The vitamin D receptor genotype predisposes to the development of calcific aortic valve stenosis

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Abstract

Objective—To test the hypothesis that vitamin D receptor polymorphism is associated with calcific aortic valve stenosis.

Design—The distribution of one polymorphism of the vitamin D receptor (BsmI B/b) was examined in 100 consecutive patients with calcific valvar aortic stenosis and compared with a control group of 100 patients (paired match for age, sex, and the presence of coronary artery disease from a total of 630 patients without calcified aortic valves). Polymerase chain reaction and restriction fragment length polymorphism were used to determine genotypes.

Results—There was a significant difference in vitamin D receptor allele and genotype frequencies between the two groups. The allele B had a higher prevalence in patients with calcific aortic stenosis (B = 0.56, b = 0.44) than in the control cohort (B = 0.40, b = 0.60) (p = 0.001).

Conclusions—There is a significant association of vitamin D receptor polymorphism with calcific aortic valve stenosis. The B allele of the vitamin D receptor is more common in patients with calcific aortic valve stenosis. It now needs to be evaluated whether other genes that control calcium homeostasis are involved in the pathogenesis of this disorder.

(*Heart* 2001;**85**:635–638)

Degenerative calcification of the aortic valve is the most common cause of valvar aortic stenosis and is becoming even more frequent with the increasing age of our population.[1] [2] Clinical factors associated with atherosclerosis are also associated with calcification of the aortic valve, and early lesions in calcific aortic valves show similarities to atherosclerosis.[3] However, approximately 50% of patients with severe aortic stenosis have no coronary artery disease. Although calcification of the aortic valve is a disease of the elderly population, there is evidence that it is not simply a consequence of aging.[4] Additional and as yet undefined factors are likely to have an important impact on degenerative calcification of the aortic valve.

The influence of genetic polymorphism of the vitamin D receptor on bone metabolism has been investigated in several studies in the past decade. BsmI polymorphism of the vitamin D receptor gene is a predictor of bone mineral mass.[5–9] As bone metabolism is not just a function of bone cells but a complex interaction of hormones and organs—bone, intestine, kidney, thyroid, parathyroid, and so on—controlling calcium homeostasis, we postulated that alterations in the latter might influence calcification of extraosseous structures such as the aortic valve. The present case–control association study (matched for age, sex, and the prevalence of coronary artery disease) was designed to test the hypothesis that the BsmI vitamin D receptor polymorphism is associated with calcific aortic valve stenosis.

Methods

STUDY POPULATION

One hundred consecutive patients with calcific aortic stenosis in whom cardiac catheterisation was undertaken formed the case group. Calcific aortic stenosis was diagnosed when calcification of the aortic valve was visible by fluoroscopy, and if the aortic valve area was $< 1.0\ cm^2$ or the mean transvalvar gradient $> 40\ mm\ Hg$, determined by invasive measurements.

A control group of 100 patients, matched for age, sex, and the presence of coronary artery disease, was formed from among 630 patients who underwent left heart catheterisation for suspected coronary artery disease and had no gradient across the aortic valve.

All patients gave written informed consent. The study was approved by the local ethics committee of the University Hospital of Aachen.

CARDIAC CATHETERISATION

All patients underwent invasive examination. Retrograde left sided cardiac catheterisation was performed using fluid filled catheters. The mean gradient across the aortic valve was determined by simultaneous measurement of aortic and left ventricular pressures. Measurements were averaged over three beats. Cardiac output was determined by thermodilution (five measurements averaged). Aortic valve area was calculated using Gorlin's equation.[10]

GENOTYPING

Genomic DNA of patients was extracted from whole blood using the QIAmp blood kit 250 (Qiagen, Valencia, California, USA). Polymerase chain reaction (PCR) of genomic DNA segments was achieved using a standard PCR thermocycler (Hybaid PCR express, Hybaid, Ashford, Kent, UK). Details of the amplification protocol have been described previously.[6] Genotyping was independently performed by two investigators.

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Table 1 Clinical characteristics of patients with calcific aortic stenosis and controls

	Calcific aortic stenosis (n=100)	Controls (n=100)
Gradient across aortic valve (mm Hg)*	59 (20)	No gradient
Aortic valve area (cm²)*	0.8 (0.3)	Not determined
Age (years)*	69.4 (9.3)	67.1 (6.7)
Sex (male/female) (n)	55/45	55/45
Coronary artery disease	48%	48%
No coronary artery disease	52%	52%
History of		
Hypertension	66%	64%
Hypercholesterolaemia	36%	63%
Smoking	18%	25%
Positive family history of coronary artery disease	31%	27%
Diabetes mellitus	15%	11%
BMI (kg/m²)*	26.8 (4.0)	26.9 (3.2)

*Mean (SD).
BMI, body mass index.

