



Original Article



# Association of Mosaic Chromosomal Alterations and Genetic Factors with the Risk of Cirrhosis

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## Abstract

**Background and Aims:** Age-related mosaic chromosomal alterations (mCAs) detected from genotyping of blood-derived DNA are structural somatic variants that indicate clonal hematopoiesis. This study aimed to investigate whether mCAs contribute to the risk of cirrhosis and modify the effect of a polygenic risk score (PRS) on cirrhosis risk prediction. **Methods:** mCA call sets of individuals with European ancestry were obtained from the UK Biobank. The PRS was constructed based on 12 susceptible single-nucleotide polymorphisms for cirrhosis. Cox proportional hazard models were applied to evaluate the associations between mCAs and cirrhosis risk. **Results:** Among 448,645 individuals with a median follow-up of 12.5 years, we identified 2,681 cases of cirrhosis, 1,775 cases of compensated cirrhosis, and 1,706 cases of decompensated cirrhosis. Compared to non-carriers, individuals with copy-neutral loss of heterozygosity mCAs had a significantly increased risk of cirrhosis (hazard ratio (HR) 1.42, 95% confidence interval (CI) 1.12–1.81). This risk was higher in patients with expanded cell fractions of mCAs (cell fractions  $\geq 10\%$  vs. cell fractions  $< 10\%$ ), especially for the risk of decompensated cirrhosis (HR 2.03 [95% CI 1.09–3.78] vs. 1.14 [0.80–1.64]). In comparison to non-carriers of mCAs with low genetic risk, individuals with expanded copy-neutral loss of heterozygosity and high genetic risk showed the highest cirrhosis risk (HR 5.39 [95% CI 2.41–12.07]). **Conclusions:** The presence of mCAs is

associated with increased susceptibility to cirrhosis risk and could be combined with PRS for personalized cirrhosis risk stratification.

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## Introduction

Cirrhosis is the end stage of progressive liver fibrosis and the leading cause of liver-related death globally.<sup>1,2</sup> In the initial stages, cirrhosis is compensated and can be intervened to prevent further deterioration. Decompensation is usually defined as the first occurrence of ascites, esophageal variceal bleeding, and in some cases, hepatic encephalopathy.<sup>3,4</sup> One challenge posed by cirrhosis is that it often goes undiagnosed at the initial stage until the patients experience decompensation. Since cirrhosis is difficult to cure and patients are prone to worsening and developing liver cancer, cirrhosis screening needs to be emphasized. Due to the relatively low incidence of cirrhosis in the general population, screening in high-risk population has the potential to improve screening outcomes.<sup>5</sup> Hence, it is crucial to enhance our understanding of the risk factors associated with cirrhosis to improve risk stratification and identify individuals at heightened risk of developing cirrhosis.

Genome-wide association studies have identified numerous risk-associated genetic loci, and the polygenic risk score (PRS), which measures the cumulative effect of these variants, has been validated as effective in predicting cirrhosis.<sup>6</sup> However, the predictive efficacy of PRS varies across strata of factors such as lifestyle and environmental exposures,<sup>7</sup> indicating that the impact of inherited factors can be influenced by non-inheritable risk factors. Hence, by exploring the elusive heritability and incorporating it with environmental fac-

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tors, the predictive accuracy of cirrhosis can be improved.

Mosaic chromosomal alterations (mCAs) are significant structural changes affecting chromosomes, observed in clonal subpopulations of cells harboring somatic mutations. They can be categorized based on copy number variations, encompassing gains, losses, and loss of heterozygosity.<sup>8,9</sup> These alterations demonstrate a pronounced age-related incidence and share similar mutational characteristics with hematological cancers and potentially other late-onset diseases.<sup>10-12</sup> While the importance of mCAs in disease pathogenesis is increasingly recognized, our comprehension of the specific types, frequencies, and consequences of acquired chromosomal anomalies in cirrhosis development remains limited. Furthermore, emerging evidence indicates that mCAs can coexist with gene mutations or even precede the detection of gene mutations several years before disease manifestations. It raises intriguing questions about the interplay and clinical implications of mCAs alongside co-occurring gene mutations during cirrhosis onset.

In this study, we harnessed detectable autosomal mCA data from the UK Biobank, and systematically investigated the association between mCAs and prospective cirrhosis events, focusing especially on expanded mCA clones (i.e., mCAs present in at least 10% of peripheral leukocyte DNA), and comprehensively tested their interactions with genetic factors.

## Methods

### Study design and population

The study population was derived from the UK Biobank, a comprehensive prospective database that enrolled over 500,000 participants aged 40–70 from 22 assessment centers between 2006 and 2010.<sup>13</sup> Social demographic information, health-related data, and blood-derived DNA samples were collected. Written informed consent was obtained from each participant, and the UK Biobank received ethical approval from the NHS National Research Ethics Service.

