**The membrane-bound O-acyltransferase domain-containing 7 variant rs641738 increases inflammation and fibrosis in chronic hepatitis B**

# Allelic differences between Han Chinese and Europeans for MBOAT7 rs641738 and its association with liver damage in chronic hepatitis B

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**Running title**: MBOAT7 and HBV

**Key words:** HBV, MBOAT7, fibrosis, inflammation steatosis

**Competing Financial Interests:** The authors declare no competing financial interests.

**Acknowledgements:** We would like to thank all the patients for their participation in this study. ME and JG are supported by the Robert W. Storr Bequest to the Sydney Medical Foundation, University of Sydney; a National Health and Medical Research Council of Australia (NHMRC) Program Grant (1053206) and Project grants (APP1107178 and APP1108422). KT is supported by a Scholarship from the Egyptian government.

**Abstract (N=177 words)**

Liver fibrosis is a polygenic disorder that supposed to share substantial genetic risk factors between the different etiologies of chronic liver diseases. MBOAT7 *rs641738* is a cirrhosis risk gene that was recently identified by genome-wide association study in alcoholic cirrhosis. Here, we explore the role of *rs641738* in two large cohorts of CHB (n=1101); of European (n=598) and Chinese ancestries (n=503). We also undertook gene expression analysis to detect the functional effects of the *rs641738* SNP on MBOAT7 expression in liver biopsy samples from CHB from both ethnic backgrounds. We demonstrated that *rs641738* impacts hepatic inflammation and fibrosis in the cohort of European (OR: 1.34, 95% CI: 1.02-2.04, p=0.03 and OR: 1.27, 95% CI: 1.07-2.83, p=0.04, respectively) but not in Chinese. It has no effect on steatosis or metabolic profile in both cohorts. We also found *rs641738* was associated with MBOAT7 expression in liver biopsies from European but not Chinese. We conclude that *rs641738* polymorphism in MBOAT7 is a novel risk variant for liver disease progression in hepatitis B, in European but not in Han Chinese populations.

## INTRODUCTION

Hepatitis B virus (HBV) infection remains a worldwide public health challenge with an estimated two billion people infected and over one million deaths annually due to fulminant hepatitis B, liver cirrhosis, or hepatocellular carcinoma (HCC) ([1]). Liver disease associated with chronic hepatitis B (CHB) is induced by inflammatory processes initiated when the host immune response attempts to eliminate the hepatitis B virus. In addition, a complex interplay between host genetics, the immune response and viral factors modulates the final outcome of hepatitis B infection ([2]).

While hepatitis B infection exists in all geographic contexts and continents, the disease is endemic with high prevalence in certain populations including in east Asia, sub-saharan Africa and around the Mediterranean basin ([3]). The reason for the variable prevalence rates is not known, but contributing factors include differences in host-virus interactions and socioeconomic or cultural practices. Accumulating evidence suggests that host genetics are important contributors to the risk of HBV persistence and progression ([4]). Hence a better understanding of host genomics in HBV infected patients is pivotal as it may allow the development of biomarkers for disease prognosis and treatment outcomes, the elucidation of mechanisms for disease pathogenesis and the identification of potential therapeutic targets ([4]).

Recently, a genome-wide association study (GWAS) identified a variant *rs641738* in the Membrane Bound O-Acyltransferase Domain Containing 7 gene (MBOAT7) to be associated with alcoholic cirrhosis ([5]). Since the pathways leading to inflammation and fibrosis severity might be similar irrespective of the inciting injury, we explored the role of MBOAT7 *rs641738* to liver disease phenotype in a large European population cohort with CHB. To test if our findings were universally applicable or particular to populations of a unique ancestry, we

conducted further analysis to determine the role of this polymorphism in a Chinese population. We subsequently undertook gene expression analysis to detect functional effects of the *rs641738* polymorphism on MBOAT7 expression in liver biopsy samples from patients with CHB of both ethnic backgrounds, supplemented by analysis of publically available datasets for expression quantitative trait loci (eQTL).

