

The use of Achilles tendon ultrasonography for the diagnosis of familial hypercholesterolemia

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Abstract

Differentiating FH from other causes of hypercholesterolemia has important clinical and therapeutic implications but is often not possible by standard clinical criteria. As accumulation of cholesterol in tendon is generally considered as pathognomonic of FH, we evaluated the sensitivity and specificity of clinical and ultrasonographic tendon characteristics using the data of 127 genetically ascertained FH and 160 controls with various lipid profiles. Upon clinical examination, none of the controls and 29% of FH individuals (17% FH women and 38% FH men) presented with xanthomata in Achilles tendons, but no female and only 6% of male FH patients also showed xanthomata in the extensor tendon of the hand. Amongst all possible quantitative parameters (thickness, breadth, section and roundness) of Achilles tendon (AT) measured by ultrasonography, the thickness presented the best receiver operating curves. AT thickness above 5.8 mm was the most useful threshold for diagnosis of FH, procuring sensitivity of 75% and specificity of 85%. Analysis of variation of AT thickness with age and sex indicated that this clinical criterion performed better in females older than 45 and in males under 45. In patients carrying the APOB-R3500Q mutation, AT-thickness appeared significantly less important compared with those carrying LDLR mutations. In conclusion, this study recommends identification of possible FH individuals amongst hypercholesterolemic patients using a criteria of AT-thickness over 5.8 mm eventually associated with a specific genetic test for APOB-R3500Q mutation. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Heterozygous familial hypercholesterolemia (FH) remains a diagnostic challenge for the clinician. In principle, detection of the causative mutations in the LDL-R or APOB genes would be the most direct and obvious diagnostic procedure of FH. But until more effective and affordable genetic methods to detect such mutations are developed, the practitioner will have to rely on clinical findings. Current clinical criteria for FH include high total cholesterol concentrations above the 95th percentile, the presence of tendon xanthomata (TX) and a history of early coronary heart disease, i.e. before age 55 in men or 65 in

women. Many practitioners, however, experience that many patients suspected of FH do not meet these criteria, especially regarding xanthomata often absent or unrecognized. The ultrasonography of Achilles tendon (AT-US) allows accurate quantitative measurement (thickness and breadth) of Achilles tendons, and several studies [1–9] have described its potency in evaluating cholesterol impregnation in the tendons. However, none of these studies has the power to evaluate precisely the performance of AT-US as a diagnostic tool for FH in comparison to the gold standard of genetic analysis. Moreover, many pitfalls limited the interpretation of the results: selection of FH patients based on presence of TX, no genetic ascertainment of FH, and inclusion of effectively treated patients with the risk that cholesterol normalization causes regression of tendon xanthomata [10].

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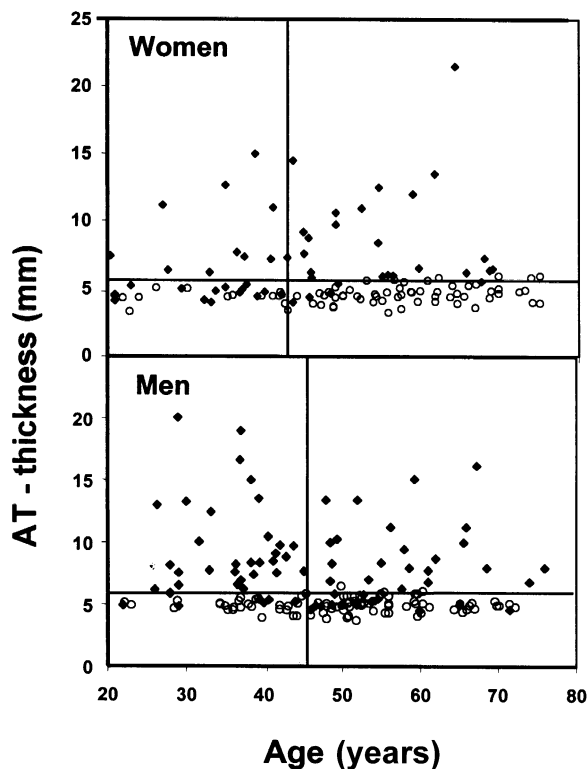


Fig. 1. Scatterplots of Achilles tendon thickness in males and females against age. Open symbols denote non-FH, and closed symbols denote FH-patients. The horizontal and vertical lines represent, respectively, for each sex the 95th percentile AT-thickness in non-FH individuals and the median age in FH individuals. The proportion of FH-individuals displaying an AT-thickness above the 95th percentile before versus above the median age were 41 versus 77% in FH-women ($\chi^2_{\text{df}} = 7.14$, $P = 0.007$) and 76% versus 58% in FH-men ($\chi^2_{\text{df}} = 2.48$, $P = 0.11$), respectively.