A total of 482,666 individuals with available mCA data were initially included in this study (application number: 64689). Participants with genotypic-phenotypic sex discrepancy (n=358) or non-white ancestry (n=27,753) were excluded from the analyses. Additionally, individuals with pre-existing hematological cancer, liver cancer, or cirrhosis at baseline (n=3,957), as well as those with incomplete information on covariates (n=1,953), were also excluded. The final analytic cohort comprised 448,645 participants (Supplementary Fig. 1).

### Exposure ascertainment

A comprehensive description of the genotyping process and arrays utilized in the UK Biobank can be found elsewhere.<sup>14</sup> In summary, DNA extracted from blood samples was genotyped using either the Affymetrix UK BiLEVE or UK Biobank Axiom arrays. Imputation was conducted using SHAPEIT3 and IMPUTE3 algorithms, utilizing merged UK10K and 1000 Genomes phase 3 panels.

The mCA detection in the UK Biobank has been previously described.<sup>8,15</sup> Based on the log<sub>2</sub> (R ratio) and B-allele frequency, identified mCAs were categorized into three groups: "copy number gain," "copy number loss," and "copy-neutral loss of heterozygosity (CN-LOH)." Any mCAs that did not fit into these types were classified as "undetermined." Rephasing was performed using Eagle2, and mCA calling utilized long-range phase information to detect allelic imbalances between maternal and paternal allelic fractions across con-

tiguous genomic segments. The mCAs were further grouped into expanded (cell fractions  $\geq 10\%$ ) and non-expanded (cell fractions  $< 10\%$ ) according to the estimated cell fractions. The mCA call sets were obtained from Return 3094 generated from UK Biobank application 19808.<sup>15</sup>

The multi-ancestry cirrhosis PRS, as established in a prior study, was applied.<sup>6</sup> This PRS comprises 12 susceptible single-nucleotide polymorphisms (SNPs) associated with cirrhosis risk, calculated by summing the number of risk alleles after multiplication by their respective weight (represented by the natural logarithm of the odds ratio [OR]). Detailed information is illustrated in Supplementary Table 1. According to quintiles of the PRS distribution among non-cirrhosis individuals, the PRS was categorized into low (lowest quintile), intermediate (quintiles – from two to four), and high (highest quintile) genetic risk.

### Outcome ascertainment

The primary outcome of interest was incident cirrhosis, defined as a composite diagnosis based on ICD-10: K70.2 (alcoholic fibrosis and sclerosis of the liver), K70.3 (alcoholic cirrhosis), K70.4 (alcoholic hepatic failure), K74.0 (hepatic fibrosis), K74.1 (hepatic sclerosis), K74.2 (hepatic fibrosis with hepatic sclerosis), K74.6 (other and unspecific cirrhosis of the liver), K72.1 (chronic hepatic failure), K72.903 (hepatic encephalopathy), K76.6 (portal hypertension), K76.7 (hepatorenal syndrome), or I85 (esophageal varices). Furthermore, compensated cirrhosis and decompensated cirrhosis, our secondary concerned outcomes, were analyzed separately. Details on the diagnoses used to define cirrhosis are presented in Supplementary Table 2. Follow-up data on health-related events and mortality were obtained through electronic connections to in-hospital admissions, the death register, and the cancer register (UK Biobank data-fields 41270, 40001, 40002, and 40006). Participants were followed up from the day they attended the assessment center until the date of diagnosis, date of death, or the last date of follow-up (30th September 2021 for England, 28th February 2018 for Wales, and 31st July 2021 for Scotland), whichever occurred first.

### Statistical analysis

To compare baseline characteristics, demographic characteristics were assessed by *t*-test for continuous variables and the Chi-squared test for categorical variables. The association between mCAs or specific mCA types and the risk of cirrhosis was evaluated using the Cox proportional hazards model, estimating hazard ratios (HRs) and corresponding 95% confidence intervals (CIs). The measure of effect was adjusted for age, age<sup>2</sup>, sex, drinking status, smoking status, the top 10 genetic principal components, and genotyping batch. The proportional hazards assumption was assessed by Schoenfeld residuals.

To quantify multiplicative interactions, a product term of the presence of mCA events and genetic risk was included. To assess the additive interaction, the relative excess risk of interaction, attributable proportion of interaction, and their 95% CIs were computed with R package epiR (version 2.0.46).

Two sensitivity analyses were conducted to assess the robustness of the findings: (1) Based on the aforementioned covariates, we adjusted for additional variables to more comprehensively control for confounding, including smoking pack-year, the Thompson deprivation index, body mass index, and diabetes status. (2) We aligned the definition of cirrhosis with that of the PRS source (ICD-10 K70.2, K70.3, K70.4, K74.0, K74.1, K74.2, K74.6, K76.6, or I85).

