# Characterization of the First PCSK9 Gain of Function Homozygote

Ana Catarina Alves PhD, Aitor Etxebarria PhD, Ana Margarida Medeiros MSc, Asier Benito-Vicente MSc, Aurélie Thedrez PhD, Maxime Passard BSc, Mikaël Croyal BSc, Cesar Martin PhD, Gilles Lambert PhD, Mafalda Bourbon PhD

Gain of function (GOF) mutations in proprotein convertase subtilisin kexin type 9 (*PCSK9*) are a rare cause of familial hypercholesterolemia (FH). We identified a child with a clinical diagnosis of FH with 2 novel putative *PCSK9* GOF missense variants (p.[(Ala62Asp)]; [(Pro467Ala)]), and no mutation in the low-density lipoprotein (LDL) receptor (*LDLR*) or in apolipoprotein B100 (*APOB*) genes. The proband was referred to the Portuguese FH Study (1) at age 11 and presented a total cholesterol of 316 mg/dl and low-density lipoprotein cholesterol (LDL-C) of 234 mg/dl on a strict diet. The phenotype presented by all *PCSK9* heterozygous carriers within this large pedigree is similar to *APOB* heterozygous carriers (LDL-C, 198.75 ± 14.98 mg/dl vs. LDL-C, 211.57 ± 42.02 mg/dl; $p = 0.227$) but significantly different than heterozygous *LDLR* carriers (LDL-C, 198.75 ± 14.98 mg/dl vs. LDL-C, 230.63 ± 76.50 mg/dl; $p = 0.012$) when comparing the relatives' phenotype in our cohort.

To characterize these variants, we first transfected HEK293 cells (which do not endogenously produce *PCSK9*) with either wild-type, p.(Ala62Asp), or p.(Pro467Ala) *PCSK9* expression vectors. The GOF p.(Asp374Tyr) *PCSK9* was used as positive control. The cellular expression and secretion patterns of wild-type and all 3 mutants were similar (not shown). We next transfected HepG2 cells with these vectors and assessed cell surface LDLR expression as well as fluorescent LDL uptake by flow cytometry (2). Compared with nontransfected cells (baseline), cells expressing wild-type *PCSK9* had reduced LDLR (−30%; $p < 0.05$), and cells expressing either p.(Asp374Tyr), p.(Ala62Asp), or p.(Pro467Ala) *PCSK9* had further reduced LDLR cell surface expression (−52%, −46%, and −56%, respectively; $p < 0.05$ vs. wild type, all) (Figure 1A). Likewise, fluorescent LDL uptake was significantly lower (−35% vs. baseline) in cells expressing any 1 of the 3 *PCSK9* variants compared with cells expressing wild-type *PCSK9* (−20% vs. baseline) (not shown). We ascertained by Western blot and enzyme-linked immunosorbent assay that *PCSK9* expression was similar in HepG2 cells expressing wild-type or each of the *PCSK9* variants (not shown). These data indicate that p.(Ala62Asp) and p.(Pro467Ala) are genuine *PCSK9* GOF variants.