The present paper presents an attempt to establish the most simple diagnosis criteria for FH based on tendon size assessed by ultrasonography.

Table 1
Descriptive data of FH and non-FH individuals

	FH men	Non-FH men	Stat	FH women	Non-FH women	Statistics
N	74	88		53	72	
Age (years)	46 ± 13	50 ± 11	<0.05	44 ± 14	51 ± 13	<0.05
BMI (kg/m ²)	26.4 ± 4.1	28.7 ± 3.4	<0.05	25.6 ± 4.5	28.3 ± 4.8	<0.05
TC (mg/dl)	410 ± 66	277 ± 64	<0.05	406 ± 77	283 ± 53	<0.05
LDL-C (mg/dl)	330 ± 65	185 ± 62	<0.05	313 ± 76	196 ± 52	<0.05
HDL-C (mg/dl)	49 ± 12	48 ± 14	NS	63 ± 16*	54 ± 18*	NS
TG (mg/dl)	156 ± 77	271 ± 209	<0.05	148 ± 104	200 ± 178*	<0.05
Tendon xanthomas	41%	0%		17%	0%	
AT breadth (mm)	17.1 ± 3.6	13.2 ± 1.7	<0.05	15.0 ± 3.9*	12.2 ± 1.8*	<0.05
AT thickness (mm)	8.5 ± 3.5	5.0 ± 0.6	<0.05	7.5 ± 3.5	4.6 ± 0.7*	<0.05
AT section (mm ²)	155.5 ± 102.4	65.5 ± 11.1	<0.05	123.7 ± 100.3	55.8 ± 13.3*	<0.05
AT roundness	0.48 ± 0.12	0.39 ± 0.09	<0.05	0.48 ± 0.11	0.38 ± 0.08	<0.05

All values are mean ± S.D. and are compared between groups by Mann–Whitney *U*-test.

* $P < 0.05$ by Mann–Whitney *U*-test when compared with the men of the same group (FH or non-FH).

2. Methods

We analyzed the tendon characteristics of 127 genetically ascertained FH individuals (FH group) who (52%) had never received or who (48%) were refractive to lipid lowering therapy (defined as LDL-C above 180 mg/dl). These individuals are the relatives of 54 probands identified by genetic testing and preliminarily recruited on the basis of high plasma TC, i.e. > 95th percentile for age and sex and a familial history of hypercholesterolemia without consideration of the presence of TX or history of early coronary heart disease. The causative mutations (and their frequency) amongst the 127 FH individuals were the R3500Q mutation of APOB or apoB3500 ($n = 9$) as well as various known [11] and novel mutations of the LDL-R: nonsense mutations E10X ($n = 2$) and C122X ($n = 52$); frameshift 2053del14 ($n = 1$) and 2451insAGAA ($n = 3$); splicing mutation 1359-1G → A ($n = 7$) and 1846-1G → A ($n = 4$); missense mutations E256K1402T ($n = 6$), K290R/C292W ($n = 26$), T413R ($n = 4$), V502M ($n = 5$), G571E ($n = 5$) and P664L ($n = 3$).

The non-FH groups consisted of individuals with a negative genetic test for ApoB3500 and LDL-R mutations and with various lipid profiles: 49 normolipidemic patients, 60 isolated hypercholesterolemia (TC > 250 mg/dl) and 51 mixed dyslipidemia (TG > 200 mg/dl and TC > 250 mg/dl).

None had any history of tears or infection of their Achilles tendons. Tendon xanthomas were searched at the usual sites [12] and were considered if the tendon appeared diffusely enlarged or deformed by one or more focal nodularities. Ultrasonography (US) of Achilles tendons (AT) was performed with an ATC-HDI-3000 using a 10 Mhz linear-array transducer, the tendon holding in maximal extension. “AT-thickness” (AT-T) and “AT breadth” (AT-B) (average for both