**Table 1.** Participant characteristics by different events

	<b>Overall population (N=448,645)</b>	<b>Cirrhosis</b>	
		<b>Case (N=2,681)</b>	<b>Control (N=445,964)</b>
Age, years, median (IQR)	58.0 (45.0–71.0)	60.0 (49.0–71.0)	58.0 (45.0–71.0)
Sex, n (%)			
Female	244,049 (54.40)	1,030 (38.42)	243,019 (54.49)
Male	204,596 (45.60)	1,651 (61.58)	202,945 (45.51)
Smoking status, n (%)			
Never	242,393 (54.03)	1,054 (39.31)	241,339 (54.12)
Former	159,554 (35.56)	1,104 (41.18)	158,450 (35.53)
Current	46,698 (10.41)	523 (19.51)	46,175 (10.35)
Drinking status, n (%)			
Never	14,471 (3.23)	103 (3.84)	14,368 (3.22)
Former	15,283 (3.41)	196 (7.31)	15,087 (3.38)
Current	418,891 (93.37)	2,382 (88.85)	416,509 (93.40)

	<b>Compensated cirrhosis</b>		<b>Decompensated cirrhosis</b>	
	<b>Case (N=1,775)</b>	<b>Control (N=446,870)</b>	<b>Case (N=1,706)</b>	<b>Control (N=446,939)</b>
Age, years, median (IQR)	60.0 (49.0–71.0)	58.0 (45.0–71.0)	60.0 (49.0–71.0)	58.0 (45.0–71.0)
Sex, n (%)				
Female	706 (39.77)	243,343 (54.45)	569 (33.35)	243,480 (54.48)
Male	1,069 (60.23)	203,527 (45.55)	1,137 (66.65)	203,459 (45.52)
Smoking status, n (%)				
Never	680 (38.31)	241,713 (54.09)	667 (39.10)	241,726 (54.08)
Former	755 (42.54)	158,799 (35.54)	698 (40.91)	158,856 (35.54)
Current	340 (19.15)	46,358 (10.37)	341 (19.99)	46,357 (10.37)
Drinking status, n (%)				
Never	73 (4.11)	14,398 (3.22)	60 (3.52)	14,411 (3.22)
Former	140 (7.89)	15,143 (3.39)	120 (7.03)	15,163 (3.39)
Current	1,562 (88.00)	417,329 (93.39)	1,526 (89.45)	417,365 (93.38)

All *p*-values for differences between groups were <0.001. IQR, interquartile range.

All *p*-values were two-sided, and *p*<0.05 was considered statistically significant. The statistical analyses were performed using R Software (version 3.6.1).

## Results

### **Identification of mCAs in the study populations**

During a median follow-up period of 12.5 years (interquartile range 11.7–13.2 years), a total of 2,681 cases of cirrhosis, 1,775 cases of compensated cirrhosis, and 1,706 cases of decompensated cirrhosis were identified (Table 1). Out of the 448,645 individuals, 15,970 (3.56%) experienced at least one autosomal event. The autosomes exhibited a total of 18,183 mCAs, with 169 mCAs detected in 136 (5.07%) individuals who developed incident cirrhosis and 18,014 mCAs found in 15,834 (3.55%) individuals without cirrhosis (Table 2). Furthermore, a higher prevalence of mCAs was also observed among cases in two other outcomes: 5.18% for compensated cirrhosis (vs. 3.55% for controls) and 4.63%

for decompensated cirrhosis (vs. 3.56% for controls) (Supplementary Table 3).

Among the autosomal events detected in the study, a total of 2,174 (11.96%) were identified as copy number gains, 3,311 (18.21%) as copy number losses, 7,662 (42.14%) as copy number neutral, and 5,036 (27.70%) had an undetermined copy number state (Table 2). Out of the 15,970 individuals with multiple copy number alterations, 14,559 (91.16%) exhibited only one mCA, while 1,411 (8.84%) individuals experienced more than one mCA event (Supplementary Fig. 2). Among the identifiable mCAs, 3,839 were expanded, comprising 677 gain events, 2,030 loss events, and 1,132 CN-LOH events.

### **Age-associated increase in prevalence of autosomal mCAs**

Due to the well-established correlation between age and the occurrence of mCAs, the prevalence of autosomal mCAs in relation to age was investigated among two distinct groups: individuals without cirrhosis and those with incident cirrho-

