Title: Effect of intensive LDL cholesterol Lowering with PCSK9 monoclonal antibodies on Tendon Xanthoma Regression in Familial Hypercholesterolemia

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Abstract: BACKGROUND AND AIMS
The effect of LDLc lowering with PCSK9 antibodies on tendon xanthomas (TX) is unknown.
METHODS
TX was measured in 24 Heterozygous familial hypercholesterolemia (HeFH) cases and in 24 HeFH controls with or without PCSK9 inhibitors for at least one year.
RESULTS
Exposure to PCSK9 inhibitors in cases was 2.96±1.33 years. LDLc decreased 80.8±7.66% in cases and 56.9±11.1% in controls. There was a decrease in maximum (-5.03%) and mean (-5.32%) TX in cases but not in controls (+3.97%, +3.16, respectively, p=.01). PCSK9 inhibitor treatment was independently associated with TX reduction.
CONCLUSION
Addition of a PCSK9 inhibitor to statin and ezetimibe resulted in a greater decrease in LDLc and TX after 3 years of treatment.

HIGHLIGHTS

1. This is the first study that assess the intensive LDL cholesterol lowering effect of PCSK9 inhibition on tendon xanthomas.

2. Compared with standard lipid-lowering therapy, the combination with PCSK9 inhibitors showed greater tendon xanthoma regression.

3. Tendon xanthoma regression was observed with mean LDL cholesterol of 60 mg/dL

4. Some patients developed asymptomatic calcifications inside the Achilles tendon

5. Considering the similarities between tendon xanthomas and atherosclerotic plaques, these results suggest that PCSK9 inhibition is highly efficient in heterozygous familial hypercholesterolemia

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1

Abbreviations

Achilles Tendon (AT)

Tendon xanthomas (TX)

Heterozygous Familial hypercholesterolemia (HeFH)

Homozygous Familial Hypercholesterolemia (HoFH)

Coronary Heart Disease (CHD)

Proprotein Convertase Subtilisin Kexin Type 9 (PCSK9)

ABSTRACT

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TX was measured in 24 Heterozygous familial hypercholesterolemia (HeFH) cases and

in 24 HeFH controls with or without PCSK9 inhibitors for at least one year.

RESULTS

Exposure to PCSK9 inhibitors in cases was 2.96 ± 1.33 years. LDLc decreased

$80.8\pm7.66\%$ in cases and $56.9\pm11.1\%$ in controls. There was a decrease in maximum (-

5.03%) and mean (-5.32%) TX in cases but not in controls (+3.97%, +3.16,

respectively, *p*=.01). PCSK9 inhibitor treatment was independently associated with TX

reduction.

CONCLUSION

Addition of a PCSK9 inhibitor to statin and ezetimibe resulted in a greater decrease in

LDLc and TX after 3 years of treatment.

3

Introduction

Tendon xanthomas (TX) are lipid deposits within certain tendons, mainly Achilles and extensors of the hands, that produce diffuse and/or focal thickening and predispose to inflammation, occasionally causing pain and impairment of function [1]. TX in presence of severe hypercholesterolemia with autosomal dominant transmission is highly specific of familial hypercholesterolemia (FH) [2] and a mutation in a LDL receptor-related gene is commonly found in this scenario [3]. Homozygous FH (HoFH) typically develop TX in the first decade of life, while TXs begin to appear after the third decade of life in 20% to 50% of affected heterozygous FH (HeFH) [2]. It is unknown why some HeFH subjects develop TX and others do not, even sharing the same pathogenic mutation [4], and may be related to interindividual variability in the inflammatory response of macrophages to oxidized LDL particles [5].

The presence of TX has important clinical implications, because they are major criterion for the clinical diagnosis of HoFH [6] and HeFH [7], and more important, subjects with TX associate higher LDL cholesterol, more intense subclinical atherosclerosis and higher risk of coronary heart disease (CHD) and, probably, deserve a more intense lipid lowering therapy [7,8].

