Eur J Vasc Endovasc Surg **20**, 379–385 (2000)
doi:10.1053/ejvs.2000.1193, available online at http://www.idealibrary.com on IDEAL®

The Role of Type III Collagen in Family Members of Patients with Abdominal Aortic Aneurysms

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Objectives: *type III collagen is responsible for the tensile strength of the aorta-wall. To determine if genetic defect in the type III collagen production is associated with familial clustering of AAA.*
Methods: *fifty-six patients with AAA and 82 first-degree family members participated. The medical and family histories were obtained. All these relatives were screened by ultrasound for AAA. In 58 relatives of 20 families, skin biopsies were taken for protein analysis to measure type III collagen production in cultured fibroblasts.*
Results: *only one new AAA was detected in a brother of a patient. Four other relatives were already known with AAA. Three AAA patients had a type III collagen deficiency, but type III collagen was normal in all family members.*
Conclusion: *type III collagen deficiency does not appear to be an aetiological factor in the development of AAA.*

Key Words: Familial abdominal aortic aneurysm; Type III collagen deficiency; Ultrasound examination.

Introduction

Abdominal aortic aneurysms (AAA) are frequently familial and recognised as a genetic disorder.[1–5] Studies using questionnaires to examine the risk for AAA of first-degree relatives of patients with AAA report an increase up to eight times that for the general population.[3,6–10] Collin *et al.*[5] combined the data of six studies in which the prevalence of AAA among siblings of patients with AAA was examined by ultrasound. He reported a prevalence of AAA of 24% in 196 male first-degree relatives and 5% in 225 female first-degree relatives of AAA patients.[11–16] Beside the greater risk to develop an AAA among siblings of patients with AAA, the risk of AAA also began at an earlier age.[16,17] Therefore several studies recommend screening the siblings or the brothers of patients with AAA.[13,14,17–19]

In 1994 we found a 29% prevalence of a positive family history for AAA by interview among 56 consecutive, unrelated patients with AAA.[20] Collagen (type I and III) is the principle component of the aortic adventitia. The tensile characteristics are attributed to type III collagen and the load-bearing characteristics to type I collagen. The tensile strength of the aortic wall can be influenced by synthesis of structurally abnormal type III collagen which constitutes the basis for the aneurysm formation.[21] A heritable syndrome with spontaneous aortic rupture is the Ehlers–Danlos syndrome type IV (EDSIV), caused by a type III collagen deficiency.[22,23] Every EDSIV family has its own type III collagen mutation, so heterogeneity between different families is very likely.[24–28] This means that if a type III collagen defect is responsible for familial AAA it is very likely that each family has its own type III collagen gene mutation.

In the present study, we performed protein analysis of type III collagen in family members of patients with an AAA. We studied in first order relatives of patients with AAA the prevalence of (a) AAA by ultrasound and (b) a type III collagen deficiency.

Patients and Methods

Patients

We studied 56 consecutive, unrelated patients with an AAA.[20] An abdominal aortic aneurysm was defined

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Table 1. Clinical and laboratory characteristics of family members with and without familial abdominal aortic aneurysm (AAA).

	Family members with familial AAA $n=38$	Family members without familial AAA $n=44$	p-value
Male/Female, n (%)	26 (68.4)/12 (31.6)	24 (54.5)/20 (45.5)	
Age, years	48 (43–63)	54.0 (46–68)	
Hypertension, n (%)	5 (13.2)	15 (34.1)	$p<0.04$
Hypercholestaeremia, n (%)	9 (23.7)	12 (27.3)	
Coronary arterial occlusive disease, n (%)§	3 (7.9)	4 (9.1)	
Peripheral arterial occlusive disease, n (%)§	1 (2.6)	1 (2.3)	
Cerebral arterial occlusive disease, n (%)§	1 (2.6)	1 (2.3)	
Diabetes mellitus, n (%)	1 (2.6)	1 (2.3)	
Current smoking, n (%)	17 (44.7)	12 (27.3)	
Past smokers, n (%)	4 (10.5)	11 (25.0)	
Pack years†	13.1 (16.3)	8.4 (12.8)	
Body mass index (kg/m^2)‡	23.3 (2.9)	26.5 (4.1)	
Total cholesterol (mmol/L)	5.7 (1.2)	5.8 (1.2)	
Triglyceride (mmol/L)	1.7 (1.9)	1.7 (1.0)	
HDL-cholesterol (mmol/L)	1.3 (0.9)	1.2 (0.4)	
LDL-cholesterol (mmol/L)●	3.8 (1.0)	3.9 (1.0)	
Diameter abdominal aneurym (cm)#	2.0 (0.8)	1.7 (0.4)	