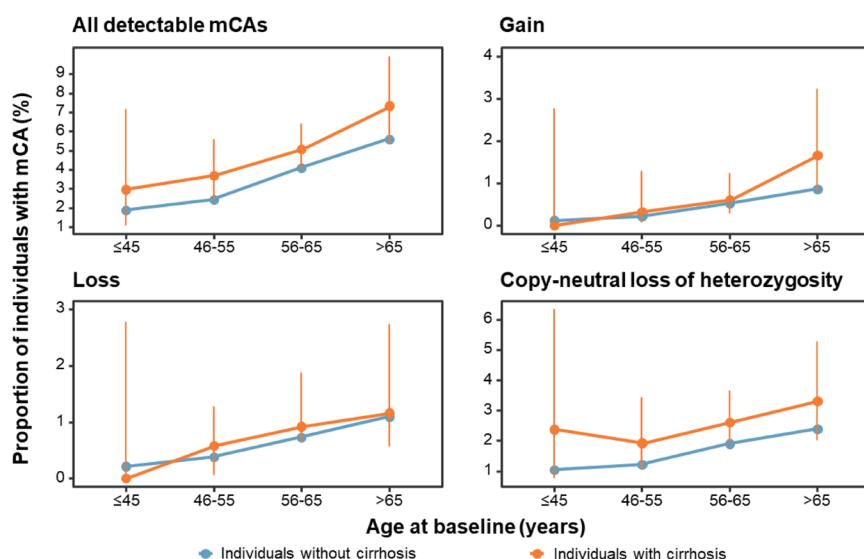
**Table 2.** Statistical description of mosaic chromosomal alterations (mCAs) by incident cirrhosis

	Overall population (N=448,645)	
	Counts of mCAs (percentage of all events)	Number of mCA carriers (prevalence)
Total number	18,183	15,970 (3.56%)
Copy number alteration types		
Gain	2,174 (11.96%)	1,934 (0.44%)
Loss	3,311 (18.21%)	2,781 (0.64%)
Copy-neutral loss of heterozygosity	7,662 (42.14%)	7,514 (1.71%)
Undetermined	5,036 (27.70%)	4,800 (1.10%)
Cirrhosis		
	Case (N=2,681)	
	Counts of mCAs (percentage of all events)	Number of mCA carriers (prevalence)
Total number	169	136 (5.07%)
Copy number alteration types		
Gain	25 (14.79%)	19 (0.74%)
Loss	32 (18.93%)	24 (0.93%)
Copy-neutral loss of heterozygosity	71 (42.01%)	69 (2.64%)
Undetermined	41 (24.26%)	36 (1.39%)
	Control (N=445,964)	
	Counts of mCAs (percentage of all events)	Number of mCA carriers (prevalence)
Total number	18,014	15,834 (3.55%)

sis. Our findings revealed a notable increase in the prevalence of mCAs with advancing age in both groups (Fig. 1). Among study participants aged 65 years or older, a total of 3,760 individuals (5.60% of the total population of 67,100) were found to carry mCAs, while only 12,210 individuals (3.20% of the total population of 381,545) exhibited mCAs ( $p=7.99\times10^{-211}$ ) among those below 65 years old. Notably, the prevalence of carriers with copy number neutral events was significantly higher among individuals with incident cirrhosis compared to those without cirrhosis ( $p=2.75\times10^{-4}$ ).

This observation was particularly pronounced in the age group older than 55 years (individuals without cirrhosis: 5,331 [2.02%] out of 263,592; individuals with incident cirrhosis: 53 [2.81%] out of 1,888;  $p=0.016$ ).

Subgroup analyses were further conducted to examine the prevalence of autosomal mCAs in relation to age (Supplementary Fig. 3). The mosaic mutation carrier rates in patients with compensated cirrhosis and decompensated cirrhosis were generally higher than those in the control groups. Notably, these analyses revealed a significant increase in the

**Fig. 1.** Associations between age and proportion of individuals with detectable autosomal mosaic chromosomal alterations (mCAs).

