Supplementary Material

Supplementary Figure 1: Slovenian universal familial hypercholesterolemia (FH) screening program

Slovenia has been implementing universal FH screening in children since 1995 as a routine part of the blood check-up at the scheduled visit of all 5-year-old children to the primary care paediatrician, recently reaching more than 90% of the 5-year-old children (of approximately 20,000) each year (Sedej et al., 2014; Klancar et al., 2015; Groselj et al., 2018, 2022). Total cholesterol (TC) was assessed as the first step of the universal FH screening program. Children with elevated TC levels were referred to the University Children's Hospital Ljubljana for further investigation. Another lipid profile was taken and routine genetic testing for the children with elevated cholesterol levels for the FH-related genes (APOB, LDLR, PCSK9) was established in 2011. In 2019, we expanded the genetic panel to 18 genes, associated with dyslipidemia. In accordance with our clinical recommendations from 2019 extra cascade screening of family members with elevated cholesterol levels is performed.
As a part of the Slovenian universal FH screening program, 664 children with elevated total cholesterol (TC) levels were referred to the Univesity Children's Hospital Ljubljana. 4 children were referred for other reasons and had elevated liver transaminase levels, and were therefore included in our study. One child was referred later as a part of LIPA sibling-cascade screening. The total number of children genetically tested for pathogenic variants in the FH-related genes (APOB, LDLR, PCSK9) and in the LIPA gene was 669. 189 children had a pathogenic variant or variant with uncertain significance (VUS) in the FH-related genes. 447 children were negative for the variants in the FH-related genes and in the LIPA gene. Three children were homozygous for a pathogenic variant in the LIPA gene - 2 were referred from a Slovenian universal FH screening program and one as a part of the sibling-cascade screening.

Supplementary Figure 2: Flowchart of the participants

TC measurement at the primary care pediatricians for all 5-year old children

LAL-D symptomatic (n = 4)  
FH screening positive (n = 664)  
LIPA sibling-cascade screening (n = 1) 

All patients with the LIPA gene sequenced (n = 669) 

Pathogenic or VUS variant in the FH-related genes (n = 189)  
Negative for variants in the FH-related genes and LIPA gene (n = 477)  
LIPA homozygous (n = 3)
Supplementary Table 1: Data on cholesterol and liver enzyme levels before and on treatment in patient 3

|                      | Before treatment | Ezetimibe | Ezetimibe + Kanuma |
|----------------------|------------------|-----------|--------------------|
| **TC (mmol/L and mg/dL)** | 7.55 ± 0.84 (292 ± 32) | 6.80 ± 0.28 (263 ± 11) | 5.11 ± 0.77 (198 ± 30) |
| **LDL-C (mmol/L and mg/dL)** | 5.83 ± 0.74 (226 ± 29) | 5.00 ± 0.28 (193 ± 11) | 3.37 ± 0.77 (130 ± 30) |
| **HDL-C (mmol/L and mg/dL)** | 1.13 ± 0.20 (44 ± 8) | 1.30 ± 0.14 (50 ± 5) | 1.18 ± 0.09 (46 ± 3) |
| **TG (mmol/L and mg/dL)** | 1.19 ± 0.35 (105 ± 31) | 1.20 ± 0.28 (106 ± 25) | 1.25 ± 0.31 (111 ± 28) |
| **AST (µkat/L)** | 1.61 ± 0.38 | 0.86 ± 0.18 | 0.60 ± 0.17 |
| **ALT (µkat/L)** | 1.55 ± 0.34 | 1.12 ± 0.30 | 0.87 ± 0.33 |
| **yGT (µkat/L)** | 0.34 ± 0.07 | 0.30 ± 0.04 | 0.26 ± 0.07 |

TC (total cholesterol), LDL-C (low-density lipoprotein cholesterol), HDL-C (high-density lipoprotein cholesterol), TG (triglycerides), AST (aspartate aminotransferase), ALT (alanine aminotransferase) and yGT (gamma-glutamyltransferase) levels for the patient 3 before treatment (6 visits), treated only with ezetimibe (2 visits) and treated with ezetimibe and Kanuma (15 visits).

Supplementary References

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