Data are given as number (n) with percentage in parentheses or as median (interquartile range).
§Coronary, peripheral, cerebral arerial occlusive disease as defined in patients and methods. †Pack years were calculated by multiplying the number of cigarette packages smoked per day by the number of years the patient smoked. ‡Body mass index was calculated as weight/height.[2] ●LDL-cholesterol was calculated by Friedewald's formula. #Diameter abdominal aneurysm as diagnosed by ultrasound or computed tomography. *Familial AAA was defined as one or more first-degree family members with an AAA mentioned by the patient and diagnosed by a physician.

as an aortic diameter greater than 3 cm in the infrarenal portion of the aorta, or a diameter of 50% greater than the normal suprarenal aorta.[29] Twenty-eight patients (median age 69 years (65–73)), three (10.7%) of whom had a type III collagen deficiency, participated in the family study. We included first-degree relatives (parents, brothers and sisters and the children) of the patients with AAA. We excluded first-degree relatives younger than 40 years or older than 85 years.

In the 28 patients (median age 71 years (66–73)) whose families did not participate, five of 96 deceased relatives had an AAA. One hundred first-degree relatives met the criteria for inclusion in the study (Fig. 2). The reasons for not participating in the study were: no family members (four), not interested (12), deceased (five), no one within the age limits (four), no family contact (one) and no response to our letters (two).

Of the 28 participating patients there were 256 (mean 3.1; range 4 to 21) first-degree relatives, 176 were alive at the time of our study and were invited for further studies. Eighty-two first-degree relatives (32%) agreed to participate and were invited to our hospital (Fig. 2). We recorded the medical and family history. We searched for the presence of aneurysms in any of their first-degree relatives.

A positive family history was defined as one or more first-degree family members with an AAA mentioned by the family member of the patient and diagnosed by a physician. In addition we recorded presence of classical risk factors like smoking habits (duration and amounts per day), the presence of hypertension (WHO criteria), diabetes mellitus and hypercholesterolaemia (diagnosed by a physician).

Furthermore, we scored atherosclerotic manifestations such as peripheral, coronary and cerebral arterial occlusive disease. Peripheral arterial occlusive disease was defined by presence of intermittent claudication confirmed by ankle/arm index <0.9 and/or decrease of >0.15 of the index after treadmill exercise testing, or ischaemic rest pain, gangrenous ulcers, or amputation for ischaemia. Coronary arterial occlusive disease was defined as the presence of myocardial infarction (WHO clinical definition plus new Q waves on electrocardiography and/or diagnostic enzyme changes). Cerebral arterial occlusive disease was defined by the presence of symptomatic cerebral vascular disease (ischaemic stroke or transient ischemic attack, WHO clinical definitions), or in case of stroke as confirmed by computed tomography. To exclude patients with an Ehlers–Danlos type IV syndrome, the emphasis in physical examination was on skin changes like ecchymoses, scarring, heavy pigmentation and easy bruisability.

After an overnight fast, venous blood samples were taken for measurement of serum lipids (total and high density lipoprotein (HDL), cholesterol and triglycerides (enzymatically), low density lipoprotein (LDL) was calculated by Friedewald's formula).[30] Ref-