Table 2 Allele and genotype frequencies of the BsmI vitamin D receptor polymorphism in patients with calcified aortic stenosis and controls

Gene polymorphism		Patients with calcific aortic stenosis (n=100)		Controls (n=100)		p Value
		n	%	n	%	
VDR Bsml allele	B	109	54.5	80	40.0	0.001*
	b	91	45.5	120	60.0	
Genotype	BB	24	24.0	20	20.0	0.023†
	Bb	61	61.0	40	40.0	
	bb	15	15.0	40	40.0	

Cases and controls were from the same geographic region.
Two sided p values: *χ^2 analysis; †analysis of variance.
VDR, vitamin D receptor.

STATISTICAL ANALYSIS

Statistical analysis was done using the computer program Graph Pad Instat tm V2.05. Data are presented as mean (SD). The differences of the frequencies of alleles and genotypes between case patients and controls were analysed by the χ^2 test and analysis of variance (ANOVA). A probability value of $p < 0.05$ was considered significant.

Results

STUDY POPULATION

The case patients represent a normal cross section of elderly patients with degenerative calcific aortic valve stenosis. Their mean (SD) age was 69.4 (9.3) years. Forty eight per cent of the case patients had additional coronary artery disease. The diagnosis of coronary artery disease was not established in all cases before cardiac catheterisation. The mean gradient across the aortic valve was 59 (20) mm Hg, corresponding to a mean valve area of 0.8 (0.3) cm . Cardiac risk factors in patients with calcific aortic stenosis were as follow: 66% had arterial hypertension, 36% had hypercholesterolaemia, 31% had a positive history of coronary artery disease, 18% were current smokers, and 15% had type 2 diabetes mellitus.

The control group was matched for age, sex, and the presence of coronary artery disease. Hence the mean age, prevalence of coronary artery disease, and distribution of male and female subjects were identical to the case group. The distribution of cardiac risk factors (with the exclusion of hypercholesterolaemia) was similar in the control group and the case group. In the control group 64% had arterial hypertension, 63% had hypercholesterolaemia, 27% had a positive history of coronary artery disease, 25% were current smokers, and 11% had type 2 diabetes mellitus. Table 1 summarises the clinical characteristics of the case and control groups.

GENOTYPES

In the case group the allele frequency was 0.54 for the B allele and 0.46 for the b allele: 24 patients were homozygous for the B allele, 15 patients were homozygous for the b allele, and 61 patients were heterozygous. In the control group the allele frequency was 0.40 for the B allele and 0.60 for the b allele: 20 patients were homozygous for the B allele, 40 patients were homozygous for the b allele, and 40 patients were heterozygous. Thus the allele frequency of the B allele was 35% higher in the case group than in the control group. This was significant (allele frequency difference in the χ^2 analysis, $p = 0.001$; genotype differences in the ANOVA analysis, $p = 0.023$). Table 2 summarises allele and genotype frequencies of the two groups.

Discussion

Aortic valve stenosis caused by degenerative calcification of the valvar structures has become the most common valvar pathomorphology requiring cardiac surgery.[1][2] Clinical factors associated with atherosclerosis are also associated with calcification of the aortic valve, but 50% of patients with severe aortic stenosis do not have coronary artery disease, and there is evidence that calcific aortic stenosis is not simply a consequence of aging.[3][4] Other factors must be important for the pathogenesis of this disease. This case–control study showed an association between a polymorphism of the vitamin D receptor gene and the prevalence of calcific aortic stenosis: there was a significant association between the B allele of the vitamin D receptor and calcific aortic stenosis; and the B allele was more frequent in case patients than in controls.

The contribution of vitamin D receptor polymorphism to bone metabolism has often been evaluated during the last decade. Vitamin D receptor polymorphism seems to predict bone density or bone mineral mass. Bone mineral mass is less in individuals with the B allele than in those with the b allele.[6–9] Furthermore, people with the B allele have more rapid bone loss with advancing age,[7] whereas those with the b allele have greater calcium absorption[11] and a better correlation between 1,25 dihydroxyvitamin D concentrations and the ^{45}Ca absorption index.[12] In addition to the lower bone mineral density, more rapid bone loss, and blunted calcium absorption, raised parathormone concentrations have been found in subjects with the BB genotype.[13] However, other studies have produced conflicting data. While one study detected an influence of the vitamin D receptor genotype on bone metabolism only in the presence of a certain genotype of the oestrogen receptor,[14] another found an inverse correlation between

vitamin D receptor genotypes and bone mineral density, indicating that vitamin D receptor polymorphism could also be a marker of linkage disequilibrium with another relevant gene.[15] Other studies were unable to detect any association between the vitamin D receptor genotypes and bone metabolism.[16–19] However, there is a high level of confidence in published reports that vitamin D receptor polymorphism is associated with bone mineral density.[20]

In this study we found an association between the B allele—which seems to predispose gene carriers to blunted calcium absorption, more rapid bone loss, reduced bone mineral density, and raised parathormone secretion—and the prevalence of calcific aortic valve stenosis. There might be several hypotheses to explain the relation between genotype and the development of aortic stenosis. Calcification of the aortic valve is believed to be a process taking years or decades.[1–4] Individuals with a slightly unfavourable bone mineral density might develop mechanisms to overcome this alteration of calcium homeostasis. Like parathormone, other hormones, proteins, or second messengers might trigger calcification of extraosseous structures like the aortic valve. Another possible explanation is the hypothesis that vitamin D receptor polymorphism is merely a marker of linkage disequilibrium with another gene involved in calcium metabolism; this as yet unknown gene might be important for osseous and extraosseous calcification. The aortic valve is likely to be one of the first extraosseous structures involved because of the high level of mechanical stress to which it is subjected.

