

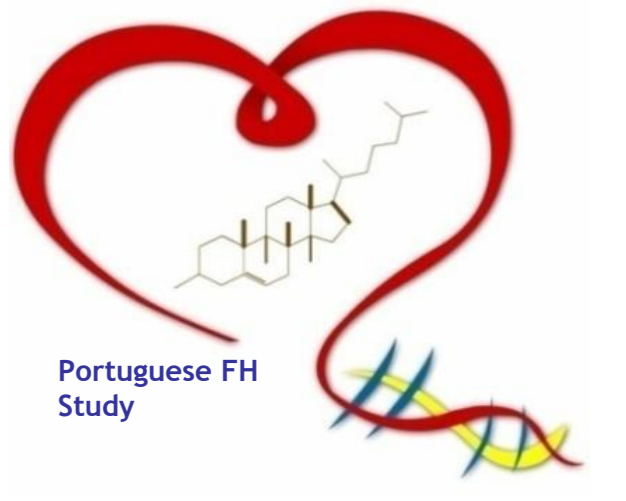
Correlation between lipid profile / cardiovascular risk and type of mutation in patients with familial hypercholesterolemia

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Introduction

Familial Hypercholesterolemia (FH) is a genetic disorder characterized by high levels of LDLc in plasma, accelerated atherosclerosis and increased risk of premature coronary heart disease. FH results from mutations in three genes involved in lipid metabolism: LDLR, APOB, PCSK9. It is known that FH patients' phenotype is heterogeneous varying with different conditions, as gene and type of mutation. Patients with mutations in the PCSK9 gene have a more severe phenotype and patients with mutations in the APOB gene have a milder phenotype, LDLR gene mutations are also associated with different clinical expressions. Consequently, the cardiovascular risk of these patients varies with the severity of the phenotype presented by each patients.

The aim of this study was to analyse the biochemical profile of patients with genetically diagnosed FH in accordance with the mutations in the genes and the different types of mutations identified in the LDLR gene to identify whether there is a correlation between these variables in Portuguese patients.

Methods

- Since 1999, blood samples were collected from 569 index patients with clinical diagnosis of FH and 1323 affected/unaffected relatives. The genetic diagnosis is based on the molecular study of LDLR, APOB and PCSK9 genes. The LDLR gene was analysed using several molecular biology techniques such as PCR, DHPLC and direct sequencing for detection of point mutations, as well as MLPA analysis for detection of large rearrangements. The fragments corresponding to the exons 26 and 29 of the APOB gene and the 12 exons of the PCSK9 were analysed by PCR and direct sequencing. Novel splicing mutations were analysed by RNA extraction of patient mononuclear cells and RT-PCR.
- The biochemical parameters, total cholesterol (TC), LDLc, HDLc, triglycerides, ApoA and ApoB) were determined for all patients by immunoturbidimetric methods and the LDL was measured directly, in automated equipment. All data were analyzed using SPSS software (version 17.0) using ANOVA and Tukey. For all tests $p < 0.05$ was considered statistically significant.

Results and Discussion

- To date has genetically identified 420 individuals (304 adults and 116 children) with a mutations in one of three genes (figure 1).
- Patients with mutations in the gene PCSK9 have LDL cholesterol levels was observed and significantly higher ($p < 0.001$) than in patients with mutations in the remaining two genes (figure 2).
- There is no significant difference in these parameters between patients with mutations in LDLR and APOB genes, although all values are higher in patients with mutations in the LDLR gene.
- Patients with nonsense mutations in the LDLR gene present values of TC, LDL and ApoB statistically higher than in patients with missense mutations (figure 2). The comparison between the other categories showed no significant changes in biochemical parameters.
- The presence of coronary heart disease (CHD) in adults with mutations in PCSK9 is 100% compared to 13%, 11% and 9% in adults with nonsense, missense and splicing mutations in LDLR gene, respectively. The age of first CHD event is different depending on the type of mutation, being lower in patients with nonsense mutations, 43 years in contrast to 48 years and 45 years for missense and splicing mutations, respectively.
- It was also verified that the phenotype of relatives is milder and in some cases without genetic diagnosis was not possible to identify these individuals.