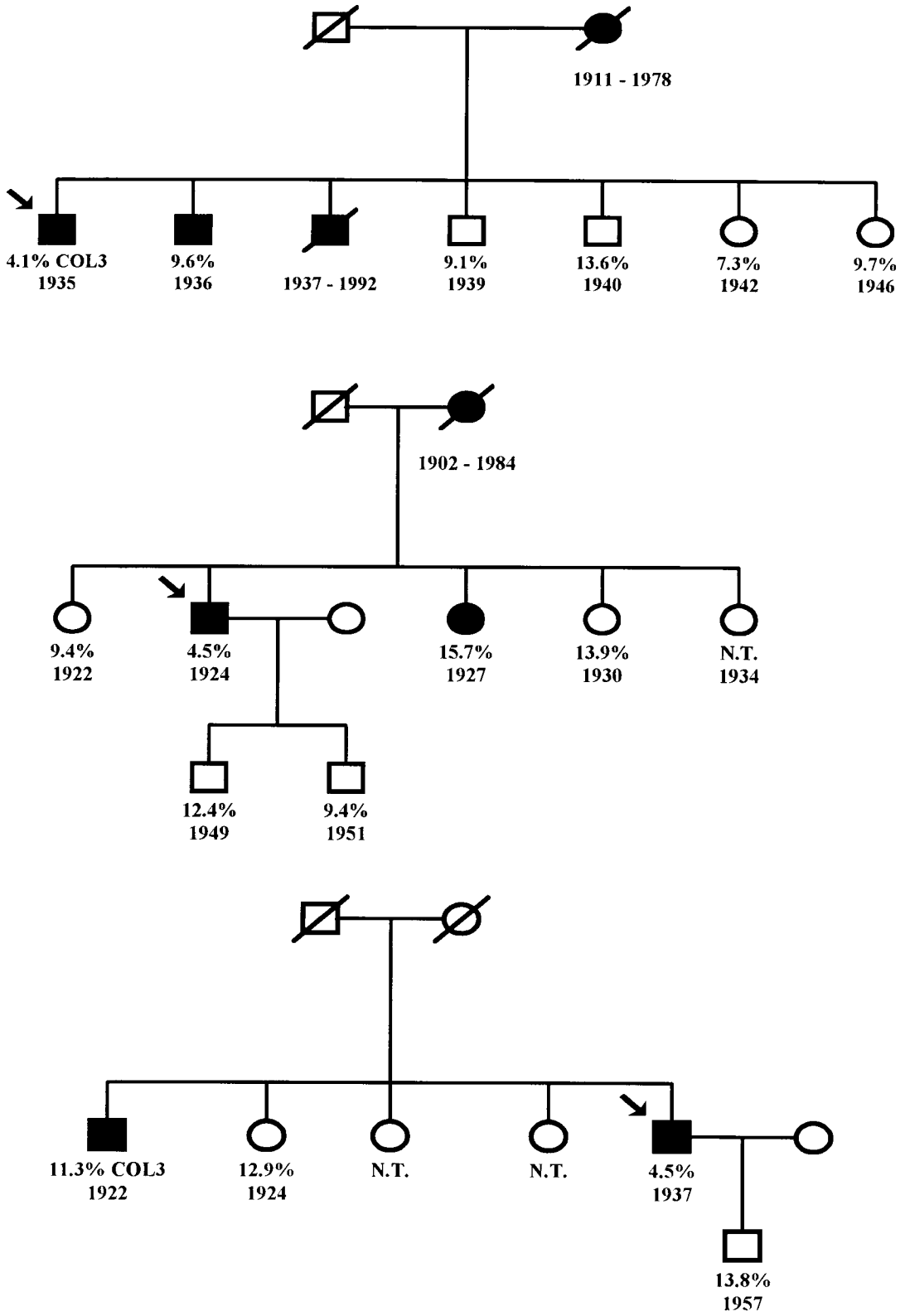

Fig. 1. The figure shows the family trees of the three index patients with a type III collagen deficiency. Black squares and circles indicates those in whom aortic aneurysm was diagnosed; arrow indicates the index patient; % col 3 = type III collagen percentage (type III collagen deficiency was defined as a type III collagen percentage less than 5% of total collagen production after protein analysis of cultured fibroblasts); date of birth and death is added; N.T. means not tested.

Flow diagram. Design of the family study and results.