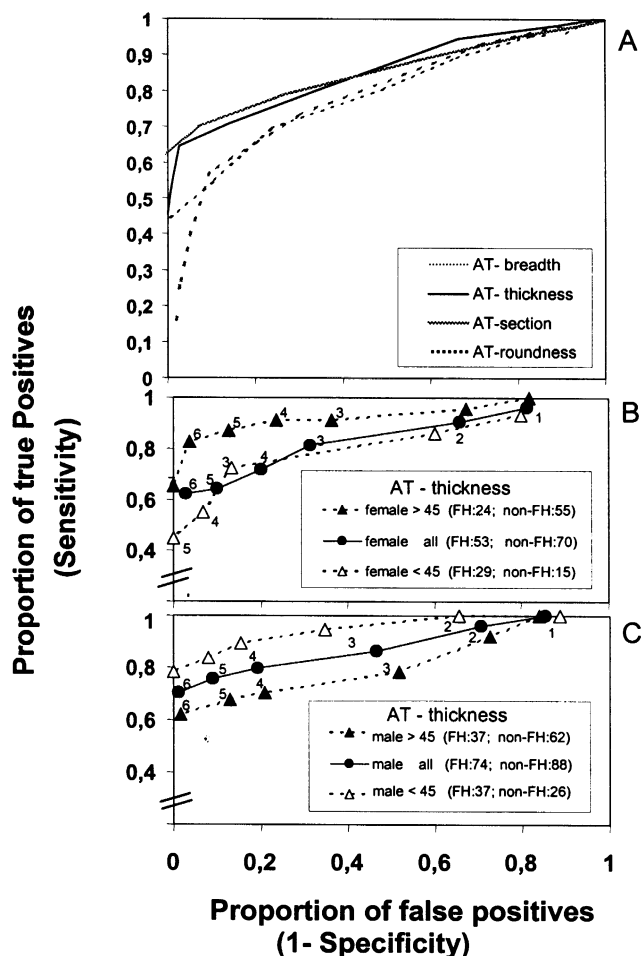


Fig. 2. ROC curves for different Achilles tendon parameters. (A) ROC curves constructed for the four AT-parameters. (B, C) ROC curves representing the AT-thickness in females and males, respectively, for selected age categories as identified in the inset. The numbered indices refer to AT-thickness values of 4.00, 4.35, 4.70, 5.05, 5.40, 5.75 and 6.10 mm, respectively.

feet) were determined from transversal scans at the point of the maximal thickening or, if the thickness was uniform in size, at the proximal part of the tendon. "AT-section" (AT-S) and 'AT roundness' (AT-R) were the averages for both sides of the product (AT-T \times AT-B) and the ratio (AT-T/AT-B), respectively. Reproducibility was tested by repeating three measurements in seven normo-cholesterolemic individuals with intervals

of 1 month. The intra-observer variation for AT-thickness and AT-breadth was 3 and 5%, respectively.

3. Results

Upon clinical examination, none of the controls and 29% of FH individuals (17% FH women and 38% FH men) presented with TX in Achilles tendons. The distribution of various quantitative AT parameters in all non-FH groups was approximately normal with no difference in mean and variance (data not shown) so that the AT characteristics of non-FH individuals were pooled for further analysis. AT parameters remained constant with age (Fig. 1 displays the AT thickness only) and tended to be greater in men than in women (Table 1) so that genders were analyzed separately. Compared with non-FH individuals, the distributions of the AT parameters were very skewed (Fig. 1) in FH subjects and showed on average significantly greater values (Table 1).

As these variables are scaled continuously and overlap between diseased and non-diseased individuals, selection of cut-off values allowing confident FH diagnosis was based on an analysis of 'receiver operating characteristic' (ROC) curves. The ROC curves representing the four different quantitative variables of Achilles tendon were constructed using all FH individuals of both sexes as the disease group and the pooled non-FH individuals as the non-disease group (Fig. 2A). The ROC curves representing AT-T and AT-S superimposed almost completely. The estimated areas under these curves (area and 95% CI calculated according to Ref. [13]) were both equal to 0.85 (95% CI = 0.81–0.90) and operated better (test with the largest area under the ROC curve is considered most accurate [13]) than the ROC curves representing AT breadth (area = 0.79; 95% CI = 0.74–0.83) and AT roundness (area = 0.78; 95% CI = 0.73–0.83). The ROC curves for AT thickness in patients separated by sex and age categories (below or above 45 years) demonstrated that the ROC curve in women (Fig. 2B) above 45 years of age (area = 0.93; 95% CI = 0.88–0.98) operated better than the ROC curve of women below 45 (area = 0.82; 95% CI = 0.67–0.96). Conversely, the ROC curves in men (Fig. 2C)