**Table 3.** Associations between autosomal mosaic chromosomal alterations (mCAs) and the risk of cirrhosis

	Number of participants with mCA events/number of participants (%)		Univariable Model <sup>a</sup>	
	Individuals with cirrhosis	Individuals without cirrhosis	HR (95% CI)	p value
<b>Cirrhosis</b>				
All detectable mCAs	136/2,681 (5.07%)	15,834/445,964 (3.55%)	1.48 (1.25–1.76)	<0.001
Gain	19/2,564 (0.74%)	1,915/432,045 (0.44%)	1.74 (1.11–2.73)	0.016
Loss	24/2,569 (0.93%)	2,757/432,887 (0.64%)	1.52 (1.02–2.28)	0.040
Copy-neutral loss of heterozygosity	69/2,614 (2.64%)	7,445/437,575 (1.70%)	1.59 (1.26–2.03)	<0.001
<b>Compensated cirrhosis</b>				
All detectable mCAs	92/1,775 (5.18%)	15,878/446,870 (3.55%)	1.52 (1.23–1.87)	<0.001
Gain	11/1,694 (0.65%)	1,923/432,915 (0.44%)	1.52 (0.84–2.76)	0.163
Loss	16/1,699 (0.94%)	2,765/433,757 (0.64%)	1.54 (0.94–2.52)	0.087
Copy-neutral loss of heterozygosity	48/1,731 (2.77%)	7,466/438,458 (1.70%)	1.68 (1.26–2.24)	<0.001
<b>Decompensated cirrhosis</b>				
All detectable mCAs	79/1,706 (4.63%)	15,891/446,939 (3.56%)	1.34 (1.07–1.69)	0.010
Gain	12/1,639 (0.73%)	1,922/432,970 (0.44%)	1.71 (0.97–3.02)	0.063
Loss	13/1,640 (0.79%)	2,768/433,816 (0.64%)	1.29 (0.75–2.23)	0.361
Copy-neutral loss of heterozygosity	40/1,667 (2.40%)	7,474/438,552 (1.70%)	1.44 (1.06–1.98)	0.021
		Multivariable Model <sup>a,c</sup>		Multivariable Model <sup>b,c</sup>
		HR (95% CI)	p value	HR (95% CI)
<b>Cirrhosis</b>				
All detectable mCAs	1.29 (1.09–1.54)	0.003	1.29 (1.11–1.49)	0.001
Gain	1.41 (0.90–2.21)	0.139	1.40 (0.95–2.04)	0.085
Loss	1.29 (0.86–1.93)	0.216	1.29 (0.92–1.80)	0.141
Copy-neutral loss of heterozygosity	1.42 (1.12–1.81)	0.004	1.41 (1.12–1.77)	0.003
<b>Compensated cirrhosis</b>				
All detectable mCAs	1.33 (1.08–1.65)	0.007	1.33 (1.11–1.59)	0.002
Gain	1.25 (0.69–2.27)	0.458	1.21 (0.72–2.03)	0.479
Loss	1.31 (0.80–2.15)	0.277	1.33 (0.88–1.99)	0.173
Copy-neutral loss of heterozygosity	1.50 (1.13–2.00)	0.006	1.47 (1.12–1.94)	0.006
<b>Decompensated cirrhosis</b>				
All detectable mCAs	1.16 (0.93–1.46)	0.188	1.19 (0.98–1.44)	0.077
Gain	1.35 (0.77–2.39)	0.297	1.36 (0.84–2.19)	0.206
Loss	1.08 (0.63–1.86)	0.783	1.10 (0.69–1.75)	0.679
Copy-neutral loss of heterozygosity	1.28 (0.94–1.75)	0.121	1.30 (0.97–1.76)	0.082

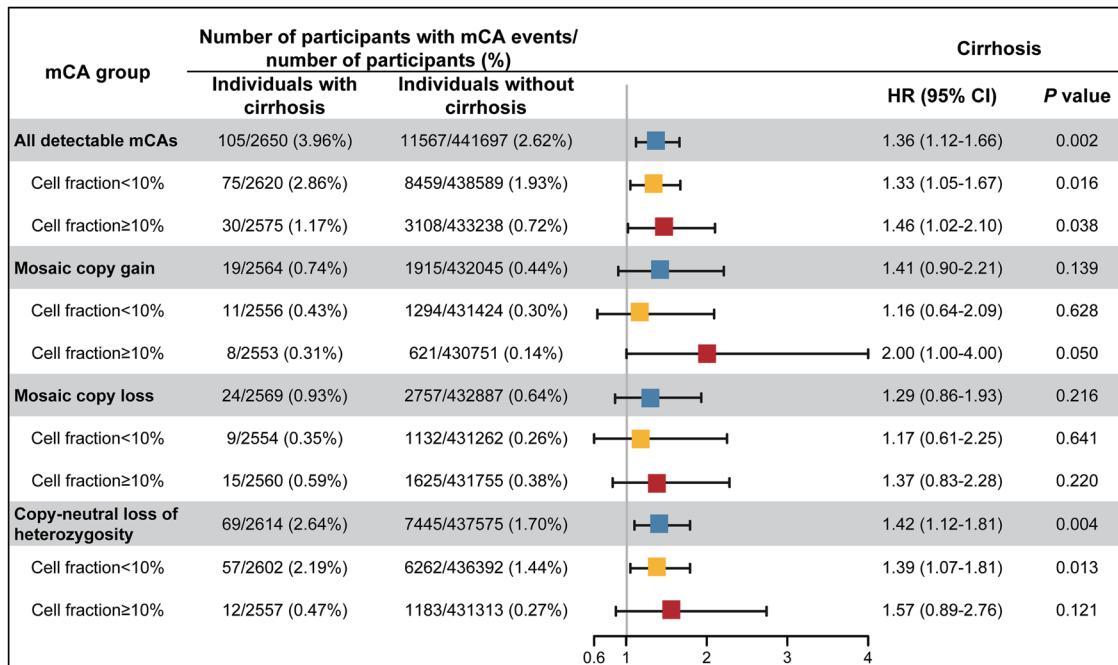
<sup>a</sup>Carrier status of mCAs: carrier vs. non-carrier of mCA. <sup>b</sup>Carrier status of mCAs: carrier of two or more mCAs vs. carrier of one mCA vs. non-carrier of mCA. <sup>c</sup>Estimates were adjusted for age, age<sup>2</sup>, sex, smoking status, drinking status, the top 10 principal components of ancestry, and genotyping batch. When evaluating the association between specific mCA types (i.e., copy-neutral loss of heterozygosity, gain, and loss) and the risk of cirrhosis, non-carriers of any mCA types were set as the reference group. HR, hazard ratio; CI, confidence interval.

occurrence of autosomal mCAs as age advanced within both subgroups.