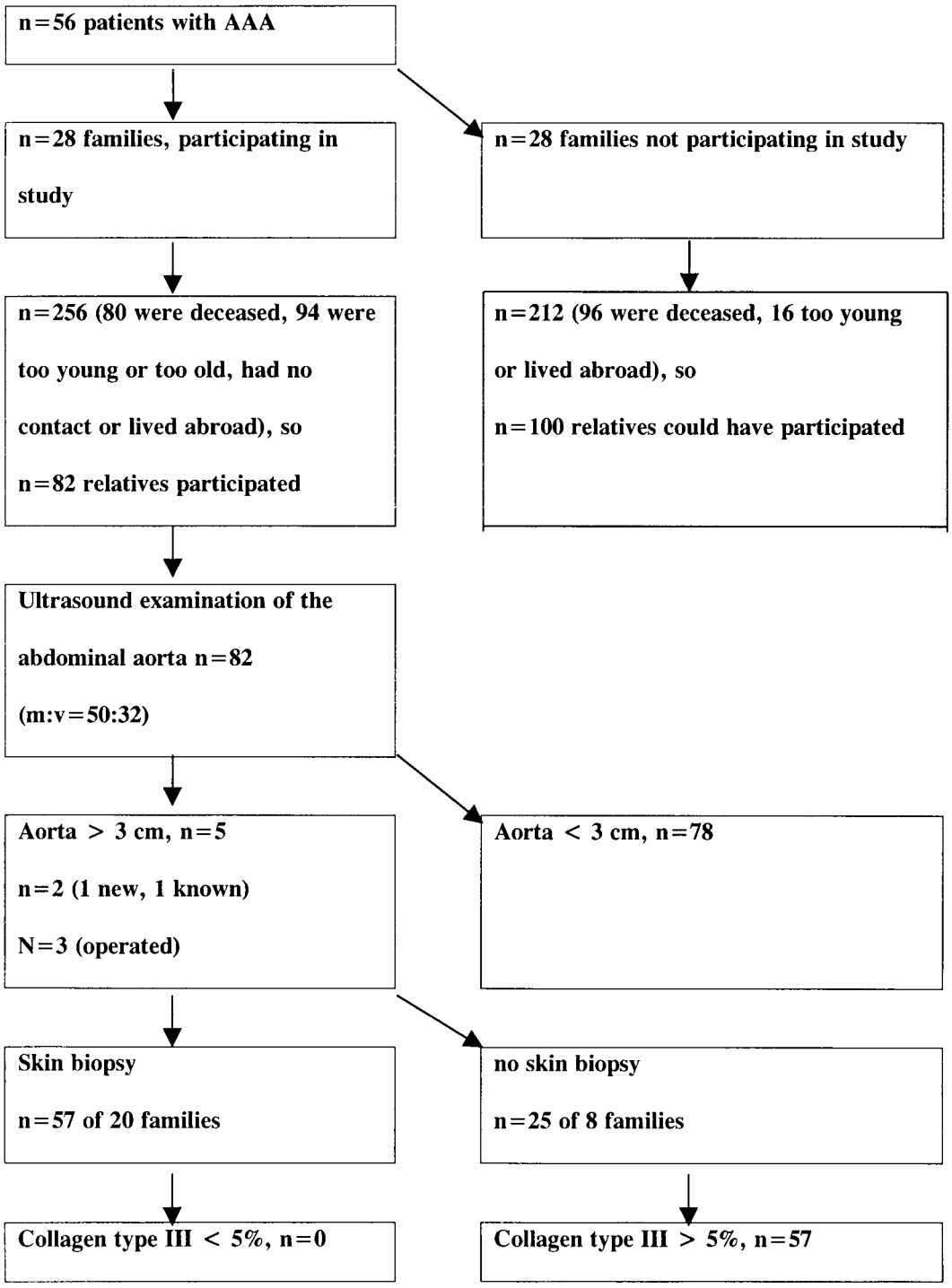

Fig. 2. Flow diagram – design of the family study and results.

erence values in our hospital are for total cholesterol <6.2 mmol/l, triglycerides <2 mmol/l, HDL cholesterol >0.9 mmol/l and/or LDL cholesterol <5.0 mmol/l.

All family members gave informed consent for these studies, which were approved by the local ethics committee.

Ultrasound examination

An experienced radiologist from our Department of Radiology performed the ultrasound scan. Abdominal aortic investigations were carried out with an Acuson 128 with a 3.5 MHz transducer C366. The maximum diameter of the infrarenal abdominal aorta was measured perpendicularly to the longitudinal axis of the aorta.

Protein analysis

Under local anaesthesia, a skin biopsy was taken for fibroblast culture from the ventral side of the lower arm in 57 members from 20 families (median age 48 years (43–59)). No skin biopsies were obtained from the other 25 family members of eight families (median age 66 years (51–71)). Control cell lines were derived from skin biopsies from 40 healthy control subjects (median age 46 years (37–51)).[20]

Collagen in cultured fibroblasts was metabolically labelled with ^{14}C and analysed by electrophoresis. Samples for electrophoresis were normalised by analysing the same amount of incorporated label for each sample.[20,31]

A type III collagen deficiency was defined as a type III collagen percentage less than 5% of total collagen production after protein analysis of cultured fibroblasts.