Macrophage-derived foam cells due to intracellular accumulation of cholesterol, extracellular cholesterol deposits and connective tissue are the main components of TX [9]. Hence, pathological characteristics of TX are similar to atherosclerosis vascular lesions, both situations being produced by an accumulation of intra and extracellular cholesterol and an inflammatory reaction surrounded by fibrosis tissue. Furthermore, major risk factors for the development of CHD are also associated with the presence of TX, including age, male gender or high LDL cholesterol [10]. These results suggested

4

that xanthomas and coronary atherosclerosis may share etiology, explaining the association between TX and CHD in FH.

Prolonged treatment with monoclonal antibodies against proprotein convertase subtiline kexin-9 (PCSK9) produce a substantial and sustained reduction in plasma PCSK9 concentration leading to an increase in hepatic LDL receptor function and to an additional 40-60% reduction in plasma LDL cholesterol on top of high dose of statins with or without ezetimibe [11,12]. Similar LDL cholesterol reductions with the two available PCSK9 inhibitors, alirocumab and evolocumab, have been observed in HeFH, and many of these patients reach normal LDL cholesterol values and lipid therapeutic goals [13,14]. However, their clinical effect on cardiovascular disease prevention in HeFH has not been proven yet.

We hypothesized that the LDL cholesterol lowering effect of prolonged PCSK9 inhibition in HeFH would induce more TX regression than high dose of potent statins. If this were the case, it would support the potential benefit of this type of drugs in a high-risk condition as HeFH.

Material and Methods

Subjects. This is a prospective case-control study, in which we selected all genetically defined HeFH with TX who began PCSK9 inhibition therapy with Alirocumab or Evolocumab from November 2012 to December 2015 at the Lipid Unit at Hospital Universitario Miguel Servet in Zaragoza, Spain. These patients had been enrolled in different open-label clinical trials and/or were assigned to active treatment in the doubled-blind phase; and the treatment assignment was known after clinical trial completion [11,13-15]. We included only those patients receiving at least 12 months of active PCSK9 inhibition treatment with monoclonal antibodies: alirocumab 75 mg or 150 mg every two weeks, evolocumab 140 mg every two weeks or evolucumab 420 mg every four weeks. Age-matched controls were genetically defined HeFH with TX studied before December 2015. All cases and controls were on high dose of potent statins with or without ezetimibe. All participants gave written consent prior to their participation in the protocol, which was approved by the Clinical Research Ethics Committee of Aragon, Spain.

Tendon xanthomas measurements. TXs were measured in the Achilles tendons using high-resolution sonography. Standardized equipment and operating procedures were used for Achilles tendon thickness measurements as previously described [16]. Persons involved in the Achilles tendon sonography and measurement were blinded to the baseline TX thickness and PCSK9 inhibitor treatment. This has been stated in the methods section. The variables of interest were mean and maximum Achilles tendon thickness bilaterally. TX was defined when Achilles tendon maximum thickness was over 5.3 and 5.7 mm in men <45 and >45 years, and over 4.8 and 4.9 mm in women <50 and >50 years, respectively. These thickness thresholds have demonstrated to be good discriminators for the TX diagnosis in HeFH [16]. TX were measured at

6

diagnosis, before PCSK9 inhibition in cases, and repeated in both groups between November 2016 and March 2017 in a follow-up visit. We also collected clinical, anthropometric and biochemical variables at baseline and follow-up visit.

Lipid and lipoprotein analysis. A lipid profile was obtained at diagnosis without lipid-lowering drugs for at least 6 weeks and at the time of sonographic visits, as previously described [3]. They included total cholesterol, calculated LDL cholesterol, HDL cholesterol, triglycerides, apolipoproteins A1 and B. Lipoprotein (a) was measured only at diagnosis.