## Methods

### Patient cohort

The study comprised two independent consecutive cohorts with histologically characterized CHB from the International Liver Disease Genetics Consortium (ILDGC) database (n=1101). The first included 598 European Caucasian patients and the second comprised a cohort of 503 Chinese patients. Details of the cohort and inclusion criteria have been reported ([6,7]). Briefly, all patients had detectable hepatitis B surface antigen (HBsAg), persistently or intermittently abnormal alanine aminotransferase (ALT) values, and serum HBV DNA >2,000 IU/mL lasting for >6 months with at least one liver biopsy prior to any therapy. Patients were excluded if they had evidence of co-infection with either hepatitis C virus (HCV), hepatitis delta virus (HDV) or human immunodeficiency (HIV) virus, had evidence of other liver diseases by standard tests or current or previous hepatic decompensation.

Ethics approval was obtained from the Human Research Ethics Committees of the Sydney West Local Health District and the University of Sydney. All other sites had ethics approval from their respective ethics committees. Written informed consent for genetic testing was obtained from all participants.

### Clinical and laboratory assessment

The following data were collected at time of liver biopsy from all patients: gender, age, ethnicity, recruitment center, alcohol intake (gms/day), body mass index (BMI), HBV-DNA level, HBe-Ag status and routine laboratory tests. Alcohol consumption was assessed by 2 separate interviews with the patient and close family members. BMI was calculated as weight divided by the square of the height ($kg/m^2$).

**Genotyping**

Genotyping for *MBOAT7 rs641738* was undertaken using the TaqMan SNP genotyping allelic discrimination method (Applied Biosystems, Foster City, CA, USA). Genotyping for *TM6SF2 rs58542926* and *PNPLA3 rs738409* was contracted to the Australian Genome Research Facility (AGRF; QLD, Australia) and samples were genotyped using the Sequenom MassARRAY system and iPLEX Gold chemistry. All genotyping was blinded to clinical variables.

**Liver Histopathology**

Liver histopathology was scored by expert pathologists according to METAVIR ([8]). Fibrosis was staged from F0 (no fibrosis) to F4 (cirrhosis). Necroinflammation (A) was graded as A0 (absent), A1 (mild), A2 (moderate), or A3 (severe). Steatosis was quantified as follows: grade 0: absent or <5% of hepatocytes involved; grade 1: 5%-33%; grade 2: 34%-66%; and grade 3: >66% of hepatocytes affected. The inter-observer agreement between pathologists was studied previously and was good ($\kappa = 77.5$) for METAVIR staging using $\kappa$ statistics ([9]).

**Statistical methods**

Statistical methods are detailed in supplementary methods. All tests were two-tailed and p values <0.05 were considered significant.

**Population genetic analysis**

Global distribution of the MBOAT7 *rs641738* among 53 populations was derived from the Human Genome Diversity Project (HGDP) browser (http://hgdp.uchicago.edu/) ([10]).

**Gene expression analysis**

To determine the functional effects of MBOAT7 *rs641738*, we undertook three analyses. In the first, we examined for the association of hepatic MBOAT7 expression according to

MBOAT7 *rs641738* genotype in two well characterized and matched cohorts of CHB patients of European (n=21) and Chinese (n=21) ancestry. The details of RT-PCR are provided in the **supplementary methods**. Next we interrogated multiple publicly available databases containing expression information from liver tissues in European ([11]) and Chinese ([12]) subjects separately.

## Results

### MBOAT7 rs641738 minor allele frequency in the HBV cohort of European ancestry

The clinical, anthropometric and biochemical characteristics of patients in the HBV cohort of European ancestry are presented in **supplementary table 1**. The genotype distribution of MBOAT7 *rs641738* was in Hardy-Weinberg equilibrium. The minor allele frequency (MAF) of MBOAT7 *rs641738* was 0.436 **(supplementary table 2)**, similar to that observed in a healthy Caucasian population from the 1000 genome project (http://browser.1000genomes.org). There was no significant difference in *rs641738* allele frequency distribution according to patient country of origin (p=0.4 for trend).

### Association between MBOAT7 rs641738 and viral, clinical and metabolic characteristics in the HBV cohort of European ancestry

Apart from the fact that subjects with the *rs641738* T allele had significantly higher GGT levels compared to subjects with CC genotype, no other significant associations were observed with any other clinical or viral characteristics. As regards the metabolic profile, data was only available in 98 subjects; in this sub-cohort, no significant associations were observed with the lipid or glycemic profile (**Supplementary Table 3**).