Statistics

Data were analysed using the statistical package SPSS for Windows 6.1. Descriptive data are given as median (interquartile range). Skewed data were logarithmically transformed. Continuous variables were tested by Student's *t*-test (for means).

As the numbers are small, percentages were compared by Fisher's Exact test. All testing was two-tailed with 0.05 as the level of significance.

Results

From 56 patients with an AAA, 28 families (50%) participated in this family study. Of these 28 families, 11 (39.3%) mentioned they had a positive family history of AAA, to compare with five out of 28 (17.9%) non-participating families ($p = 0.14$).

Eighty-two first-degree relatives of 28 families (2.9 relative per family) were enrolled in the study. The flow diagram shows the study design and results.

To exclude other risk factors we compared the known risk factors in aneurysm disease in family members with and without familial AAA (Table 1). The mean age in the group of family members with familial AAA (median age 48 years (43–63)) is not significantly lower than in the family group without familial AAA (median age 54 years (46–68)). In the family group with familial AAA significantly less relatives mentioned that they were treated for hypertension ($p<0.04$). Only a few people had coronary, peripheral, and/or cerebral arterial occlusive diseases, explained by the young age of this group.

In the participating family group with 11 positive family histories, seven relatives with an AAA (three brothers, three fathers and one mother) were deceased, two (brother, mother) did not participate in the study but were treated for AAA in a hospital elsewhere. These nine relatives are from different families. Of these nine families one brother, 60 years old, was diagnosed in a hospital elsewhere for an AAA, diameter 3.7 cm and one brother (age 58 years), has been operated on for AAA. Another brother (age 65 years) and sister (age 68 years) from two different families have also been operated on for AAA.

Since results from family histories are unreliable, ultrasound examination of the abdominal aorta was performed in all 82 family members. We detected in only one relative, a 75-year-old brother of a patient, an aortic diameter of 3.4 cm in September 1998. By April 1999 the AAA had expanded to 5.8 cm. He was successfully operated. Before this finding there was no other first-degree relative known in this family with an AAA.

So, in this group, five of 82 (6.1%) first-degree relatives had been diagnosed with an AAA, one of them newly detected.

When we add the family history and ultrasound examination results, 12 of the 28 families had a positive family history for AAA or proven AAA by ultrasound.

Seventeen brothers cooperated in the study, 3 (17.6% (95% confidence interval, 4–43%) (age 58, 59 and 74 years)) had an AAA. In the sister group one (age 66 years) of 22 (4.5% (95% confidence interval, 0–23%))

had an AAA. No sons or daughters of the AAA patients had an AAA. Only one parent was alive during the study, this father had no AAA.

Type III collagen was measured in 57 individuals from 20 families. Although three index patients of these 20 families had a collagen type III deficiency, there were no first-degree relatives with a decreased secretion of type III collagen (Fig. 1). All type III collagen measurements in relatives were within the normal range so no further analyses were carried out.

Protein analysis of 40 healthy control subjects showed no type III collagen deficiency (9.7(SD $\pm$ 3.7)).[20] The type III/I collagen ratio showed no decrease with age, so it is unlikely that the lower mean age of the control group has affected the results.

Discussion

Based on family history and ultrasound screening, 18% of the brothers of index patients had an AAA in our study. In a recent study, van der Graaf *et al.*[32] detected a new AAA in 26 out of 210 brothers of AAA patients, 13 individuals were already known to have an AAA, this resulted in an overall prevalence of 17%.

We found only one new AAA by ultrasound examination. The other family members were already known with an AAA, some were deceased, others had been operated on or were followed by a surgeon.

Whether more relatives will develop AAA in the future is an interesting question. The reason we allowed the family members to enrol from the age of 40 years is because Baird *et al.*[16] concluded that the risk of AAA began earlier and increased more rapidly for siblings of AAA probands. In our study we could not corroborate this conclusion, because the mean age in the group with familial AAA is not significantly lower than in the group without familial AAA.