Table 2
Effect of mutation type on clinical characteristics

	Nonsense and frameshift LDL-R mutations	Missense and splicing LDLR mutations	ApoB3500
N	58	60	9
Sex (F/M)	27/31	23/37	3/6
LDL-C (mg/dl)	340 \pm 78	314 \pm 58*	272 \pm 57*
AT thickness (mm)	9.1 \pm 3.7	7.4 \pm 3.3*	5.9 \pm 1.8*
AT thickness >5.8 mm	51 (88%)	42 (70%) ^S	2 (22%) ^S

* $P < 0.05$ by Mann-Whitney U -test when compared with the first group. ^S $P < 0.05$ by χ^2 test when compared with the first group.

below 45 years of age (area = 0.96; 95% CI = 0.90–1.00) operated better than the ROC of men above 45 (area = 0.81; 95% CI = 0.73–0.89). A cut-off value of 5.8 mm of AT thickness, which is close to the 95th percentile of non-FH women and FH men, yielded diagnosis with 95% specificity and about 80% sensitivity in older women and in younger men.

When stratified (Table 2) in three groups according to the severity of the mutation, FH individuals with null LDL-R had thicker Achilles tendon as well as a greater LDL-C concentration. Individuals with ApoB3500 mutation had the lowest prevalence of thicker tendons.

4. Discussion

A test based on measurements of AT thickness is presented as a valid and practical alternative for diagnosing FH. The procedure can be widely applicable for routine clinical practice and is highly reproducible, as shown by other authors [1–7] and ourselves. It yields a higher specificity than a test based on cholesterol level. Cholesterol accumulation in tendon is indeed considered essentially pathognomonic for familial hypercholesterolemia with the exception of some conditions that are rare and easily differentiated from FH on clinical and biochemical grounds, i.e. type III hyperlipoproteinemia [14] cerebrotendinous xanthomatosis [15], overproduction of apolipoprotein B [16], and beta-sitosterolemia [17]. It is evidently most important to interpret AT thickening as cholesterol impregnation only after exclusion of other disorders, i.e. acute tendonitis, trauma or gout tophi.

The AT thickness cut-off at 5.8 mm was the most useful threshold for diagnosis of FH, procuring a sensitivity of 75% and a specificity of 85%. Decreasing the cut-off value of AT thickness increased the sensitivity, but the gain was minimal, relative to the important loss in specificity.

The population prevalence of FH is estimated to be 0.002, but the a priori probability of FH in individuals with cholesterol above the 95th percentile may approach 4% (considering the reasonable assumption that 95% of FH individuals have cholesterol above the 95th percentile). Evidence of personal or familial history of coronary heart disease or of hypercholesterolemia at childhood also increases the a priori probability of FH. So, for a priori probability at 4, 10 and 25%, the positive predictive value (PPV) may be as high as 33, 56 and 80%, respectively, if discriminatory thresholds of 5.8 mm for AT thickness were applied (sensitivity 70%, specificity 94%). The PPV can even be higher if the tests are limited to women above 45 years or men below 45 years, as the sensitivity is much greater (about 84%).

We observed indeed that AT thickening occurred later in women than men and that a trend to a larger proportion of AT thickening was observed in men before age 45 (see Fig. 1). This last observation was surprising given that tendon size is expected to increase with age due to cholesterol accumulation. It is likely that men with the thickest tendons have the most severe prognosis and therefore died earlier, as suggested by previous studies [18]. The actual genetic defect is another confounding factor, yet more insidious than age and sex because it is a priori inaccessible to the practitioner. Some mutations, such as apoB3500 resulting in a lesser severity of disease [19], appeared to have less thickening of AT tendons so that patients with these mutations may be poorly identifiable by AT-US. A different distribution of mutation in another cohort may in turn, result in different ROC curves, and the ROC curve presented here thus may not apply to other populations.

Contrarily to some previous studies [4–6], we did not evaluate any changes in echogenicity because in our experience, it is far less accurate and reproducible than quantitative measurements. For most sonographers, this evaluation is indeed very subjective as no tissue at this site can serve reliably as a reference to qualify the echogenicity of the tendon. In addition, the tendon often may be interpreted as being hypoechogenic if the transducer is not placed strictly parallel to fibers.

5. Conclusion

AT thickness assessed by ultrasonography, will be most helpful for clinical diagnosis of heterozygous FH outside specialized centers. An AT thickness above 5.8 mm was a most useful threshold for diagnosis of FH. However, this clinical criterion performed better in females older than 45 and in males under 45. Additional genetic testing for the ApoB3500 mutation could or should complete the proposed test, since a great proportion of FH due to this mutation may not be effectively identified by AT thickness measurements.

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