### MBOAT7 rs641738 and liver histology in the HBV cohort of European ancestry

We next assessed the impact of *rs641738* on hepatic histological features including inflammation, fibrosis and steatosis. The distribution of *rs641738* genotypes according to histological features are depicted in **Figure 1.** In multivariate ordinal regression analysis adjusted for covariates including age, gender, T2DM, recruitment centre, *PNPLA3* and *TM6SF2* genotypes, HBe-Ag status, MBOAT7 *rs641738* was associated with the severity of

necroinflammation (β=0.085±0.043, 95% CI: 0.023-0.173, p=0.03) and fibrosis (β=0.079±0.031, 95% CI:0.023-0.225, p=0.04). In further analysis subdividing the cohort into those with mild necroinflammation (A0–1) and severe necroinflammation (A2–A3), again the *rs641738* T allele was independently associated with severe necroinflammation (OR: 1.34, 95% CI: 1.02-2.04, p=0.03) in multiple logistic regression analysis adjusting for the same variables (**Table 1**). Similarly for fibrosis, *rs641738* T allele was associated with significant fibrosis (≥ F2) (OR: 1.27, 95% CI: 1.07-2.83, p=0.04) (**Table 1**). Hepatic steatosis scoring was only available in 373 (62%) subjects; in those, *rs641738* T allele has no impact on steatosis grade (β=0.044±0.014, 95% CI: 0.006-0.376, p=0.5) or moderate/severe steatosis (S2-S3) (OR: 1.06, 95% CI: 0.53-1.88, p=0.9).

**MBOAT7 rs641738 minor allele frequency in the HBV cohort of Chinese ancestry**

To replicate our results, we tested whether *rs641738* was associated with histological features in Chinese patients with CHB. In this cohort of 503 patients, the genotype distribution of MBOAT7 *rs641738* was in Hardy-Weinberg equilibrium. The MAF was dramatically lower than in Europeans (MAF=0.24), an observation that was similar in a Chinese population from the 1000 genome project (MAF=0.22-0.24). To examine for the detailed global allele frequency distributions of MBOAT7 *rs641738*, we next compared its allele frequencies in 53 world populations. Intriguingly, MBOAT7 *rs641738* showed dramatic differences in derived allele frequency (T allele) with the highest frequencies in Europe and South Asia, followed by North and southern Africa and lowest in East Asia (**Figure 2**).

Apart from the fact that subjects with *rs641738* T allele had significantly higher alkaline phosphatase levels, compared to subjects with CC genotype, no differences in clinical,

anthropometric, viral or biochemical parameters including circulating lipids and blood glucose were detected across MBOAT7 *rs641738* genotypes (**Supplementary Table 6**).

**MBOAT7 rs641738 is not associated with liver injury in the HBV cohort of Chinese ancestry**

We next assessed the impact of *rs641738* on histological features including inflammation, fibrosis and steatosis in the HBV cohort of Chinese ancestry. In contrast to the observed association of *rs641738* with liver injury in Europeans, this was not replicated in the Chinese cohort. The distribution of *rs641738* genotypes according to histological features (inflammation, fibrosis and steatosis grade) are depicted in **Figure 3.**

MBOAT7 *rs641738* was not associated with the severity of necroinflammation (β=0.059±0.049, 95% CI: 0.026-0.167, p=0.1), fibrosis (β=0.043±0.035, 95% CI: 0.016-0.219, p=0.4) or steatosis (β=0.035±0.012, 95% CI: 0.017-0.179, p=0.5) (**Supplementary Table 7**). In a further analysis, MBOAT7 *rs641738* was not associated with severe necroinflammation (OR: 1.16, 95% CI: 0.97-1.68, p=0.1), significant fibrosis (≥ F2) (OR: 1.09, 95% CI: 0.87-1.85, p=0.4) or moderate/severe steatosis (S2-S3) (OR: 1.05, 95% CI: 0.8-2.28, p=0.6) (**Table 2**).

To further explore for a potential explanation for the differential effects on liver histology between European and Chinese CHB cohorts, we repeated the analysis using different genetic models; the same results were obtained. The same results were also obtained when subjects with HBe-Ag negative or positive disease or high (i.e. equal or higher than median) or low HBV-DNA levels were analyzed separately (**data not shown**).

We also performed a power analysis using the G*power program ([13]) based on Cohen's method ([14]). The European and Chinese sample sizes revealed >98% and >95% power to detect a

significant association ($\alpha<0.05$), respectively, given an effect size index of 0.2 (which corresponds to a weak to moderate gene effect), hence the possibility that this negative association was due to lack of power is less likely.