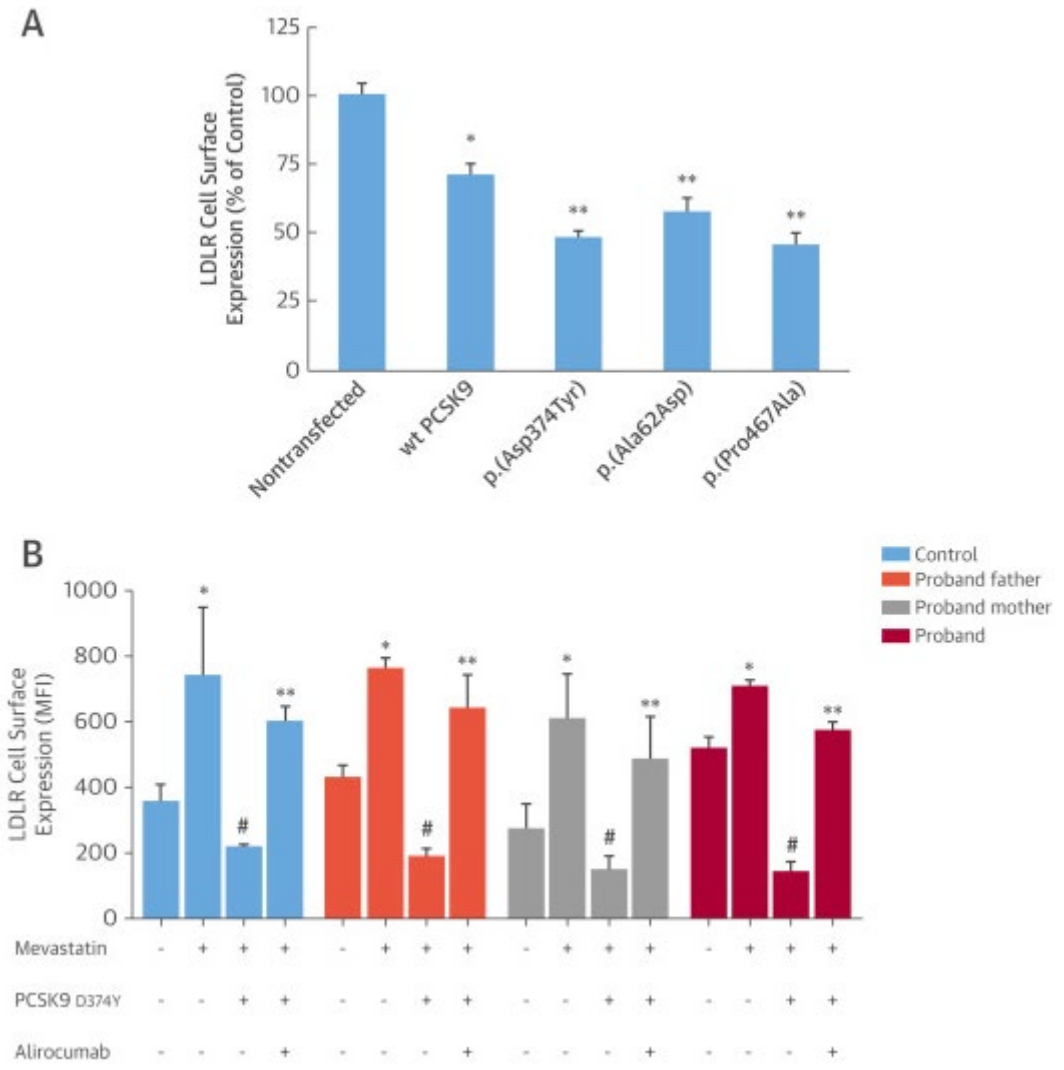


Figure 1. Characterization of 2 Novel PCSK9 Gain of Function Variants. (A) Cell surface LDLR expression in HepG2 transfected with wild-type, p.(Asp374Tyr), p.(Ala62Asp), and p.(Pro467Ala) PCSK9 variants. *p < 0.05 versus nontransfected; **p < 0.05 versus wild-type PCSK9. (B) Cell surface LDLR expression in lymphocytes from a control donor, the father (PCSK9-Pro467Ala carrier), the mother (PCSK9-Ala62Asp carrier), and the proband (PCSK9-Ala62Asp and PCSK9-Pro467Ala compound heterozygote carrier). Values represent the mean ± SD of triplicate determinations. *p < 0.05 versus no statin treatment; #p < 0.05 versus mevastatin only treatment; **p < 0.05 versus mevastatin + PCSK9-Asp374Cys. LDLR = low-density lipoprotein receptor; MFI = mean fluorescence intensity (arbitrary units).

To ascertain the functionality of the LDLR in patients carrying p.(Ala62Asp) and/or p.(Pro467Ala) PCSK9 variants, we performed a series of analyses of their lymphocytes. Statin treatment similarly increased baseline LDLR expression at the surface of lymphocytes isolated from the proband, her heterozygous parents, and a normolipidemic donor. Recombinant PCSK9 similarly reduced LDLR expression by as much as 75% to 85% in statin-treated lymphocytes, irrespective of the donor. The PCSK9 inhibitor alirocumab reversed these effects in each experimental condition (Figure 1B). The cellular uptake of fluorescent LDL in those cells paralleled the levels of cell surface

LDLR expression in control and the patient's lymphocytes alike (not shown). Thus, the LDLR of the proband and of her parents is normally expressed and fully functional, underscoring the causative link that exists between their FH phenotype and their PCSK9 mutations. It is noteworthy that despite ongoing statin treatment, circulating PCSK9 levels of the proband, her father, and her mother were found within the normal range for age and sex at 148, 209, and 250 ng/ml, respectively (3). This further underpins that both PCSK9 p.(Asp62Ala) and p.(Pro467Ala) are bona fide GOF variants.

We fully characterize here the first compound heterozygote FH patient with 2 PCSK9 GOF variants. The experiments conducted on the proband's lymphocytes clearly suggest that this patient should respond particularly well to a treatment combining a statin and a PCSK9 inhibitor. This child (now 15 years old) is currently treated with atorvastatin 10 mg/day, with her last LDL-C levels at 88 mg/dl. However, as seen in other FH children, the phenotype is always milder than in adulthood (4). Because her phenotype can worsen over time, these novel therapeutic options (a statin with a PCSK9 inhibitor) will probably allow her to maintain her LDL values below target levels for high-risk patients. This patient will be very interesting to follow in the coming years.

Acknowledgments

Thanks to all patients and relatives for participating in the Portuguese FH Study, to Dr. Goreti Lobarinhas for excellent medical support, to Joana Canilho and Rocío Alonso for excellent technical assistance, and to Prof. A. Gómez-Muñoz for flow cytometry facilities.

REFERENCES

1. Medeiros AM, Alves AC, Bourbon M. Mutational analysis of a cohort with clinical diagnosis of familial hypercholesterolemia: considerations for genetic diagnosis improvement. Genet Med 2015 May 28 [E-pub ahead of print].

2. Etxebarria A, Benito-Vicente A, Alves AC, Ostolaza H, Bourbon M, Martin C. Advantages and versatility of fluorescence-based methodology to characterize the functionality of LDLR and class mutation assignment. PLoS One 2014;9:e112677.

3. Lambert G, Petrides F, Chatelais M, et al. Elevated plasma PCSK9 level is equally detrimental for patients with nonfamilial hypercholesterolemia and heterozygous familial hypercholesterolemia, irrespective of low-density lipoprotein receptor defects. J Am Coll Cardiol 2014;63: 2365–73.

4. Medeiros AM, Alves AC, Aguiar P, Bourbon M, for the Pediatric Investigators of the Portuguese Familial Hypercholesterolemia Study. Cardiovascular risk assessment of dyslipidemic children: analysis of biomarkers to identify monogenic dyslipidemia. J Lipid Res 2014;55:947–55.