Biochemical and genetic evaluation of dyslipidemia in a population sample from São Brás de Alportel - Algarve

V Francisco[1,6], M Barreto da Silva[2,6], AC Alves[1,6], P Rasteiro[3], E Sousa[3],
AM Vicente[1,6], AP Gil[2], F Mendonça[4], A Fernandes[3], F Horta Correia[5], CM Dias[2], M Bourbon[1,6]

[1] DPSDC, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa;
[2] DEP, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa [3] Laboratório de Saúde Pública Dra. Laura Ayres, Faro
[4] ARS Algarve , [5] Centro Saúde S. Brás Alportel [6] Center for Biodiversity, Functional & Integrative Genomics (BioFIG)

Introduction

Dyslipidemia is an important cardiovascular risk factor and represents a serious public health problem in our society. The causes of dyslipidemia can be environmental, genetic or as a consequence of other conditions. The prevalence of this risk factor has not been determined in our population and a national epidemiologic study of prevalence of cardiovascular risk factors, is being conducted. In the scope of this project, a pilot study has been performed. Samples were collected in association with INSEF (Inquérito Nacional de Saúde com Exame Físico).

Aims

The aim of this study was the biochemical and genetic evaluation of dyslipidemia, in a random sample of 221 individuals registered at the Health Centre of São Brás de Alportel, Algarve.

Methods

From 221 inquired individuals, 198 had donated a sample for DNA extraction and biochemical studies. Biochemical parameters were analysed by automated methods. Genetic studies for the Low density lipoprotein receptor (LDLR) and Apolipoprotein B (APOB) were performed by PCR, sequencing and Multiplex ligation-dependent probe amplification (MLPA). sdLDL was also analysed by electrophoresis with Lipoprint® (Quantimetrix Corp).

Figure 1. Map of Algarve region, indicating the location of S. Brás de Alportel.

Results

According to the European Cardiology Society guidelines 64,5% of the population analysed had hypercholesterolaemia (TC>190mg/dl or LDL> 115mg/dl). Ten individuals who presented a severe phenotype characteristic of Familial Hypercholesterolaemia (FH) ((TC>290mg/dl or LDL> 190mg/dl, family history of CVD and/or hypercholesterolaemia). Were selected for genetic study (TC=268,5±23,3mg/dL; LDL-c=196,7±13,8mg/dL; HDL-c=51,3±18mg/dL; TG=137,7±54,5mg/dL; sdLDL= 48,0±8,99).

Three possible alterations causing disease in LDLR gene were found, p.Gly76Trp and p.Arg633Cys, described before in the Portuguese and English population, and p.Cys176Cys that could result in a splicing defect. None of the alterations were found in a panel of 100 normolipidemic individuals and further studies are requested to prove pathogenicity. One case of extremely low values of HDL (3mg/dL) was detected, and molecular studies of APOA1 (the major protein component of the HDL particles) and ABCA1 (involved in reverse cholesterol transport) genes are being performed to exclude the diagnosis of Tangier Disease.

Table 1 – Biochemical Characterization of INSEF individuals who presented a severe phenotype

Identification	Glicose mg/dL	Total Cholesterol mg/dL	HDL-Cholesterol mg/dL	LDL-Cholesterol mg/dL	TG mg/dL	Apo-AII (mg/dL) **	Apo-CII (mg/dL) **	Apo-CIII (mg/dL) **	ApoE (mg/dL) **	sdLDL (mg/dL) **	Alteration	Lipoprint Profile
INSEF 9	90	304	91	200	66	39,6	6.4	12.19	6.71	46.42	R 612C (c. 1897 C>T)	A
INSEF 48	166	240	35	190	155	27.50	7.28	12.67	5.68	41.74		B
INSEF 54	115	257	34	202	94	26.60	3.51	5.68	3.02	40.73		A
INSEF 59	105	235Δ	51	171Δ	91	28.20	3.43	9.47	3.18	38.77		A
INSEF 98	98	269	43	190	254	32.10	10.07	17.38	5.24	58.35		B
INSEF 112	97	301	61	220	195	39.40	9.60	17.82	4.43	60.88	C167C (c. 590 C>T)	A
INSEF 123	109	269	41	211	140	32.60	6.68	11.44	3.15	62.30	G55W (c. 226 G>T)	B
INSEF 141	91	271	50	205	123	33.50	5.02	12.08	4.60	43.14		B
INSEF 197	95	254	38	189	134	30.30	5.42	9.35	3.18	46.18		A
INSEF 199	479	285	69	189	125	37.50	7.89	16.89	5.14	41.46		B

