Author's response to reviews

Title: Familial hypercholesterolemia mutations in Petrozavodsk: no similarity to St. Petersburg mutation spectrum

Authors:

Tatiana Yu. Komarova (ktu17@yandex.ru)
Victoria A. Korneva (vikkorneva@mail.ru)
Tatiana Yu. Kuznetsova (eme@karelia.ru)
Alexandra S Golovina (ale4086@yandex.ru)
Vadim B. Vasilyev (vadim@biokemis.ru)
Michail Yu. Mandelshtam (michail@MM13666.spb.edu)

Version: 3 Date: 10 October 2013

Author's response to reviews:

St. Petersburg
October, 10, 2013

Dear Editor and Dear Reviewers,

Thank you very much for your comments allowing our manuscript becoming better.

The manuscript was read by the USA resident, specialist in human molecular genetics, who made corrections to written English and improved the quality of the language.

The research reported was approved by Ethics committees both of Petrozavodsk State University and Institute of Experimental Medicine (St.Petersburg) and was in compliance with the Helsinki declaration. All persons included into the study gave written informed consent for the study performed as stated in the Methods section of the manuscript.

Authors affiliations of Victoria A. Korneva and Tatiana Yu. Kuznetsova were updated to fit modern name of their Department.

Point by point reply to reviewer comments:

Dr. Mathilde Varret:

1. All mutations mentioned in the text were re-designated according to the
Human Genome Variation Society recommendations (http://www.hgvs.org/mutnomen). Table 1 provides, besides modern names of mutations also conventional nomenclature after Yamamoto to help readers associate the mutations described herein with previously described variants.

2. All mutation discussed in the text were checked by several programs allowing predictions of their possible causative effects in disease development, including now not only SIFT, but also by PolyPhen, PROVEAN and Mutation Taster. The results of mutation effect in silico analysis are combined in Table 4 (Additional file 3 supplied with the manuscript).

3. The predictions about disease-causing ability of missense variants p.Ser447Cys and p.Leu646Ile by different computer algorithms are in discordance. We believe both these variants to be pathogenic and we cannot explain the presence of only heterozygous FH phenotype in patient number 19 (compound heterozygote); however it is an experimental fact. Variant p.Asn640=, considered in the paper as neutral polymorphism does not modify the gravity of the disease in subject No. 23 carrying the predicted disease-causing mutation p.Ser447Cys. Sequencing of all LDLR exons in patients 19, 23, 72 and 91 has not revealed other disease-causing mutations, besides described in Table 2.

4. Total of 18 sequence variations were found in the collection analyzed; the misprint about 19 variants was corrected. There were two carriers of c.925_931del7 mutation and of c.2191delG mutation in the families analyzed; the discrepancy between Table 1 and Table 2 is liquidated.

5. According to all mutation effect prediction algorithms (Sift, PolyPhen, Mutation Taster and PROVEAN) the variant p.Leu511Ser is not tolerated (in contrast to other mutations where predictions depends on the program used). Indeed, this mutation causes the substitution of polar serine residue for non-polar leucine residue what may cause protein misfolding. Besides, this mutation was identified in Italian patient with familial hypercholesterolemia (however, also without proven effect on protein function).

6. p. Pro539= variant was found at a frequency of 5% when we used enlarged cohort of donors from Petrozavodsk, with the exactly the same frequency as in FH patients. This discrepancy is liquidated.

7. It is not surprising to find Petrozavodsk FH sample different from St.Petersburg sample because populations of the cities is formed to the great extent by migrants from other regions and so far ares genetically very complex. However, because several mutations were recurrent in St.Petersburg we expected that these mutations may occur wider in Russia, including Petrozavodsk. However, this was not the case and the heterogeneity of familial hypercholesterolemia in Russia is even greater than we expected beginning the Petrozavodsk FH study.
Dr. George Dedoussis:

1. The language was corrected by USA resident, specialized in human molecular genetics.

2. Out of 80 probands probable disease-causing mutations were found in 10 persons: such low percent of mutation carriers may be in part due to non-efficacy of the SSCP analysis, however, we believe that this low percent is mostly due to the fact that patients with possible FH and not only with definite FH according to Dutch criteria were included into the study.

3. Results about of p.Gly20Arg mutation are not confusing; the carrier of this mutation was bearing c.925_931del7 mutation, well characterized disease-causing allele FH North Karelia inherited from her father without p.Gly20Arg mutation. The facts that p.Gly20Arg was inherited from the healthy mother and did not modify the heterozygous FH phenotype in the daughter are sufficient to claim that p.Gly20Arg is a neutral mutation.

Sincerely,

Michail Mandelshtam, Corresponding author