#### **Significant association between mCAs and cirrhosis risk**

We proceeded to assess the relationship between autosomal mCAs and the risk of cirrhosis, and the results are presented in Table 3. Compared with non-carriers of any

mCAs, the presence of mCA events showed a significant association with an increased risk of cirrhosis, especially with two or more mCAs (HR 1.29 [95% CI 1.11–1.49]). Further investigation through analyses of specific mCA types indicated that the impact of mCAs on cirrhosis primarily originated from CN-LOH. Compared to non-carriers, individuals carrying copy number neutral mCAs exhibited a significantly higher risk of developing cirrhosis (HR 1.42 [95% CI 1.12–



**Fig. 2. Associations of different types of mosaic chromosomal alterations (mCAs) with the risk of cirrhosis.** Estimates were adjusted for age, age<sup>2</sup>, sex, smoking status, drinking status, the top 10 principal components of ancestry and genotyping batch. The cell fraction could not be estimated for 4,298 individuals who had undetermined mCA merely. Non-carriers of any mCA types were set as the reference group. HR, hazard ratio; CI, confidence interval.

1.81]). Subsequent analyses demonstrated a significant impact of CN-LOH on the risk of compensated cirrhosis (HR 1.50 [95% CI 1.13–2.00]). However, the effect of CN-LOH on the risk of decompensated cirrhosis was only marginally significant (HR 1.30 [95% CI 0.97–1.76]). These results remained consistent when adjusted for smoking pack-year, Thompson deprivation index, body mass index, and diabetes status for extra and redefined cirrhosis (Supplementary Tables 4 and 5).

Considering the potential variation in cell fractions, the impact of mCAs on the risk of cirrhosis was evaluated across two different cell fraction groups. Notably, expanded mCAs displayed a much stronger association with cirrhosis risk than non-expanded ones (HR 1.46 [95% CI 1.02–2.10] vs. 1.33 [95% CI 1.05–1.67], Fig. 2). Similar associations were found in mCA type-specific analyses. We observed that expanded and non-expanded mCAs had similar effects on the risk of compensated cirrhosis (HR 1.42 [95% CI 0.90–2.24] vs. 1.37 [95% CI 1.04–1.81], Supplementary Table 6). Inversely, the presence of expanded mCAs showed a much stronger association with the risk of decompensated cirrhosis than non-expanded ones (HR 1.58 [95% CI 1.03–2.44] vs 1.10 [95% CI 0.80–1.50], Supplementary Table 7), especially for individuals carrying copy number neutral mCAs (HR 2.03 [95% CI 1.09–3.78] vs. 1.14 [95% CI 0.80–1.64]).

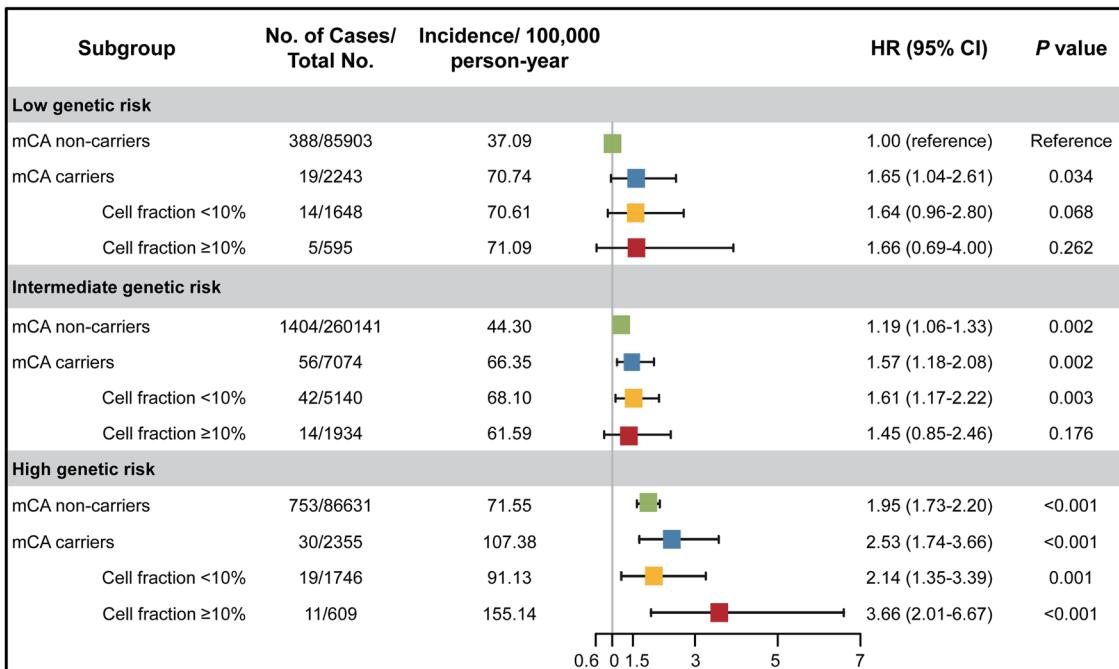
#### Joint and interaction effect of mCAs and PRS on the incident cirrhosis risk

