
| Bias                         | 9              | Describe any efforts to address potential sources of bias  | Matching of cases and controls for sex, age, alcohol exposure |

|                        |     |   |  |
|------------------------|-----|---|--|
| Study size             | 10  | Explain how the study size was arrived at   | Pg 13, 15  |
| Quantitative variables | 11  | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why  | Pg 11-12   |
| Statistical methods    | 12  | (a) Describe all statistical methods, including those used to control for confounding   | Pg 11-12   |
|                        |     | (b) Describe any methods used to examine subgroups and interactions   | Pg 12  |
|                        |     | (c) Explain how missing data were addressed   | Listwise deletion for logistic regressions, otherwise all available data were used.  |
|                        |     | (d) If applicable, explain how matching of cases and controls was addressed   | Matching for age and sex at recruitment (Pg 9).  |
|                        |     | (e) Describe any sensitivity analyses   | NA   |
| <b>Results</b>         |     |   |  |
| Participants           | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | Potentially eligible – unknown.<br><br>Confirmed eligible and included – 2047. (Pg 13) and 6980 (Pg 11)<br><br>Follow-up – NA. |
|                        |     | (b) Give reasons for non-participation at each stage  | Criteria outlined on pp.9-11, or patient declined to participate.  |
|                        |     | (c) Consider use of a flow diagram  | Not considered necessary.  |
| Descriptive data       | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders  | Pg 13-15, Tables 1-5, Supp Table 3.  |
|                        |     | (b) Indicate number of participants with missing data for each variable of interest   | See Tables and Table Legends   |
| Outcome data           | 15* | Report numbers in each exposure category, or summary measures of exposure   | Tables 1-5   |
| Main results           | 16  | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make   | Tables 1-5   |

|                          |    |  |   |
|--------------------------|----|--|---|
|                          |    | clear which confounders were adjusted for and why they were included   |   |
|                          |    | (b) Report category boundaries when continuous variables were categorized  | N/A, except for Figure 1 (see Figure)                                   |
|                          |    | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period   | N/A   |
| Other analyses           | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses   | Comparisons by country of recruitment, Supp Table 4 and Supp Fig 1      |
| <b>Discussion</b>        |    |  |   |
| Key results              | 18 | Summarise key results with reference to study objectives   | Pg 17-20  |
| Limitations              | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias                 | Pg 21   |
| Interpretation           | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | Pg 22   |
| Generalisability         | 21 | Discuss the generalisability (external validity) of the study results  | p. 21, (consistency between two sources of data (GenomALC, UK Biobank). |
| <b>Other information</b> |    |  |   |
| Funding                  | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based              | Pg 4  |

\*Give information separately for cases and controls.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.