Genetic diagnosis. *LDLR* and *APOB* genes were analyzed for mutations with Lipochip® (Progenika-Grifols, Spain) [17]. Samples in which the microarray did not uncover a genetic defect undergo further analysis of large rearrangement by quantitative fluorescence-based multiplex PCR and, if negative, sequencing of the promoter region, the 18 exons and flanking intronic regions of *LDLR*.

Statistical analyses. Numerical variables with normal distribution were expressed as mean ± standard deviation and those with skewed distribution are expressed as median [percentile 25 − percentile 75]. We used *t*-Student or Mann-Whitney tests for assessing differences among two-categories quantitative variables while chi-squared test was used for categorical variables. Differences in paired variables were calculated with the *t*-test for paired samples or with the Wilcoxon test as appropriated. To identify those variables associated with TX thickness percentage variation we applied multiple linear regression by including age, sex, LDL cholesterol after treatment, baseline TX thickness, treatment with PCSK9 inhibitors and years with PCSK9 inhibitors as independent variables.

Results

A total of 48 HeFH subjects, 24 cases and 24 controls, were included in the study. The

clinical and biochemical characteristics at the time of the first Achilles tendon

measurement are presented in **Table 1**. Mean age was 50 years in both groups. They did

not differ in any clinical or biochemical characteristics, including major CHD risk

factors as gender, smoking, diabetes or hypertension. The past history of statin use was

also similar in cases and controls.

Table 2 describes lipid values at diagnosis without lipid lowering drugs and in

the last visit during the follow-up. All subjects were on stable dose of atorvastatin 40-80

mg/day or rosuvastatin 20-40 mg/day at the follow-up visit. All 24 subjects in the case

group and 22 in the control group were on combined treatment with ezetimibe. The

exposure to PCSK9 inhibitors in cases was 2.96 ± 1.33 years. Lipid parameters

substantially improved at the follow-up visit. LDL cholesterol decreased from 326 ± 60.4

mg/dL to 61.0 ± 20.2 mg/dL ($-80.8\pm7.66\%$, $p <.001$) in HeFH subjects with PCSK9

inhibitors and from 314 ± 65.0 mg/dL to 131 ± 26.9 mg/dL ($-56.9\pm11.1\%$, $p <.001$) in

HeFH in the control group. The mean percentage reduction in LDL cholesterol was

higher with PCSK9 inhibitors ($p <.001$). Similar differences between groups were

observed in total cholesterol, non-HDL cholesterol and apolipoprotein B. No

differences were found between cases and controls in triglycerides, HDL cholesterol

and apolipoprotein A1.

AT measurements were higher in cases than in controls at diagnosis and at the

follow-up (**Table 2**). However, there was a significant decrease in the maximum and

mean AT thickness in cases that was not observed in controls, who presented a slight

but non-significant increase during the follow-up. The mean percentage change in

8

maximum and mean TX thickness in cases was -5.03% and -5.32%, respectively. In contrast, there was a non-significant increase in controls (+3.97% and +3.16, respectively), with significant differences between groups (p=.01) (**Figure 1**, panels A and B). Interestingly, 3 patients who were under PCSK9 inhibitor treatment developed calcifications inside the AT (**Figure 1,** panels C and D) while none in the control group. All subjects in the PCSK9 inhibitor group showed some TX regression, except three subjects: a male with only 12 months of exposure to PCSK9 inhibitors and two subjects with large TX at baseline (maximum AT 10.3 mm and 9.1 mm). These subjects were the oldest within PCSK9 inhibitor group, they were 69 and 72 years old at the time of the follow-up. In the regression analysis, the PCSK9 inhibitor treatment was independently associated with percentage reduction of TX thickness after adjustment for age, gender, baseline TX thickness and baseline LDL cholesterol, Beta -6.081, 95% confidence interval (-10.431 – (-1.730)), p =0.007. There was a non-significant trend of the association of a higher regression of TX with lower LDL cholesterol levels at follow-up.

