Frequency of LCT-13910C/T and LCT-22018G/A single nucleotide polymorphisms associated with adult-type hypolactasia/lactase persistence among Israelis of different ethnic groups

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Primary lactase deficiency (PLD), the physiological decline of lactase, is associated with the LC-13910C/T and LCT-22018G/A polymorphisms. PLD is the most common phenotype in humans and varies widely as a function of ethnicity. Israel is a multiethnic country. We analyzed the genetic frequencies of PLD in different Israeli ethnicities.

Ethnicity-related frequencies were analyzed in 439 Israelis: Ashkenazi (n=96), Iraqi (n=96), Moroccan (n=96) Jews and Bedouin-Arabs (n=151). DNA was extracted from leukocytes; LCT-13910C/T, -22018G/A and -13915T/G (in Bedouin-Arabs) polymorphisms were analyzed by PCR-restriction fragment length polymorphism analysis. There was a significant association between ethnicity and genotype in both polymorphic LCT SNPs (-13910C/T and -22018G/A). Prevalence of the CC (LCT-13910C/T) genotype associated with adult hypolactasia was 97%, 93%, 83% and 82% among Bedouin-Arabs and Iraqi, Ashkenazi and Moroccan Jews, respectively. The prevalence of the GG (LCT-22018G/A) adult hypolactasia genotype among those groups was identical to that of the CC genotype in each group, except for Iraqi-Jews, of which only 83% carried the GG genotype. The prevalence of heterozygous and homozygous genotypes associated with lactase persistence (CT, TT for -13910C/T and GA, AA for -22018G/A) was 3%, 7%, 17% and 3%, 17%, 18% for Bedouin-Arabs, Ashkenazi, Iraqi and Moroccan Jews, respectively. A significant correlation between SNPs was found. PLD prevalence is high among different ethnic groups in Israel and varies between ethnicities. The prevalence of the -13915*G allele, indicative of lactose persistence in African and Arab populations, was 41% in the Bedouin-Arabs group. Lactase persistence genotype prevalence was found to vary between Israeli ethnicities (4–18%). SNPs (-13910C/T and -22018G/A) showed significant correlation in detecting genotype prevalence in Israeli Jews. We suggest adjusting nutritional recommendations accordingly.

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1. Introduction

Lactase non-persistence, termed also Lactose intolerance and adult-type hypolactasia, is the genetically programmed down-regulation of lactase enzyme activity in the intestinal wall after weaning, resulting in inability to digest lactose (Swallow, 2003). Affected patients are unable to cleave and absorb the ingested lactose adequately, leading to bloating, abdominal pain, flatulence, abdominal cramping and diarrhea (Heyman, 2006). PLD is detectable at a relatively young age; however, the onset and extent is variable worldwide (Wang et al., 1998). PLD is the most common phenotype in humans, with worldwide frequencies around 65%. Although the prevalence is very high, there is great variation (2–100%) between different ethnic backgrounds and populations (Ingram et al., 2009; Mattar et al., 2009).

Current indirect conventional diagnosis of PLD is usually done through hydrogen breath testing (HBT), measuring the H2 concentration in the exhaled air after an oral load of lactose (Fernandez-Banares and Gassull, 1994). This method is not completely reliable (especially at young age), due to intestinal bacteria composition, gastrointestinal background diseases, smoking, antibiotic treatment prior to the breath test, dietary history, etc. Furthermore, this form of testing is associated with unpleasant symptoms in positively diagnosed patients (Waud et al., 2008).