Three AAA patients had an abnormality in type III collagen production. Affected as well as unaffected relatives had normal type III collagen in these cases. Because the type III collagen contents were normal and there were no large families with multiple surviving affected relatives we did not perform segregation analysis in this group. In a previous study we described segregation of the gene for type III procollagen (COL3AI gene) and the presence of AAA in a single family, using a DNA repeat marker in intron 25. The COL3A1 gene segregated with the disease in this family, but due to the small size of the family the LOD score was only 1.5 (for significant linkage LOD >3 is required).

Several polymorphisms and mutations in COL3A1 have been reported in AAA patients, but all these alterations were rare and only found in single families.[33–38] Verloes *et al.*[28] indicated that the most likely explanation for occurrence of AAA in families is a dominant inheritance with reduced penetrance. Our families are in keeping with this mode of inheritance, but the number of participating families is too small for statistical calculations.

In conclusion, brothers of AAA patients are at high risk to develop an AAA. There is insufficient evidence to assume that a type III collagen defect is an important etiologic genetic factor in the development of familial AAA. In only a few families a type III collagen defect may be responsible for AAA. Because in most families the number of surviving affected individuals is small and the aetiology is complex, the best approach towards localising the gene or genes involved is to study multiple pairs of affected siblings.

References

1 Clifton MA. Familial abdominal aortic aneurysms. *Br J Surg* 1977; **64**: 765–766.
2 Tilson MD, Seashore MR. Fifty families with abdominal aortic aneurysms in two or more first-order relatives. *Am J Surg* 1984; **147**: 551–552.
3 Johansen K, Koepsell T. Familial tendency for abdominal aortic aneurysms. *JAMA* 1986; **256**: 1934–1946.
4 Loosemore TM, Child AH, Dormandy JA. Familial abdominal aortic aneurysms. *Royal Soc Med* 1988; **81**: 472–473.
5 Collin J, Walton J. Is abdominal aortic aneurysm familial? *BMJ* 1989; **299**: 493.
6 Webster MW, St.Jean PL, Steed DL, Ferrell RE. Abdominal aortic aneurysm: results of a family study. *J Vasc Surg* 1991; **13**: 366–372.
7 Norrgard Ö, Rais O, Ängquist KA. Familial occurrence of abdominal aortic aneurysms. *Surgery* 1984; **95**: 650–656.
8 Powell JT, Greenhalgh. Multifactorial inheritance of abdominal aortic aneurysm. *Eur J Vasc Surg* 1987; **1**: 29–31.
9 Cole CW, Barber GG, Bouchard AG *et al.* Abdominal aortic aneurysm: consequences of a positive family history. *CJS* 1989; **32**: 117–120.
10 Darling RC, Brewster DC, Darling RC *et al.* Are familial abdominal aortic aneurysms different? *J Vasc Surg* 1989; **10**: 39–43.
11 Collin J. The Oxford Screening program for aortic aneurysm and screening first-order male siblings of probands with abdominal aortic aneurysm. *Ann N Y Ac Sci* 1996; **800**: 36–43.
12 Bengtsson H, Norrgard O, Angquist A *et al.* Ultrasonographic screening of the abdominal aortic among siblings of patients with abdominal aortic aneurysms. *Br J Surg* 1989; **76**: 589–591.
13 Webster MW, Ferrel RE, St Jean PL *et al.* Ultrasound screening of first-degree relatives of patients with an abdominal aortic aneurysm. *J Vasc Surg* 1991; **13**: 9–14.
14 Adams DCR, Tulloh BR, Galloway SW *et al.* Familial abdominal aortic aneurysm: prevalence and implications for screening. *Eur J Vasc Surg* 1993; **7**: 709–712.
15 Fitzgerald P, Ramsbottom D, Burke P *et al.* Abdominal aortic aneurysm in the Irish population: a familial screening study. *Br J Surg* 1995; **82**: 483–486.
16 Baird PA, Sadovnik AD, Yee IML, Cole CW, Cole L. Siblings risks of abdominal aortic aneurysm. *Lancet* 1995; **346**: 601–604.