To evaluate the combined impact of mCAs and genetic factors, a PRS for cirrhosis was constructed using 12 SNPs (Supplementary Table 1). The density plots of the PRS demonstrated a noticeable shift in distribution towards higher values among individuals who developed cirrhosis, as opposed to those who did not (Supplementary Fig. 4). Notably, individu-

als with a higher genetic risk exhibited an elevated susceptibility to cirrhosis compared to those with a lower genetic risk. Supplementary Table 8 shows that the HRs for intermediate and high genetic risk were 1.18 [95% CI 1.06–1.32] and 1.94 [95% CI 1.72–2.19], respectively. Moreover, the risk of incident compensated and decompensated cirrhosis also displayed a graded escalation corresponding to increasing genetic risk.

The overall incidence of cirrhosis was found to increase in a dose-response manner with both mCAs and genetic risk (Fig. 3). Compared to individuals without mCAs and with a low genetic risk, individuals with mCAs and a high genetic risk exhibited the highest risk of developing cirrhosis (HR 2.53 [95% CI 1.74–3.66]). Similar results were found in the association of CN-LOH and genetic categories with the risk of cirrhosis (Supplementary Table 9). Particularly, in comparison to non-carriers of CN-LOH at low genetic risk, individuals with expanded CN-LOH and a high genetic risk showed the highest cirrhosis risk (HR 5.39 [95% CI 2.41–12.07]). Furthermore, the presence of expanded mCAs further amplified this effect. Specifically, out of 609 individuals carrying expanded mCAs and a high genetic risk, 11 developed cirrhosis (incidence rate: 155.14 per 100,000 person-years), whereas out of 85,903 individuals without mCAs and with a low genetic risk, 388 developed cirrhosis (incidence rate: 37.09 per 100,000 person-years; HR 3.66 [95% CI 2.01–6.67]).

In addition, for groups of expanded gain abnormalities with an intermediate genetic risk, the relative excess risk of interaction was 2.87 [95% CI 0.44–5.31], which suggested that there would be 2.87 relative excess risks because of the additive interaction, accounting for 95% [95% CI 89–100%] of the risk of cirrhosis (Supplementary Table 10). Null interaction effects were observed between PRS and autosomal mCAs on the risk of compensated cirrhosis (Supplementary Table 11).



**Fig. 3. Risk of cirrhosis according to mosaic chromosomal alterations (mCAs) and genetic categories.** Estimates were adjusted for age, age<sup>2</sup>, sex, smoking status, drinking status, the top 10 principal components of ancestry and genotyping batch. The cell fraction could not be estimated for 4,298 individuals who had undetermined mCA merely. Non-carriers of any mCA types in the low genetic risk category were set as the reference group. HR, hazard ratio; CI, confidence interval.

## Discussion

This study presents groundbreaking findings that provide novel evidence for the link between mCAs and increased susceptibility to cirrhosis, with the primary source attributed specifically to CN-LOH, particularly for expanded mCAs. Additionally, we have identified a synergistic interaction effect between mCAs and genetic susceptibility, resulting in an amplified risk of cirrhosis. These findings enhance our comprehension of the genetic predisposition to cirrhosis and emphasize the potential benefits of integrating mCAs into existing risk assessment methods, such as PRS for precision improvement. All of these may enhance cirrhosis screening initiatives and provide valuable insights into the intricate etiology.

mCAs have variable effects on an individual's health and disease development, depending on the specific mutation type, the extent of mosaicism, and the affected tissues or organs. Our study is, to our knowledge, the first to establish a significant link between mCAs and the occurrence of cirrhosis, with an adjusted HR of 1.46. This effect size is comparable to that observed in digestive system infections (HR 1.46 vs 1.51) and greater than those in respiratory and genitourinary system infections (HR 1.46 vs 1.25).<sup>16</sup> However, it is weaker than the effects observed on the incidence of chronic lymphocytic leukemia and myeloid leukemia.<sup>17</sup> Notably, we observed a stronger and more robust association of mosaic mutations with compensated cirrhosis than with decompensated cirrhosis, implying that mCAs may mainly affect the early development of cirrhosis. However, the development and progression of cirrhosis are complex processes, and the role of mosaic mutations in decompensated patients may be masked by other disease or behavioral factors, such as chronic liver disease, alcohol and drug abuse, and viral infection. More studies are needed to reveal the possible mechanisms in the future.