In total, in contrast to the effect *rs641738* on liver injury in patients infected with CHB of European descent, it had no effect on those of Chinese descent.

**rs641738 genotype associates with MBOAT7 mRNA expression in liver biopsies from European but not in Chinese patients**

To reconcile the above findings, we investigated the functional impact of *rs641738* on hepatic MBOAT7 expression in liver biopsies from two well characterized sub-cohorts of Europeans and Chinese patients, correcting for difference in MAF between both groups (n=21 per each group). Apart from the fact that Europeans had a higher BMI, the baseline characteristics of both groups were matched and are summarized in **supplementary table 8**; the sub-cohort were also matched with the overall cohort. In this analysis, *rs641738* genotype was associated with hepatic MBOAT7 expression in liver biopsies from European but not Chinese subjects (**Figure 4**), further supporting our genetics observations.

Finally, we explored multiple publicly available eQTL databases. Consistent with our data, in a genome-wide eQTL mapping of human liver samples from Caucasian patients, *rs641738* was associated with hepatic MBOAT7 expression ($p= 3.65x10^{-12}$) ([12]), while in a set of Chinese liver tissue samples, at the 5% FDR level, no association with hepatic MBOAT7 expression with was reported ([12]).

## Discussion

To our knowledge, this is the first study to explore the role of polymorphisms at MBOAT7 *rs641738* on the liver phenotype of patients with CHB. We demonstrate that *rs641738* impacts hepatic inflammation and fibrosis in Europeans with CHB. These observations were not replicated in a cohort of Chinese ancestry, a finding supported by publically available MBOAT expression eQTL datasets from liver, as well as our data on MBOAT7 expression in liver biopsies from Chinese and European patients with chronic hepatitis B.

In the European cohort, subjects carrying the MBOAT7 *rs641738* T minor allele demonstrated an increased risk of hepatic inflammation and fibrosis. This effect was independent of other risk factors including age at time of biopsy, gender, HBe-Ag status, T2DM, recruitment centre, and other related risk variants, i.e. PNPLA3 *rs738409* and TM6SF2 *rs58542926* genotype. These findings in CHB for Europeans are consistent with what has recently been reported in alcoholic and non alcoholic fatty liver diseases and recently by us in Caucasians with hepatitis C (manuscript submitted) ([5,15]). In contrast, no similar effects according to MBOAT7 *rs641738* genotype were present in those of Chinese descent either with regard to liver histological phenotype or hepatic MBOAT expression. To our knowledge, no other studies have explored the role of MBOAT7 to liver injury in Chinese population for any liver disease. Collectively, these data, in the context of other published reports ([5,15]) suggest that *rs641738* is a novel risk variant for hepatic inflammation (and consequently fibrosis), in Europeans across multiple liver diseases.

Notably, both European and Caucasian cohorts had a balanced distribution of fibrosis stage (~60% and 55% with ≥ F2 in the European and Chinese cohorts, respectively), age and

gender indicating that this is unlikely a cause for the discrepancy in results. This suggests that either the MBOAT7 variant examined herein might not be a risk gene for HBV-related liver injury in Chinese populations, or that genetic heterogeneity may be an additional explanation for the negative results. In the latter case, other risk variants rather than *rs641738* in the MBOAT7 gene could be a risk variant for liver injury in Chinese, however, it should be noted that no association between any variant and hepatic MBOAT7 expression in Chinese cohort from available eQTL dataset was reported ([12]). Further fine mapping studies in Chinese populations could help in resolving this issue. As the variation in genotypic relative risk across different populations may also be attributed to interactions with unmeasured factors that are unique to specific populations, another intriguing but untested possibility raised by the present findings is that MBOAT7 *rs641738* might influence hepatic inflammation and fibrosis in Chinese populations, only according to specific liver disease etiologies. Further studies to investigate the role of MBOAT7 *rs641738* in other liver disease etiologies are thus required.

Notably, there are ethnic differences in the allele frequency of the MBOAT7 *rs641738* polymorphism between European (MAF=~ 0.45) and East Asian populations (~0.24), in the healthy population from the 1000 genome project as well as in the present cohorts. Hence, to explore if sample size is likely to explain the negative associations observed by us in Chinese, we undertook power analysis. This confirmed that both cohorts had > 95% power to detect an effect size index of 0.2 (which corresponds to a weak to moderate gene effect). However, our cohorts were not powered to detect an effect size index of 0.1 (which corresponds to a weak gene effect), so the possibility that *rs641738* could have a very weak effect in Chinese cannot be ruled out.

