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Genetic variation in alcohol metabolizing enzymes among Inuit and its relation to drinking patterns

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ABSTRACT

Background: Variation in genes involved in alcohol metabolism is associated with drinking patterns worldwide. We compared variation in these genes among the Inuit with published results from the general population of Denmark and, due to the Asian ancestry of the Inuit, with Han Chinese. We analyzed the association between gene variations and drinking patterns among the Inuit.
Methods: We genotyped 4162 Inuit participants from two population health surveys. Information on drinking patterns was available for 3560. Seven single nucleotide polymorphisms (SNPs) were examined: ADH1B arg48his, ADH1C ile350val, ADH1C arg272gln, ALDH2 glu504lys, ALDH2 5′-UTR A-357G, ALDH1B1 ala86val and ALDH1B1 arg107leu.
Results: The allele distribution differed significantly between Inuit and the general population of Denmark. A protective effect on heavy drinking was found for the TT genotype of the ALDH1B1 arg107leu SNP (OR = 0.59; 95% CI 0.37–0.92), present in 3% of pure Inuit and 37% of Danes. The ADH1C GG genotype was associated with heavy drinking and a positive CAGE test (OR 1.34; 95% CI 1.05–1.72). It was present in 27% of Inuit and 18% of Danes. The Asian genotype pattern with a high frequency of the ADH1B A allele and an ALDH2 gene coding for an inactive enzyme was not present in Greenland.
Conclusions: ADH1C and ALDH1B1 arg107leu SNPs play a role in the shaping of drinking patterns among the Inuit in Greenland. A low frequency of the ALDH1B1 arg107leu TT genotype compared with the general population in Denmark deserves further study. This genotype was protective of heavy drinking among the Inuit.

1. Introduction

Alcohol related somatic, psychiatric and social consequences are major public health problems in many indigenous populations, including the Inuit in Greenland. In Greenland, the average yearly consumption of alcohol has for the last 20 years been equivalent to 12 l of pure alcohol per person aged 15 and above (Statistics Greenland, 2013) but binge drinking is prevalent (Jeppesen and Larsen, 2008).

Alcohol is metabolized by two isoenzymes, i.e., alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH), coded from different gene loci (Hempel et al., 1984; Yoshida et al., 1984; Bosron and Li, 1986; Eriksson et al., 2001). Variations in alcohol metabolizing enzymes have been associated with a higher frequency of alcohol problems (Tolstrup et al., 2008), higher prevalence of binge drinking (drinking five drinks or more per occasion (Husemoen et al., 2008)) and alcohol-induced hypersensitivity (Linneberg et al., 2010). In East Asians, an allele of ALDH encoding inactive acetaldehyde dehydrogenase is prevalent. Carriers of this allele experience malaise and flushing when drinking, and it has consistently been shown that such individuals binge-drink less often and have lower risk of alcoholism compared with individuals with functional ALDH (Harada et al., 1982; Higuchi et al., 1994, 1996; Muramatsu et al., 1995; Luczak et al., 2006; Zintzaras et al.,

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2006). This allele has not been observed in a general white population (Tolstrup et al., 2008), but it is unknown if it exists in Inuit. However, neither typical nor atypical flushing has been reported from Greenland.

The ancestors of the Inuit migrated from Siberia to Alaska as late as 2000 years ago and arrived in Greenland from arctic Canada within the last millennium (Gulløv, 2004; Reich et al., 2012). The distribution of the genetic variations in ADH and ALDH among Inuit and related indigenous populations of Alaska and Siberia is largely unknown. Among Alaska Natives entering treatment for alcoholism the allele distributions of ADH and ALDH were similar to those of a general sample of Yupik Eskimos, and not significantly different among seven indigenous populations in the study. The distribution of alleles did not resemble the typical Asian distribution (Segal, 1999). Other studies showed that the inactive ALDH2 allele was absent among Alaska Natives, Siberian Eskimos and Chuckchi (Thomasson et al., 1992; Avksentyuk et al., 1994).

The purpose of the present study was to compare the variation in genes that are involved in alcohol metabolism among the Greenland Inuit with published results from the general population of Denmark and Han Chinese. Specifically, we wanted to test the hypothesis that the Inuit carried the allele of ALDH encoding inactive acetaldehyde dehydrogenase which has been found among East Asians. Secondly, we wanted to analyze if the association between such genes and drinking patterns among the Inuit were similar to those reported in the literature and thus to determine the role of genetics in drinking behaviour in this indigenous population.

2. Methods

The data were collected as part of two population surveys in Greenland. Only participants categorized at enrolment as indigenous Greenlanders (synonym: Inuit) based on the primary language of the participant and self-identification were included in the analyses. In 1999–2001, data ($N = 1299$) were collected by interview and clinical examination in six towns and villages on the west coast of Greenland as part of a general population health survey with a participation rate of 67% (Bjerregaard et al., 2003). In 2005–2010, data ($N = 2885$) were collected by interview and clinical examination in 24 towns and villages as part of a general population health survey with a participation rate of 67% (Bjerregaard, 2011). Information about at least one of the genetic markers was available from 4162 participants (99.5%).