The association with cirrhosis, especially with decompensated cirrhosis, can be explained principally by CN-LOH, which

refers to a genomic event where there is a loss of one of the two copies of a chromosomal region without any accompanying change in DNA copy number.<sup>18</sup> Recent investigations have provided compelling evidence on the link of CN-LOH to an elevated vulnerability to infection, myeloid malignancies, and coronary artery disease.<sup>19-21</sup> Furthermore, Midorikawa Y et al. utilized SNP arrays to perform allelic gene dosage analysis on 36 hepatocellular carcinomas and uncovered a direct association between CN-LOH events and an increased risk of hepatocellular carcinoma.<sup>22</sup> The observed effect size indicates that mosaic mutations of CN-LOH may play a vital role in the development of liver disease and cancer.

In the realm of potential biological mechanisms, it is assumed that mCAs may function as an intrinsic hallmark, indicative of accumulated DNA damage.<sup>23</sup> Especially when the cell content of the mosaic mutation exceeds 10%, it may indicate a high level of clonal expansion of abnormal cells, thereby causing a more pronounced deleterious effect. These consequential activities have been strongly implicated in both the initiation and progression of organ fibrosis.<sup>24</sup> Moreover, in the context of hepatitis progression, there has been an observed phenomenon of mosaic mutations contributing to a chronic inflammatory response, particularly in the presence of predominant stimuli. This leads to an intensified activation of proximal immune and fibrogenic pathways.<sup>25</sup> Chronic liver injury, caused by factors such as viral hepatitis, alcohol abuse, or metabolic diseases, triggers a wound-healing response that can be dysregulated by mosaic mutations. This dysregulation may result in an excessive accumulation of scar tissue,<sup>26</sup> contributing to the development of early cirrhosis. Mosaic mutations, including CN-LOH, as a complete set of pathogenic molecular lesions, disease, and prognosis markers, can impact genes involved in the regulation of fibrogenesis, a process by which fibrosis forms in the liver at an early stage.<sup>27</sup> In a synchronous colorectal cancer study,

the most frequent mutation cluster region was 16p11.2-p11.1 (59.5%), where CN-LOH was significantly associated with polyclonal synchronous colorectal cancers ( $p=0.038$ ).<sup>28</sup> CN-LOH was also associated with the duplication of oncogenic mutations with concomitant loss of the normal allele in myeloid malignancies.<sup>21</sup> Therefore, combining mosaic mutations and genetic mutations is a more accurate way to achieve precise prediction of disease.

Our findings may shed profound light on the intricate interplay between hereditary genomic variations inherited from parents and acquired genomic changes resulting from cumulative DNA damage. This underscores their synergistic influence on the initiation of cirrhosis. In the academic context, while the PRS approach has undeniably advanced the identification of cirrhosis high-risk subpopulations, our study conspicuously highlights the necessity of combining both PRS and mCAs to improve the precision of cirrhosis risk assessment. Moreover, mCAs could serve as an efficient and cost-effective biomarker in practical clinical applications as they can be derived from the SNP array employed in traditional PRS construction, without the need for additional tests.

Our analysis had some limitations. Primarily, our study solely captured mCAs at baseline levels. Considering the dynamic alterations of mCAs, relying on a solitary time-point measurement might introduce potential disparities in the observed effects concerning cirrhosis risk. Secondly, the current count of identified mCA events remains insufficient to precisely estimate the impact exerted by genetic or environmental factors on these specific genomic alterations. Finally, the limitation of a single center restricted our ability to conduct additional investigations on the impact of mCAs on cirrhosis risk across diverse populations.

## Conclusion

In conclusion, our findings present compelling evidence of a heightened susceptibility to cirrhosis among individuals with autosomal mCAs. This substantiates the impactful role of genetic determinants in liver disease progression to a significant extent. Furthermore, these results offer novel insights that contribute to a more accurate prognosis of cirrhosis.

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## Conflict of interest

The authors have no conflict of interests related to this publication.

## Author contributions

Study design (CS, QZ, XG, ML), project supervision (CS, QZ, LJ, FJ, QW), data interpretation (XG, LZ, XX, CY), data analysis (XW, YY, CT, JY, YD) and draft writing (XG, LZ, ML). All authors reviewed or revised the draft and approved the submitted draft.

## Ethical statement

The UK Biobank study received approval from the National Information Governance Board for Health and Social Care and the National Health Service Northwest Multi-Centre Research Ethics Committee (Ref: 11/NW/0382). Written informed consent was obtained from each participant.

## Data sharing statement

The data supporting the findings of this study are available from the UK Biobank (<https://www.ukbiobank.ac.uk/>). Restrictions apply to the availability of these data, which were used under license for the current study (Project ID: 64689). Data are available for bona fide researchers upon application to the UK Biobank.

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