Discussion

In this study, genetically confirmed HeFH subjects with TX were studied after receiving intense lipid-lowering therapy with high dose of potent statins with or without ezetimibe and either alirocumab or evolocumab, two PCSK9 inhibitors. The evolution of TX was analyzed by high-resolution ultrasonography. An age-matched HeFH group receiving high dose of potent statins with or without ezetimibe but without PCSK9 inhibitors was used as control group. No significant benefit of lipid-lowering therapy was observed on TX size in the control group. In contrast, there was a significant regression of TX associated with PCSK9 inhibitors.

Considering that HeFH with TX associate a 3.2-fold higher risk of CVD [10], this association is independent of traditional risk factors, and TX composition share many characteristics of vascular atherosclerotic plaques, our results would indicate that intense lipid-lowering achieving LDL cholesterol concentrations of approximately 60 mg/dL is able to regress not only chronic lipid deposits in tendons but possibly, other deposits in other territories, as vascular walls. There is not clinical evidence of atherosclerosis regression in HeFH with PCSK9 inhibitors, although resolution of xanthelasmas has been also observed with alirocumab in one HeFH patient [18]. The parallel evolution of TX with CHD is very well documented in HoFH where TX development usually precedes clinical CHD, and LDL apheresis effectively regress TXs and vascular lesions [19].

We observed a 5% reduction in TX thickness with PCSK9 inhibitors during a mean treatment period of almost 3 years. No previous case-control studies have analyzed TX regression in HeFH. The unique atherosclerosis regression trial in humans published so far with PCSK9 inhibition is GLAGOV [20]. In GLAGOV, evolocumab treated patients reduced LDL cholesterol level from 93 mg/dL to 36.6 mg/dL and

10

coronary total atheroma volume, the primary efficacy parameter, decreased 0.95% in 76 weeks [20]. This regression in GLAGOV is lower than the observed in our study. Intra and extracellular cholesterol comprise up to 50% of TX composition [21], usually in a greater extent than in coronary arteries [22], and this different proportion, and a larger exposure to the drugs may explain the differences in regression in both territories. Furthermore, the large differences in the methodology, type of patients and location of lipid deposits between both studies should be considered and the comparison taken with caution.

TXs are not usually modified with conventional lipid-lowering agents. Isolated cases of reduction of TX with statins and probucol have been reported [23,24] but this study is the first to compare two different LDL cholesterol levels achieved with two different lipid-lowering approaches. As occurs with coronary lesions [25], mean LDL cholesterol concentrations of 130 mg/dL are not associate with TX regression which was achieved with mean LDL cholesterol of 60 mg/dL. Interestingly, some patients developed AT calcifications in the PCSK9 inhibitor group. It is well described that intense LDL cholesterol lowering with statins reduces coronary plaque volume, lipid content and intravascular inflammation, with a simultaneous increase of dense calcium volume [26]. A similar phenomenon probably explains the calcification observed in some of our patients.

In conclusion, among HeFH patients with TX treated with potent statins, the addition of a PCSK9 inhibitor resulted in a greater decrease in LDL cholesterol and TX thickness after 3 years of treatment.

CONFLICT OF INTEREST

11

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14

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Table 1. Baseline clinical and biochemical characteristics of study subjects with or without PCSK9 inhibitors[a].