It is noteworthy that *rs641738* controls hepatic expression of MBOAT7 in Europeans but not in Chinese, a result consistent with publically available eQTL data and with the previously reported variation in expression levels of many other genes between human populations ([16,17]). This variation among populations could be explained by the fact that the contribution of an allele in any given population depends upon its allele frequency ([16]), but more importantly by population-specific genotypic effects, as reported previously ([17]). On the other hand, further detailed evolutionary analyses would be required to understand the major forces shaping the genetic diversity of MBOAT7 between Europeans and Chinese. Such analyses of population genetic histories may aid in explaining the frequently observed inconsistency in genetic associations for complex diseases between different ethnic populations ([18]).

The detailed functional mechanisms of MBOAT7 action are still not completely known. MBOAT7 (Lysophospholipid acyltransferase) belongs to the remodeling pathway of phosphoinositides (Land's cycle) that attaches arachidonic acids (AA) to lysophosphatidylinositol ([19]) to reduce free AA levels. Free AA induces apoptosis ([20]), which is a potent trigger of hepatic inflammation and fibrosis ([21]) and amplifies the inflammatory response in macrophages and other immune cells ([22]). Thus, upregulation of MBOAT7 in response to a hepatic insult might be a compensatory or adaptive mechanism, similar to that observed in other contexts such as intrauterine growth restriction and shock ([23]).

In conclusion, we provide evidence that MBOAT7 *rs641738* contributes to hepatic inflammation and fibrosis in European but not Chinese patients with chronic hepatitis B. The reasons for this differential effect are unknown, but warrants further evolutionary analyses of MBOAT7 variants in Chinese populations with CHB and also those with other liver diseases such as chronic hepatitis C, alcoholic and non alcoholic fatty liver disease.

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**Table 1. Independent predictors of moderate/severe steatosis (≥S2), severe necroinflammation (A2–A3) and significant fibrosis (≥F2) by logistic regression analysis in the HBV patient cohort of European ancestry (n=598).**

| | Moderate/severe steatosis (≥S2) | | | Severe necroinflammation (A2–A3) | | | Significant fibrosis (F2-F4) | | |
|---|---|---|---|---|---|---|---|---|---|
| | **OR** | **95% CI** | ***P* value** | **OR** | **95% CI** | ***P* value** | **OR** | **95% CI** | ***P* value** |
| **Age, years** | 1.04 | 1.01-1.06 | 0.001 | 1.008 | 0.97-1.03 | 0.3 | 1.05 | 1.01-1.08 | 0.001 |
| **Gender, male** | 1.81 | 0.86-3.82 | 0.1 | 1.6 | 1.05-2.7 | 0.01 | 1.4 | 1.1-2.2 | 0.01 |
| **Diabetes** | 1.89 | 1.32-4.15 | 0.001 | 1.32 | 0.66-2.63 | 0.4 | 1.25 | 0.72-2.18 | 0.4 |
| **BMI, Kg/m²** | 1.15 | 1.06-1.24 | 0.0001 | 1.006 | 0.95-1.06 | 0.8 | 0.99 | 0.94-1.05 | 0.9 |
| **HBV-DNA ($Log_{10}$ IU/mL)** | 0.88 | 0.75-1.03 | 0.1 | 0.954 | 0.87-1.03 | 0.2 | 0.94 | 0.86-1.02 | 0.1 |
| ***MBOAT7* Genotype** | 1.06 | 0.53-1.88 | 0.9 | 1.34 | 1.02-2.04 | 0.03 | 1.27 | 1.07-2.83 | 0.04 |