Δ under treatment * Reference values for Hyperchoelsterolemia and Hypertriglyceridemia according to the European Cardiology Society guidelines : TC>190mg/dl or LDL> 115mg/dl; TG >150mg/dl
**Reference values for RX Daytona: Apo AII (23.0-34.5 mg/dL): Apo CII (1.60-4.20 mg/dL); Apo C-III (5.50-9.50 mg/dL); ApoE (2.70-4.50 mg/dL) and sdLDL (10.70-48.70 mg/dL); high CV risk>35mg/dl)

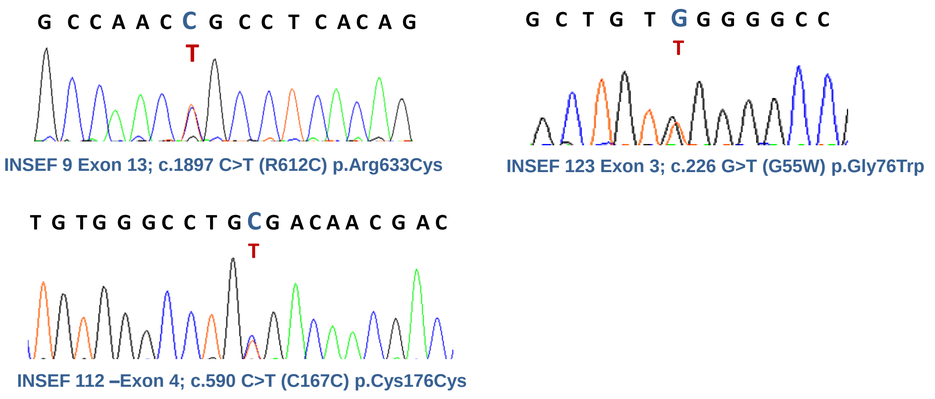
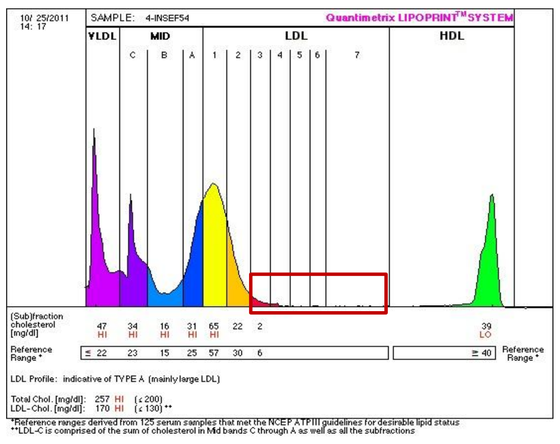


Figure 2 - Sequence of part of LDLR exon 3, 4 and 13 of index case where the base pair substitution can be seen . Alteration p.Arg633Cys was found in a English woman, living in Portugal and the alteration is already described in the English population. p.Gly76Trp was not found in a panel of 100 normolipidemic individuals and is conserved between species, but family studies are required as well as functional studies since this alteration is new in the LDLR. Alteration p.Cys176Cys could result in a splicing defect and should be further studied.

a)



b)

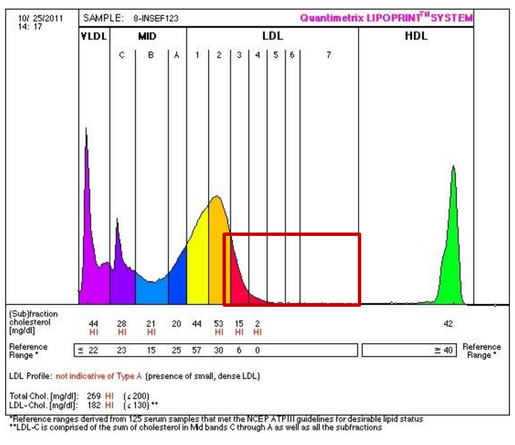


Figure 4. Example of LDL profiles obtained with Lipoprint® System.
a) example of a non-atherogenic LDL profile A– INSEF 54; **b)** example of an atherogenic LDL profile B– INSEF123. The fractions inside the red rectangle correspond to sdLDL fractions. All patients have sdLDL above cut off for CV risk (method RX Daytona) but only 5 have profile B by Lipoprint system, what shows a difference in methodology, one is quantitative and the other is qualitative. INSEF 123, who present an alteration in exon 3 has an atherogenic by both methods.

Discussions and Conclusion

Biochemical results are in accordance with other studies. The prevalence of FH in Europe is estimated in 1/500 but this small sample shows that prevalence could be greater. For more relevant conclusion on biochemical and genetic results, a larger sample is required.

Acknowledgements:
Vânia Franciso was funded by project grant FCT PIC/IC/83020/2007 and this work was founded by project grant FCT PIC/IC/83020/2007.

SPGH November 2011