2.1. Genotyping

The following single nucleotide polymorphisms (SNPs) were examined in 4112–4151 participants each: ADH1B arg48his (rs1229984), ADH1C ile350val (rs698), ADH1C arg272gln (rs1693482), ALDH2 glu504lys (rs671) (Hempel et al., 1984; Yoshida et al., 1984), ALDH2 5′-UTR (untranslated region, A to G conversion located at −357 from starting codon) (rs886205) (Chou et al., 1999; Harada et al., 1999; Fischer et al., 2007), ALDH1B1 ala86val (rs2228093) (Hsu and Chang, 1991; Sherman et al., 1993), and ALDH1B1 arg107leu (rs2073478) (Hsu and Chang, 1991; Sherman et al., 1993). We chose to analyze these genotypes because they were previously shown in several studies to be associated with alcohol metabolism or suggested to be associated with drinking patterns (Husemoen et al., 2008; Tolstrup et al., 2008). DNA was collected from buffy coats obtained from whole EDTA blood samples. Buffy coats were stored at −80 °C until extraction. Nucleic acid extraction was done using the patented sbeadex™ technology (surface-coated superparamagnetic beads) (KBioscience, Hoddesdon, UK). Genotyping was performed by KASPar® technology (KBioscience, Hoddesdon, UK).

2.2. Drinking patterns

Information on alcohol consumption and drinking patterns was collected by a self-administered questionnaire and was available for 3560 participants. The data included an estimate of consumption (drinks per week), a modified CAGE questionnaire (Zierau et al., 2005) and an estimate of binge drinking. Participants who indicated not having consumed alcohol within the last 12 months were categorized as non-drinkers. For other participants, the habitual weekly consumption was calculated the number of days per week the respondent usually drank alcohol (reported as specific number of days or if not available the following frequency categories: daily/almost daily; 3–6 times per week; 1–2 times a week; less often) multiplied by the number of drinks consumed on the last occasion (Bjerregaard and Becker, 2013). From this information participants were classified as heavy drinkers if they reported a weekly consumption above 21 drinks (men) or 14 drinks (women). The CAGE questionnaire is a short four-item questionnaire to detect alcoholism (Mayfield et al., 1974). The original CAGE questionnaire, however has not worked optimally in a Danish population and the questions have therefore been modified to include the following six questions (Zierau et al., 2005). The test has been validated in hospital patients (Zierau et al., 2005), and consists of six items:

1. Have you, within the last year, felt you should cut down on your drinking?
2. Have people, within the last year, annoyed you by criticizing your drinking?
3. Have you, within the last year, felt bad or guilty about your drinking?
4. Have you, within the last year, had a drink first thing in the morning to steady your nerves or get rid of a hangover (eye opener)?
5. Do you drink alcohol outside mealtimes on weekdays?
6. How many days a week do you drink alcohol?

Two positive answers in questions 1–5 or one positive answer in questions 1–5 plus alcohol drinking more than 3 days per week (question 6) define a positive test result. Binge drinking was defined from answers to the question "Within the past year, how often did you drink more than 5 drinks on the same occasion?" Answers "more than once a week" and "once a week" counted as positive. Information on binge drinking was only available for the 2005–2010 survey.

2.3. Confounders

Information on confounders was obtained by interview. Ancestry was based on questions about the ethnicity and place of birth of parents and grandparents. A population group with the assumed least admixture of non-Inuit genes was defined as those born in remote East or North Greenland and having four Inuit grandparents. This population was used to characterize the allele distribution in "least admixed" Inuit (Table 2 and Fig. 1). A related but not identical measure of "social remoteness" was participants who were born and currently lived in remote East or North Greenland. Age, sex, education categorized as no education, short education, and medium or long education, and social remoteness were chosen as confounders for model 2 in Table 3 because these variables were significantly associated with drinking patterns and genetic admixture.

2.4. Statistical analyses

Deviations from the Hardy–Weinberg equilibrium were tested in Excel by chi-squared analyses with 1 df. All other analyses were conducted using SPSS version 21. Associations between ADH and ALDH gene variants with non-drinking, heavy drinking, positive CAGE and binge drinking were analyzed in unadjusted logistic regression models (model 1) and adjusted for age, sex, education and social remoteness (model 2). Weekly consumption (no. of drinks) was analyzed in General Linear models adjusted for the same confounders. Models were tested for interaction with gender but there was no interaction with gender in any of the models. The simultaneous analysis of 16 statistical models (four SNPs and four outcomes) calls for a Bonferroni adjustment of significance levels; a p value of 0.05 corresponds after Bonferroni correction to $p/n = 0.003$. The p values in Table 3 were not Bonferroni adjusted.

2.5. Ethical considerations

The studies were ethically approved by the Commission for Scientific Research in Greenland. Participants gave their written consent after being informed about the study orally and in writing.

3. Results

The study base consisted of 4162 Inuit with information about at least one of the SNPs. Among these, 531 were born in East or North Greenland and reported having four Inuit grandparents. These were considered the least admixed Inuit. Information about alcohol consumption was available for 3560 participants. Table 1 shows the characteristics of the study populations. Non-drinkers made up 17% among men and 23% among women, heavy drinkers 14% among men and 11% among women; 35% and 22%, respectively, were CAGE positive, and 48% and 35% were binge drinkers.

All genotypes were in Hardy–Weinberg equilibrium except for the ALDH1B1 ala86val variant ($P = 0.0007$). The two ADH1C variants were in complete linkage disequilibrium. The inactive ALDH2 variant was not found in any of the participants. Table 2 shows the distribution of genotypes of six SNPs in Inuit with presumed little admixture and in the general population of Greenland; for comparison, a general Danish population and Alaska Yupik Eskimos were included in the table. ALDH2 5′ URT showed no allele