**Table 2. Independent Predictors of moderate/severe steatosis (≥S2), severe necroinflammation (A2–A3) and significant fibrosis (≥F2) by logistic regression analysis in the HBV patient cohort of Chinese ancestry (n=503).**

| | Moderate/severe steatosis (≥S2) | | | Severe necroinflammation (A2–A3) | | | Significant fibrosis (F2-F4) | | |
|---|---|---|---|---|---|---|---|---|---|
| | **OR** | **95% CI** | ***P* value** | **OR** | **95% CI** | ***P* value** | **OR** | **95% CI** | ***P* value** |
| **Age, years** | 1.01 | 0.99-1.04 | 0.8 | 1.03 | 1.007-1.07 | 0.01 | 1.02 | 1.004-1.04 | 0.01 |
| **Gender, male** | 1.16 | 0.64-2.11 | 0.6 | 1.44 | 0.72-2.89 | 0.3 | 2.007 | 1.26-3.17 | 0.007 |
| **Diabetics** | 1.18 | 1.02-1.37 | 0.02 | 1.14 | 0.29-4.46 | 0.8 | 1.3 | 0.56-3.01 | 0.5 |
| **BMI, Kg/m²** | 1.74 | 1.22-2.82 | 0.01 | 0.96 | 0.9-1.02 | 0.2 | 1.003 | 0.95-1.05 | 0.8 |
| **HBV-DNA ($Log_{10}$ IU/mL)** | 1.005 | 0.95-1.06 | 0.8 | 1.45 | 1.23-1.71 | 0.0001 | 1.27 | 1.13-1.42 | 0.0001 |
| ***MBOAT7* Genotype** | 1.05 | 0.8-2.28 | 0.6 | 1.16 | 0.97-1.68 | 0.1 | 1.09 | 0.87-1.85 | 0.4 |

**Figures:**

**Figure 1: Association of *rs641738* genotype with steatosis degree (A), necroinflammation (B) and fibrosis stage (C) in the European cohort (n=598)**. P-values are univariate and provided for the dominant model of inheritance, unless otherwise indicated.

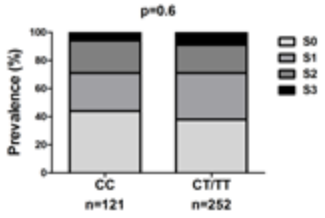
**Figure 2: Global distribution of the MBOAT7 *rs641738* single-nucleotide polymorphism (SNP) in different populations** Human Genome Diversity Project (HGDP) browser (**http://hgdp.uchicago.edu/**). The derived allele [T] is the risk allele.

**Figure 3: Association of *rs641738* genotype with steatosis degree (A), necroinflammation (B) and fibrosis stage (C) in the Chinese cohort (n=503)**. P-values are univariate and provided for the dominant model of inheritance, unless otherwise indicated.

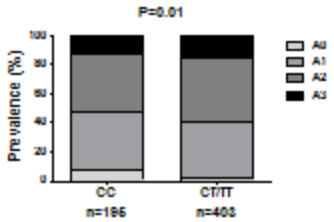
**Figure 4: Correlation between *MBOAT7 rs641738* genotype and hepatic MBOAT7 mRNA levels** in Europeans (n=21) (A) and Chinese (n=21) (B).