	Subjects with PCSK9 inhibitors N = 24	Subjects without PCSK9 inhibitors N = 24	*P*
Age, years	49.8±9.01	49.9±9.08	0.987
Men, n (%)	15 (62.5)	12 (50.0)	0.383
Current smokers, n (%)	5 (20.8)	4 (16.7)	
Non-smokers, n (%)	9 (37.5)	11 (45.8)	0.834
Former smokers, n (%)	10 (41.7)	9 (37.5)	
Cardiovascular disease, n (%)	6 (25.0)	2 (8.3)	0.121
Carotid plaque, n (%)	13 (54.2)	10 (41.7)	0.386
Previous statin treatment, years	10.7±8.23	6.8±6.28	0.08
Diabetes, n (%)	0	1 (4.2)	0.312
Hypertension, n (%)	4 (16.7)	7 (29.2)	0.303
Body Mass Index, Kg/m2	27.5±3.56	25.9±4.47	0.163
Corneal arcus, n (%)	11 (45.8)	14 (58.3)	0.386
Lipoprotein(a), mg/dL	67.4±58.1	34.2±37.6	0.023
Glucose, mg/dL	93.6 ± 13.0	95.4±14.7	0.657
Hemoglobin A1c, %	5.24±1.06	5.40±0.043	0.594
C-reactive protein, g/L	1.60 (0.70-3.50)	1.07 (0.30-2.00)	0.159
Creatinine, mg/dL	0.87±0.13	0.84±0.11	0.347
APOE Genotype E3/3, n (%)	16 (66.7)	16 (66.7)	
APOE Genotype E3/2, n (%)	5 (20.8)	4 (16.7)	
APOE Genotype E3/4, n (%)	2 (8.3)	3 (12.5)	0.958
APOE Genotype E2/4, n (%)	1 (4.2)	1 (4.2)	
APOE Genotype E2/2, n (%)	0	0	

[a] Numerical variables with normal distribution are expressed as mean ± standard deviation and those with skewed distribution are expressed as median [percentile 25 – percentile 75]. *t*-Student-t or Mann-Whitney or chi-square tests were used as appropriate.

Table 2. Lipid profile and Achilles tendon measurements at diagnosis and follow-up visit in subjects with and without PCSK9 inhibitors[a].

	WITH PCSK9 INHIBITORS (N=24)				WITHOUT PCSK9 INHIBITORS (N=24)				p^c
	Diagnosis	Follow-up	Δ %	p^b	Diagnosis	Follow-up	Δ %	p^b	
Total cholesterol, mg/dL	408±67.2	132±24.7	-66.4±8.65	<0.001	387±70.0	208±35.8	-45.2±11.0	<0.001	<0.001
Triglycerides, mg/dL	162±134	103±54.6	-15.5±48.2	0.020	128±79.4	105±46.7	-5.04±40.4	0.033	0.424
HDL cholesterol, mg/dL	46.4±11.3	52.1±11.7	16.2±24.3	0.015	50.0±13.6	55.7±16.1	13.7±24.6	0.030	0.728
Non-HDL cholesterol, mg/dL	357±67.0	80.9±25.0	-76.6±8.90	<0.001	337±72.2	139±30.2	-61.1±8.10	<0.001	<0.001
LDL cholesterol, mg/dL	326±60.4	61.0±20.2	-80.8±7.66	<0.001	314±65.0	131±26.9	-56.9±11.1	<0.001	<0.001
Apolipoprotein A, mg/dL	143±27.2	149±19.9	10.9±24.5	0.182	132±29.3	164±34.7	24.1±19.0	<0.001	0.088
Apolipoprotein B, mg/dL	205±45.1	61.5±17.8	-68.9±10.2	<0.001	188±49.5	107±25.2	-41.4±13.0	<0.001	<0.001
Maximum TX thickness, mm	8.596±3.30	8.120±3.05	-5.02±8.12	0.011	6.582±1.76	6.845 ± 2.05	3.97±13.2	0.192	0.007
Mean TX thickness, mm	8.204±3.18	7.704±2.89	-5.32±9.17	0.012	6.342±1.79	6.500 ± 1.84	3.16±13.2	0.417	0.013

[a] Data expressed as mean±SD. [b] p refers to differences between diagnosis and follow-up time points; they were calculated by paired *t*-test. [c] p refers to differences among subjects with and without PCSK9 inhibitors; they were calculated by *t*-test or Mann-Whitney as appropriate

Figure legend.

Panels A and B: Images of Achilles tendon xanthoma before and after 3.2 years of PCSK9 inhibition treatment

Panel C and D: Images of Achilles tendon calcification in two patients that appeared after PCSK9 treatment

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