Prevalence of Cholesteryl Ester Storage Disease

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Cholesteryl ester storage disease (CESD) is an autosomal recessive chronic liver disease caused by lysosomal acid lipase (LAL) deficiency. The gene is located on chromosome 10q23.2-q23.3, and the enzyme is essential for triglycerides and cholesteryl ester hydrolysis in lysosomes. CESD is characterized by hypercholesterolemia, hypertriglyceridemia, HDL deficiency, and abnormal lipid deposition in many organs. In the liver this results in hepatomegaly caused by hepatic steatosis and fibrosis that can lead to micronodular cirrhosis. Disease onset takes place during childhood or adolescence. Males and females are affected in about equal numbers. Patients rarely reach the age of 30. Biochemically, the disorder is recognized by largely reduced lysosomal acid lipase activity. Complete absence of LAL activity causes Wolman Disease, which is normally fatal within the first 6 months of life. Several groups have identified mutations in the LAL gene underlying CESD and Wolman disease. Mutations causing Wolman disease produce an enzyme with no residual activity or no enzyme at all, whereas CESD-causing mutations encode for LAL which retains some lipase activity, however E8SJM does not cause Wolman Disease because 2% to 4% of normally spliced LAL is present in homozygote carriers. The vast majority of CESD patients described to date are E8SJM carriers. This observation provided the possibility to obtain a good estimate for the prevalence of CESD in the general population by performing a genetic E8SJM screening.

We therefore screened for the mutations in a test cohort of 1152 individuals from the PROCAM (PROspective CARDiovascular Münster)-Study of North-Western Germany and 2 validation cohorts, 1 from the city of Münster (PROCAM, n=478) and 1 from the MEMO-Study cohort (n=376), which is a follow-up of the WHO-MONICA project from Augsburg (Southern Germany). The study was approved by an institutional review committee, and informed consent was obtained from all participants. EDTA blood was collected and organized in pools of 8 individuals using equal amounts of leukocytes from each participant. In case a pool of 8 was tested positive, each participant’s DNA was individually extracted using standard procedures and subjected to an individual genotyping test. No further analysis was performed on negative pools.

Pooled DNA screening was facilitated using a previously published allele-selective polymerase chain reaction (PCR) procedure. The reaction (50 μL volume) was carried out in a single tube using the following protocol: 0.015 μmol/L wild-type selective primer (5’-AACCCCAAATGCACTCC-TGGAAATGACTCCC-3’), 0.2 μmol/L mutation (E8SJM) selective primer (5’-TCTGATGTTGATTTCATCGG-CCCAATGCACTCTGGAATGTCATT-3’), 0.1 μmol/L common primer (5’-CACATACCTTGGATTAGCTCTCA-GTT-3’) with 12.5 μmol/L d-NTP in a standard PCR buffer. The PCR initial denaturation step (95°C, 30 sec) was followed by 47 cycles of 94°C (30 sec), 64°C (45 sec), and 72°C (45 sec). The PCR product(s) were separated by agarose gel electrophoresis, ethidium bromide stained, and photographed after transillumination with UV light. A 219-bp fragment indicated the presence of the wild-type allele (Figure A, No. 1), whereas a 239-bp fragment identified the mutant allele (Figure A, No. 3). Both bands were simultaneously present in heterozygote individuals (Figure A, No. 2; Figure C, No. 2) or if a pool of 8 contained at least 1 mutant of its 16 alleles (Figure B, No. 2 and 5). Two known LAL polymorphisms (thr[-6]pro and gly2arg) were monitored and recorded for future studies using a previously published method.

DNA from all E8SJM carriers was subjected to sequencing analysis for further confirmation, using a previously reported protocol. Plasma lipids and lipoproteins were determined from fasted blood samples by CDC-controlled laboratory procedures. A current literature review revealed that about half of all reported CESD cases so far were E8SJM carriers. Therefore, a screening for this mutation should yield a fairly reliable estimate of the CESD frequency in the population. With the use of our allele-selective PCR pool-screening procedure, 3 geographically different cohorts (from Northern and Southern Germany) were analyzed for the presence of E8SJM. In all 3 cohorts, comprising 2023 individuals, 10 heterozygous carriers were identified (allele frequency: 0.0025). In the test cohort from Northern Germany the allele frequency was 0.0026 (Eastern Westphalia, 6 carriers in 1152), and in the validation cohorts it was 0.0020 (Münster, 2 in 495) and from Southern Germany 0.0026 (Augsburg, 2 in 376). The combined E8SJM allele frequency of 0.0025 translates into a
carrier frequency of about 1 in 200 or 5000 per million in the general population.

Assuming Hardy-Weinberg equilibrium, the homozygote carrier frequency can be estimated to 6 per million. Applying these results to the German general population, about 91 E8SJM homozygotes aged 18 years or younger would be expected. Under the assumption that this mutation represents about 50% of all CESD-causing mutations, the prevalence of CESD (homozygotes or compound heterozygotes) among German newborns is estimated at 25 per million, or a total of 135 cases under the age of 18. This estimate is in apparent conflict with the small number of CESD cases reported in the literature. Even after considering a higher E8SJM prevalence in CESD, for example Lohse et al. found E8SJM carriers in 70% of Czech CESD patients, 13 or more cases per million newborns would be expected.

Because most of the reported E8SJM carriers are of European or North American origin, it can be expected that this mutation strongly impacts CESD formation in these countries. Furthermore, a large number of family studies reported so far have never identified a CESD-free E8SJM homozygote. We interpret these findings as evidence for a high penetrance of the mutation and conclude that the here identified disparity between expected and reported cases indicates that CESD should be largely under-diagnosed in Europe and North America.

We therefore suggest that CESD should more often be considered as a differential diagnosis in liver diseases of unknown (nonalcoholic steatohepatitis or NASH) or known (alcoholic steatohepatitis) origin and in dyslipidemic patients with combined hyperlipidemia and low HDL-cholesterol (Familial Combined Hyperlipidemia). Awareness of the disease combined with efficient diagnostic tools should facilitate the correct diagnosis and therapy of CESD.

Disclosures

References


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