**Figure 1:**

**A)**


p=0.6
Prevalence (%)
100
80
60
40
20
0
S0
S1
S2
S3
CC
n=121
CT/TT
n=252

**B)**


P=0.01
Prevalence (%)
100
80
60
40
20
0
A0
A1
A2
A3
CC
n=186
CT/TT
n=403

**C)**

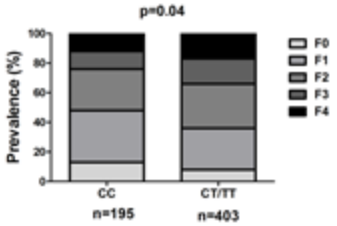

p=0.04
Prevalence (%)
100
80
60
40
20
0
F0
F1
F2
F3
F4
CC
n=195
CT/TT
n=403

**Figure 2:**

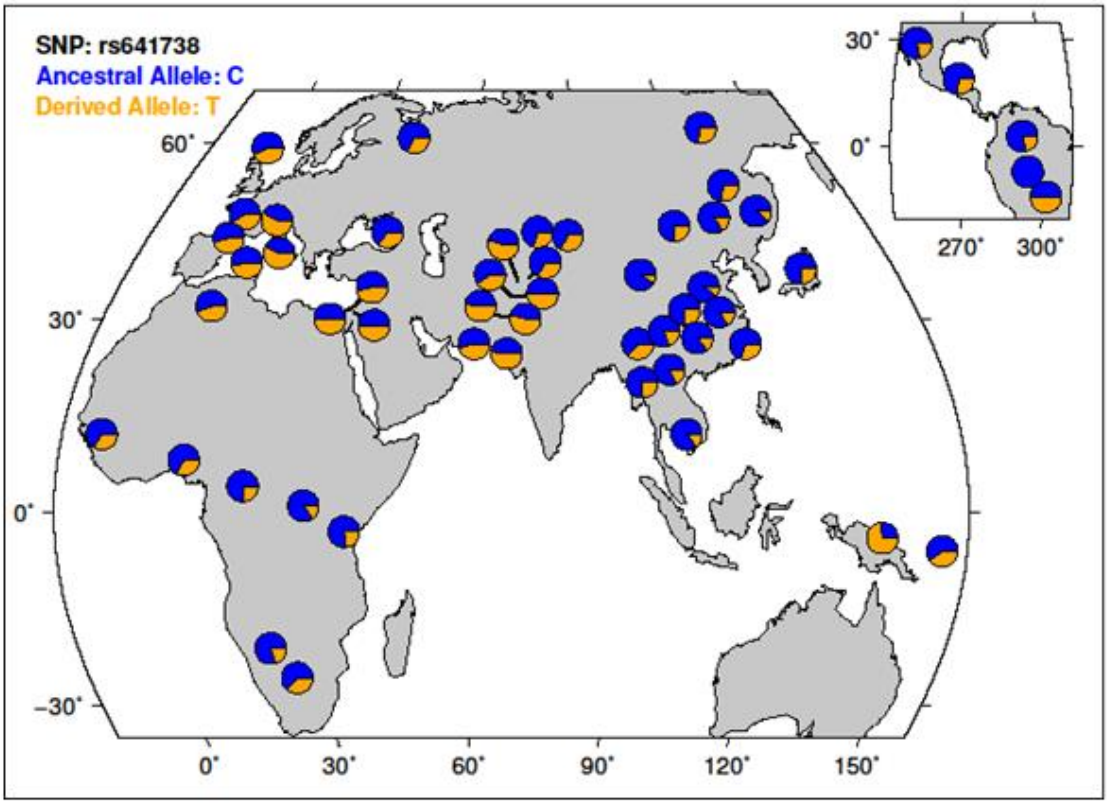
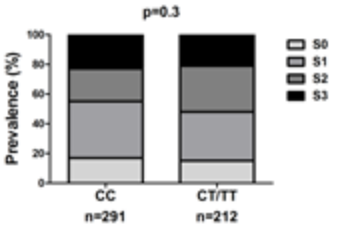
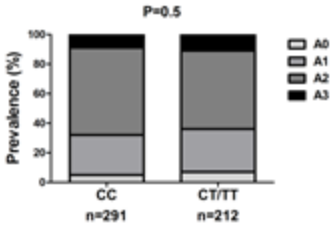

SNP: rs641738
Ancestral Allele: C
Derived Allele: T
60°
30°
0°
−30°
0°
30°
60°
90°
120°
150°
30°
0°
270°
300°

Figure 3:

A)


p=0.3
Prevalence (%)
100
80
60
40
20
0
CC
n=291
CT/TT
n=212
S0
S1
S2
S3

B)


P=0.5
Prevalence (%)
100
80
60
40
20
0
CC
n=291
CT/TT
n=212
A0
A1
A2
A3

C)

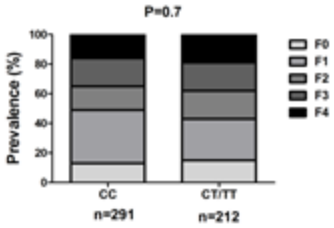
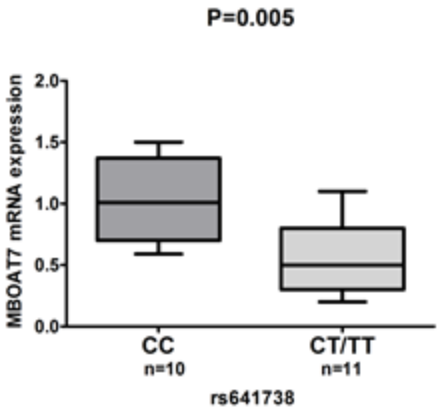

P=0.7
Prevalence (%)
100
80
60
40
20
0
CC
n=291
CT/TT
n=212
F0
F1
F2
F3
F4

Figure 4:

A) Europeans




B) Chinese