Recent studies identified two PLD-associated single nucleotide polymorphisms (SNPs) upstream of the lactase gene locus within introns 13 and 9 of the minichromosome maintenance type 6 gene (MCM6): LCT-13 910C/T and LCT-22 018G/A (Ennatah et al., 2002; Kuokkanen et al., 2003). These polymorphism variants function in vitro as cis regulatory elements capable of enhancing differential transcriptional
activation of the lactase promoter, consistent with a causative role in the mechanism of lactase persistence/non-persistence phenotypes in humans (Olds and Sibley, 2003). These polymorphisms were found to highly correlate with PLD as detected by other biochemical and pathological methods (Högenauer et al., 2005; Mattar et al., 2008; Sibley, 2004). In fact, such correlation was evident in various study cohorts including Italians, Germans, Finns, Brazilians, Indians and other populations (Almon et al., 2007; Babu et al., 2010; Mattar et al., 2009; Nagy et al., 2009; Schirru et al., 2007). Recently, other variants adjacent to the most common variant C/T13910 have been identified in nomadic pastoralist and non-pastoralist groups from Africa and the Middle East using the indirect lactose tolerance test (LTT) (Ingram et al., 2007; Tishkoff et al., 2007). Of these newly identified variants, T/G13915, has been found in Bedouin populations in Saudi Arabia and in Jordan and in several pastoralist and non-pastoralist groups and was identified as the founder mutant variant of lactase persistence in an urban Saudi population using sequence and biochemical tests (Imtiaz et al., 2007). The Israeli Bedouin population is of the same origins; thus, this founder SNP (T/G13915) was also tested in this population. 

Adult-type hypolactasia prevalence has been previously studied in Israeli ethnicities using the conventional HBT methodology. However, the HBT has many limitations in determining the true prevalence of PLD (Waud et al., 2008). We now determined the prevalence of PLD in different Israeli ethnic groups through molecular analysis of the three MCM6 PLD-associated SNPs.

2. Materials and methods

2.1. Study population

This study was approved by the local ethics committee. The study population consisted of 439 healthy Israelis undergoing routine genetic carrier screening tests, who gave their informed consent to participate anonymously in genetic population studies. The tested individuals were of the following ethnicities: Bedouin Arabs (n = 151), and Jews of Iraqi (n = 96), Moroccan (n = 96) and Ashkenazi (n = 96) ancestries. We included in the study only individuals whose 4 grandparents were of the same self-reported ethnic origin.

2.2. Polymerase chain reaction-restriction fragment length polymorphism analysis

DNA was extracted from leukocytes and PCR-restriction fragment length polymorphism analysis was analyzed with light modification to the previously described method by Bulhões et al. (2007). For the 13915T/G variant forced RFLP reaction was done. Briefly, LCT-13910C/T, LCT-22018G/A and -13915T/G polymorphisms were analyzed by PCR-restriction fragment length polymorphism using relevant primers. Primers designed for forced RFLP for the -13915T/G polymorphisms were F: TGCTCATACGACCATGGAAT, R AAAAAAAAAACTTTGAGGCCAG. Primers spanning the LCT-13910, -22018 and -13915 regions, were used in a polymerase chain reaction (PCR): each reaction was denatured at 95 °C for 15 min, followed by amplification of 35 cycles of 95 °C for 40 s, 62 °C for 1 min and 72 °C for 40 s. The PCR product of LCT-22018G/A was digested with BstUI at 37 °C for 3 h, resulting in one fragment of 371 bp (the GA genotype), two fragments of 238 and 133 bp (the GG genotype) and three fragments of 371, 238, and 133 bp (the TT genotype). The PCR product of LCT-13910C/T was digested with FastDigest BsmFI at 37 °C for 30 min, resulting in one fragment of 386 bp (the CC genotype), two fragments of 238 and 148 bp (the TT genotype) and three fragments of 386, 238, and 148 bp (the CT genotype). The PCR product of -13915T/G (intron 13 of adjacent gene (MCM6) amplified PCR region-13895 to -14115) was digested with BseII at 60 °C for 1 h, resulting in one fragment of 231 bp (the TT genotype), two fragments of 200 and 31 bp (the GT genotype) and one fragment of 200 bp (the GG genotype) (Fig. 1). The restriction analysis fragments were visualized on a 1.5% agarose gel stained with ethidium bromide, running at 90 V for 40 min.

2.3. Statistical analysis

SPSS program was used for Chi-testing. P < 0.05 was considered as statistically different.