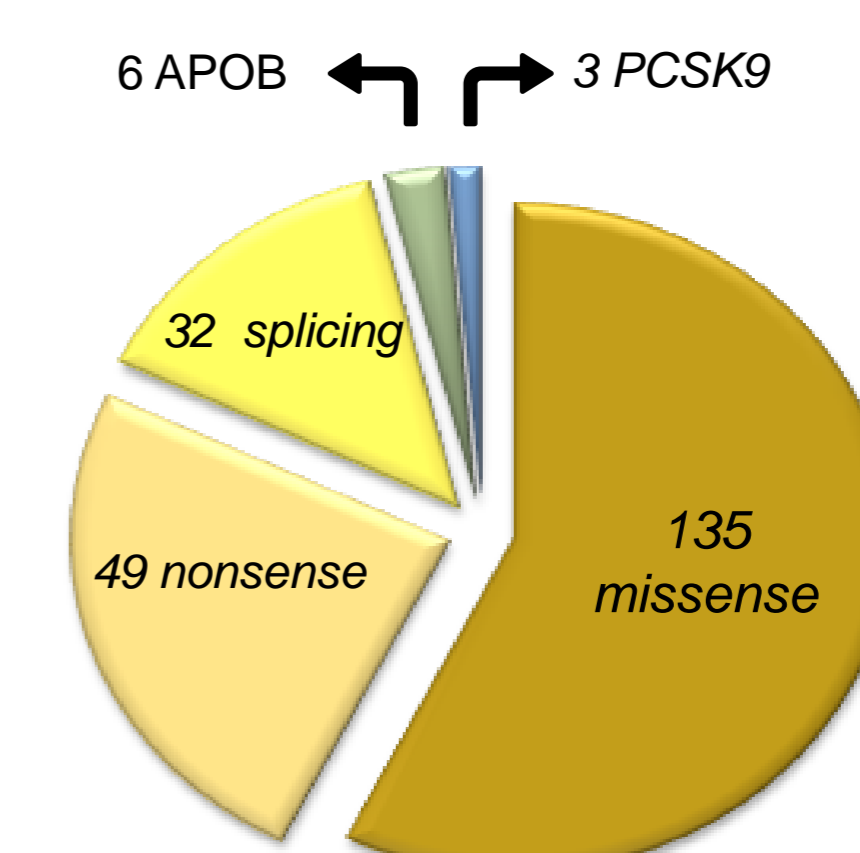


Figure1 – type of mutations found in patients identified

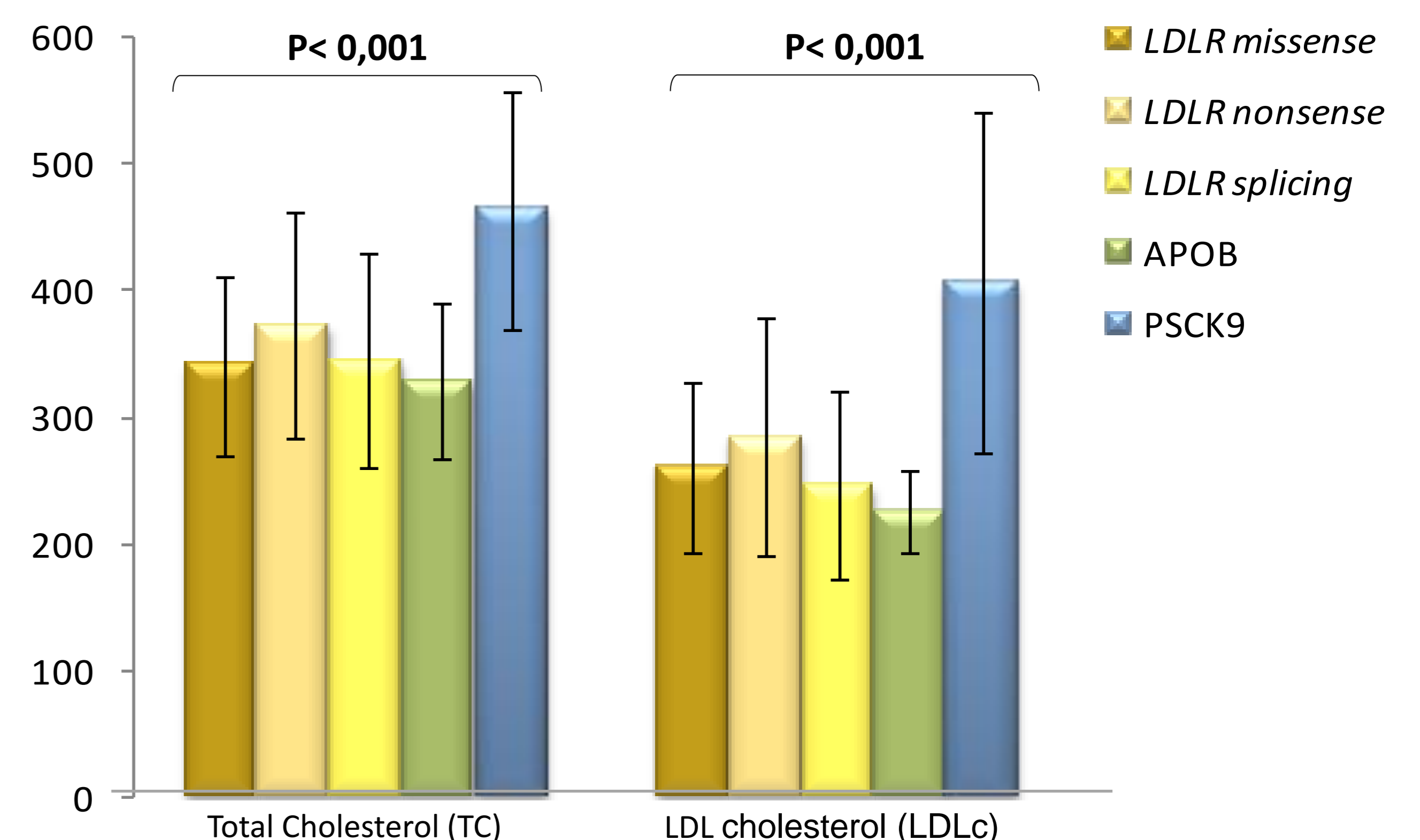


Figure 2 – Mean TC and LDLc, with respectively, standard deviations in adults group.

Conclusion

Patients with mutations in the PCSK9 gene have a more severe phenotype than patients with mutations in LDLR and APOB genes and for these reason a higher cardiovascular risk. In Portuguese FH patients were not statistically significant differences between the phenotype of patients with mutations in LDLR and APOB genes. The type of mutation (in different genes or different mutations in LDLR) is important in determining cardiovascular risk in individual patients.

Since only 20% of patients with FH reach the age of 70 years, the type of mutation (in different genes or different mutations in LDLR) must be analysed to determine the cardiovascular risk of each patient.