17 Adamson J, Powell JT, Greenhalgh RM. Selection for screening for familial aortic aneurysms. *Br J Surg* 1992; **79**: 897–898.
18 Bengtsson H, Sonesson B, Länne T *et al.* Prevalence of abdominal aortic aneurysm in the offspring of patients dying from aneurysm rupture. *Br J Surg* 1992; **79**: 1142–1143.
19 Jaakkola P, Kuivaniemi H, Partanen K *et al.* Familial abdominal aortic aneurysms: screening of 71 families. *Eur J Surg* 1996; **162**: 611–617.
20 Keulen van C, Akker van de E, Pals G, Rauwerda JA. The role of type III collagen in the development of familial abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 1999; **18**: 65–70.
21 Loosemore TM, Child AH, Dormandy JA. Familial abdominal aortic aneurysms. *J R Soc Med* 1988; **81**: 472–473.
22 Beighton P. Lethal complications of the Ehlers–Danlos Syndrome. *BMJ* 1986; **3**: 656.
23 Superti-Furga A, Steinmann B, Ramirez F, Byers PH. Molecular defects of type III procollagen in Ehlers–Danlos syndrome type IV. *Hum Genet* 1989; **82**: 104–108.
24 Barabas AP. Heterogeneity of the Ehlers–Danlos Syndrome. *BMJ* 1967; **2**: 612.
25 Byers PH, Holbrook KA, Barsh GS, Smith LT, Bornstein P. Altered secretion of type III procollagen in a form of type IV Ehlers–Danlos syndrome: biochemical studies in cultered fibroblasts. *Lab Invest* 1981; **44**: 336–341.
26 Superti-Furga A, Steinmann B, Ramirez F, Byers PH. Molecular defects of type III procollagen in Ehlers–Danlos syndrome type IV. *Hum Genet* 1989; **82**: 104–108.
27 Majumder PP, St Jean PL, Ferrel RE, Webster MW. On the inheritance of abdominal aortic aneurysm. *Am J Hum Genet* 1991; **48**: 164–170.
28 Verloes A, Sakalihasan N, Loulischer L, Limet R. Aneurysms of the abdominal aorta: familial and genetic aspects in three hundred thirteen pedigrees. *J Vasc Surg* 1995; **21**: 646–655.
29 Johnston KW, Rutherford RB, Tilson MD *et al.* Suggested standards for reporting on arterial aneurysms. *J Vasc Surg* 1991; **13**: 444–450.
30 Friedewald WT, Levy RI, Fredickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**: 499–502.
31 Leski ML, Steward O. Protein synthesis within dendrites ionic and neurotransmitter modulation of synthesis of particular polypeptides characterized by gel electrophoresis. *Neurochemical Research* 1996; **21**: 681–690.
32 Van der Graaf Y, Akkersdijk GJ, Hak E, Godaert GL, Eikelboom BC. Results of aortic screening in the brothers of patients who had elective aortic aneurysm repair. *Br J Surg* 1998; **85**: 778–780.
33 Anderson DW, Edwards TK, Ricketts MH *et al.* Multiple defects in type III collagen synthesis are associated with the pathogenesis of abdominal aortic aneurysms. *Ann NY Acad Sci* 1996; **800**: 216–228.
34 Anderson DW, Thakker-Varia S, Stolle CA. Phenotypic overlap between familial aneurysms and Ehlers–Danlos syndrome type IV resulting from a type III procollagen gene mutation. *Ann N Y Acad Sci* 1996; **800**: 294–298.
35 Kontusaari S, Tromp G, Kuivaniemi H, Ladda RL, Prockop DJ. Inheritance of an RNA splicing mutation ($G^{+1\ IVS20}$) in the type III procollagen gene (COL3A1) in a family having aortic aneurysms and easy bruisability: phenetypic overlap between familial arterial aneurysms and Ehlers–Danlos Syndrome type IV. *Am J Hum Genet* 1990; **47**: 112–120.
36 Kontusaari S, Tromp G, Kuivaniemi H, Romanic AM, Prockop DJ. A mutation in the gene for type III procollagen (COL3A1) in a family with aortic aneurysms. *J Clin Invest* 1990; **86**: 1465–1473.
37 Tromp G, Wu Y, Prockop DJ *et al.* Sequencing od cDNA from 50 unrelated patients reveals that mutations in the triple-helical domain of type III procollagen are an infrequent cause of aortic aneurysms. *J Clin Invest* 1993; **91**: 2539–2545.
38 Powell JT, Adamson J, MacSweeney STR *et al.* Influence of type III collagen genotype on aortic diameter and disease. *Br J Surg* 1993; **80**: 1246–1248.

Accepted 6 July 2000