3. Results

We found significant association between ethnicity and genotype (Table 1); Bedouins show statistically significant difference from Jews of Ashkenazi, Iraqi and Moroccan ancestries. The prevalence of the CC (LCT-13910C>T) genotype associated with adult hypolactasia varied among different Israeli ethnicities and was 97%, 83%, 93% and 82% among Bedouins and Jews of Ashkenazi, Iraqi and Moroccan ancestries, respectively. These frequencies were almost identical for the prevalence frequencies of GG (LCT-22018G/A) adult hypolactasia genotype among Bedouins (97%), Moroccans (82%) and Ashkenazi (83%). However, Iraqi Jews, exhibited a lower prevalence of 83% compared to 93% in the CC (LCT-13910C>T) genotype. The prevalence of heterozygote and homozygote CT and TT and GA, AA genotypes for -13910C>T and -22018G/A (associated with lactase persistence) was 3%, 17%, 7% and 18% and 3%, 17%, 17% and 18% for Bedouins and for Ashkenazi, Iraqi and Moroccan Jews, respectively. Among Bedouins and Iraqi Jews no homozygotes (TT and AA for the -13910C>T and -22018G/A, respectively) were found. Among Moroccans and Ashkenazi Jews, 1% and 2% were found to be homozygous (TT and AA for the -13910C>T and -22018G/A, respectively). The prevalence of the different genotypes, namely adult hypolactasia, lactase persistent heterozygotes and homozygotes for both the -13910C>T and the -22018G/A polymorphisms were almost identical in Bedouins and in Moroccan and Ashkenazi Jews. However, Jews of Iraqi ancestry exhibited different prevalences for the -13910C>T and -22018G/A genotypes. The prevalence of the lactase persistence homozygotes was identical and significantly (P < 0.05) correlated for both genotypes (-13910C>T and -22018G/A). Recently, -13915G/T was found to be a more reliable and wide spread SNP correlating with the adult lactase persistence phenotype in the African and Arab population (Ingram et al., 2007). Thus, we analyzed the -13915G/T genotype in Fig. 1. RFLP of SNP-13915.
Other less common SNPs have been reported in different populations (Snook et al., 1976) and of Israeli Arabs (Gilat et al., 1970, 1971). PLD in majority of Arabs originating from the Mediterranean basin to the prevalence of PLD in Arab populations show high prevalence of BHT test: 24% Jordanian Bedouins and 75% among urban/agricultural published regarding the frequencies of PLD in Bedouins; Hijazi et al. (97%). Several studies with inconsistent results were previously published comparing to Ashkenazi and Moroccan Jews (16 ethicities: Bedouins and Iraqi Jews show lower prevalence (3 prevalence of lactase persistence was relatively low and variable among Bedouins; the homozygote TT was 59% of the population (Table 2).

4. Discussion

The multi-ethnic Israeli population includes descendants of Europeans, Asian, Africans and Arabs. PLD prevalence, although high worldwide, is highly dependent on ethnicity. It has been proven that 2 ethnicities compared to the prevalence previously published using standard biochemical and phenotypic diagnostics. We assume that these differences are partly due to the differences in methods used, definition of origin and reliability of indirect methods. In accordance with studies in other populations (Bodlaj et al., 2006; Bulhões et al., 2007), we also demonstrated statistically significant correlation between the genotypes of C/T-13910 and G/A-22018 in Israeli subjects from different ethnicities, suggesting that both molecular tests can be used for the diagnosis of adult-type hypolactasia in Israeli Jews of various origins and to serve in determining nutritional recommendations and strategies. For Israeli Arab Bedouins, testing for the -13915*G allele is the appropriate method of analysis.

4.1. Conclusions

PLD is the most common phenotype in humans and varies widely as a function of ethnicity. Israel is a multiethnic country. We found that PLD prevalence is high among different ethnic groups in Israel and varies between ethnicities. Lactase persistence genotype prevalence was found to vary between Israeli ethnicities (4–18%). Both SNPs (C/T-13910 and G/A-22018) showed significant correlation in detecting genotype prevalence in Israeli Jews of various origins. We suggest that in the Israeli population, testing for the (C/T-13910 and G/A-22018) SNPs in Jews and for the -13915*G SNP in Arab Bedouins is the preferred genotyping procedure for determining PLD. We suggest adjusting nutritional recommendations accordingly.

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References


