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Letters

Characterization of the First *PCSK9* Gain of Function Homozygote

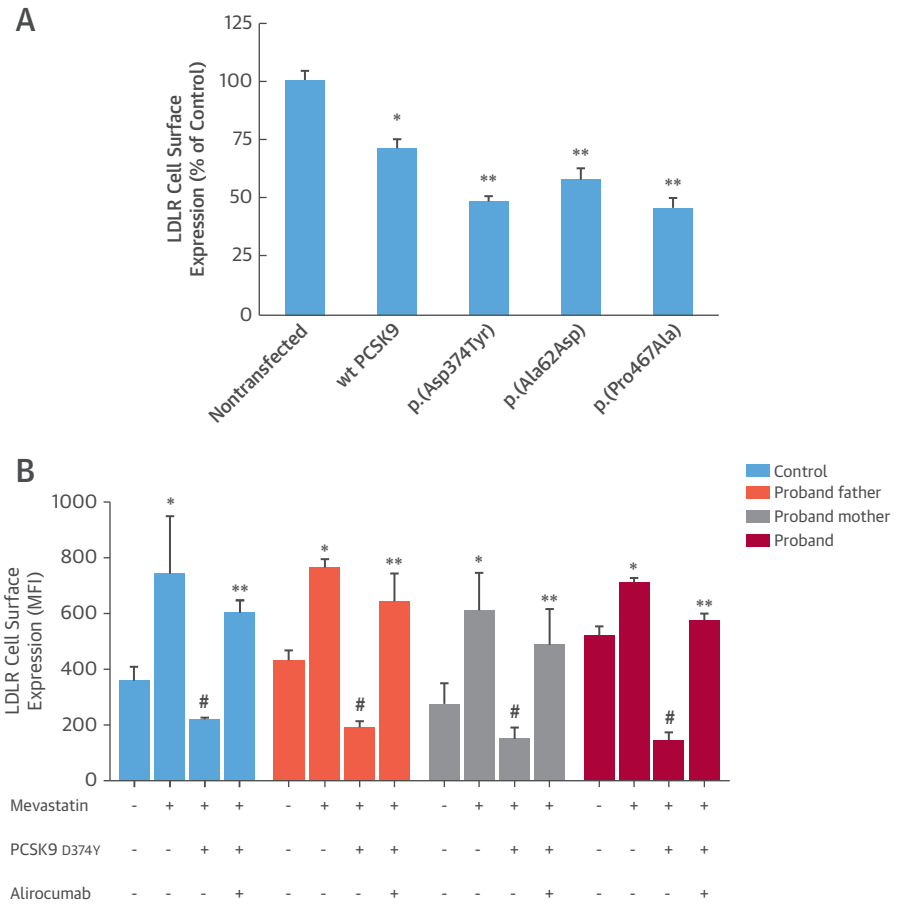
Gain of function (GOF) mutations in proprotein convertase subtilisin kexin type 9 (*PCSK9*) are a rare cause of familial hypercholesterolemia (FH). We identified a child with a clinical diagnosis of FH with 2 novel putative *PCSK9* GOF missense variants (p.[(Ala62Asp)]; [(Pro467Ala)]), and no mutation 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.

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.

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).

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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.

Prediction of Long-Term Survival After Liver Transplantation for Familial Transthyretin Amyloidosis

Familial transthyretin amyloidosis (ATTR) is a rare, life-threatening, autosomal dominant disease involving mainly the heart and the peripheral nervous system due to a point mutation of the transthyretin (*TTR*) gene. By removing the main source of the mutated *TTR*, liver transplantation (LT) has become the standard treatment for ATTR (1). Because the demand for liver grafts exceeds the number of available organs and because new treatments have recently emerged, screening patients at high risk of death after LT is critical (2).

We identified 215 consecutive patients who underwent LT between 1993 and 2011. The diagnosis was made by the observation of both amyloid deposits in biopsy specimens and a *TTR* mutation. The pre-operative evaluation included physical examination, electrocardiography, echocardiography, autonomic dysfunction score, and polyneuropathy disability score (PND) calculation. The primary study endpoint was all-cause mortality after LT. The prognostic model predicting the individual probability of death within the first 5 years after LT was developed from the Cox proportional hazards model and was internally validated using bootstrapping.

At the time of LT, patients' median age was 43 years, 61% were men, and 69% carried the *Val30Met* mutation. There were 81% patients in New York Heart Association (NYHA) functional class I, 40% had conduction disorders, 36% had a ≥12-mm interventricular septum on the echocardiograms, and the median left ventricular ejection fraction was 65%. All patients presented with neurological manifestations of ATTR: isolated sensory disturbances (PND I: 61%), difficulties with walking (PND II: 22%), and the need for cane(s) to walk (PND III: 17%). The vegetative score was abnormal in 82%, and 59% had orthostatic hypotension.

Over a median follow-up of 5.9 years after LT, 84 patients died, and cardiac events were the leading cause of death (38% of all deaths). The significant pejorative factors were PND score ≥III (hazard ratio [HR]: 1.75; 95% confidence interval [CI]: 1.04 to 2.96; $p = 0.036$), orthostatic hypotension (HR: 2.26; 95% CI: 1.39 to 4.22; $p = 0.001$), NYHA functional class >I (HR: 2.25; 95% CI: 1.18 to 4.27; $p = 0.014$), QRS duration ≥120 ms (HR: 1.90; 95% CI: 1.05 to 3.43; $p = 0.035$), thickened interventricular septum (for each millimeter: HR: 1.12; 95% CI: 1.04 to 1.20; $p = 0.002$).

The individual probability of death at 5 years was calculated as $P_{\text{death at 5 years}} = 1 - 0.735^{e(\text{coeff sum} - 1.27810)}$, where coeff sum = (0.57423 × PND score ≥III) + (0.77339 × orthostatic hypotension) + (0.91192 × NYHA functional class >I) + (0.60378 × QRS ≥120 ms) + (0.14589 × [interventricular septum thickness − 6]). Risk can be computed using the online calculator. The calibration slope was 0.89 (95% CI: 0.64 to 1.15), the C-index of Harrel was 0.68 (95% CI: 0.45 to 0.88), and the concordance probability estimate was 0.71 (95% CI: 0.67 to 0.75). The area under the receiver-operating characteristic curve for the 5-year survival was 0.80, and significant differences of survival were found according to the 5-year death risk (**Figure 1**). Pre-operative identification of a high-risk profile (risk >50%) was retrospectively documented in 40 of 215 patients (19%).

The risk score was built from variables that measured the cardiac and neurological status regardless of mutation type. Therefore, our proposed score should be useful to gauge the risk of patients with rare variants of *TTR* and to take into account the phenotypic variability encountered among patients with a similar mutation. The study population was representative of a region where ATTR is not endemic and the 74% 5-year survival of our patients in line with the 77% previously reported (3). Previous reports