We are aware that no firm pathophysiological conclusions can be drawn from a genetic association study. Nevertheless an interaction between genes controlling calcium metabolism or calcification and the development of calcific aortic stenosis is intriguing and requires further study.

LIMITATIONS

In the light of recently suggested criteria that should be met by a high quality association study,[21] this study had certain limitations. It is proposed that association studies should have large sample sizes, small p values, and report associations that make biological sense and alleles that affect the gene product in a physiologically meaningful way. Furthermore, they should include an initial study with a subsequent independent replication, and the association should be observed both in family based and population based studies. The author of these recommendations admitted that most studies do not meet all these criteria.[21] Our study included a relatively small number of case and control patients, a limitation which is mitigated by the fact that case and control patients were carefully phenotyped and matched. Furthermore, the results of our study have not yet been analysed in a second replication study. Thus it is desirable that our results should be confirmed in a larger study in the near future. The difference in the allele frequencies between the case and control

groups reported in this study was high at 0.14, and is similar to differences of alleles reported in other genetic association studies.[22–24] Moreover the reported association makes biological sense and alleles affect the phenotype in a physiologically meaningful way.

CONCLUSIONS

The vitamin D receptor genotype is associated with the prevalence of calcific aortic stenosis. This is the first report on a genetic association with aortic valve stenosis. Further larger studies are required to prove the influence of the vitamin D receptor gene on the development of calcific aortic stenosis.

1 Passik CS, Ackermann DM, Pluth JR, *et al*. Temporal changes in the causes of aortic stenosis: a surgical pathological study of 646 cases. *Mayo Clin Proc* 1987;**62**:119–23.
2 Carabello BA, Crawford FA. Valvular heart disease. *N Engl J Med* 1997;**337**:32–41.
3 Otto CM, Kuusisto J, Reichenbach DD, *et al*. Characterization of the early lesion of "degenerative" valvular aortic stenosis: histologic and immunohistochemical studies. *Circulation* 1994; **90**:844–53.
4 Stewart BF, Siscovick D, Lind BK, *et al*. Clinical factors associated with calcific aortic valve disease. *Am J Cardiol* 1997; **29**:630–4.
5 Morrison NA, Yeoman R, Kelly PJ, *et al*. Contribution of transacting factor alleles to normal physiological variability: vitamin D receptor polymorphisms and circulating osteocalcin. *Proc Natl Acad Sci USA* 1992;**89**:6665–9.
6 Morrison NA, Qi JC, Tokita A, *et al*. Prediction of bone density from vitamin D receptor alleles. *Nature* 1994; **367**: 284–7.
7 Ferrari S, Rizzoli R, Chevalley T, *et al*. Vitamin-D-receptor gene polymorphisms and change in lumbar-spine bone mineral density. *Lancet* 1995;**345**:423–4.
8 Sigurdsson G, Magnusdottir DN, Kristinsson JO, *et al*. Association of BsmI vitamin-D receptor gene polymorphism with combined bone mass in spine and proximal femur in Icelandic women. *J Intern Med* 1997;**241**:501–5.
9 Karkoszka H, Chudek J, Strzelczyk P, *et al*. Does the vitamin D receptor genotype predict bone mineral loss in haemodialysed patients? *Nephrol Dial Transplant* 1998;**13**: 2077–80.
10 Gorlin R, Gorling SG. Hydraulic formula for calculation of the area of the stenotic mitral valve, other cardiac valves, and central circulatory shunts. *Am Heart J* 1951;**41**:1.
11 Kiel DP, Myers RH, Cupples LA, *et al*. The BsmT vitamin D receptor polymorphism (bb) influences the effect of calcium intake on bone mineral density. *J Bone Miner Res* 1997;**12**:1049–57.
12 Dawson-Hughes B, Harris SS, Finneran S. Calcium absorption on high and low calcium intakes in relation to vitamin D receptor genotype. *J Clin Endocrinol Metab* 1995;**80**:3657–61.
13 Ferraris S, Manen D, Bonjour JP, *et al*. Bone mineral mass and calcium and phosphate metabolism in young men: relationship with vitamin D receptor allelic polymorphisms. *J Clin Endocrinol Metab* 1999;**84**:2043–8.
14 Willing M, Sowers M, Aron D, *et al*. Bone mineral density and its change in white women: estrogen and vitamin D receptor genotypes and their interaction. *J Bone Miner Res* 1998;**13**:695–705.
15 Houston LA, Grant SF, Reid DM, *et al*. Vitamin D receptor polymorphism, bone mineral density, and osteoporotic vertebral fracture: studies in a UK population. *Bone* 1996;**18**: 249–52.
16 Garnero P, Borel O, Sornay-Rendu E, *et al*. Vitamin D receptor gene polymorphisms are not related to bone turnover, rate of bone loss, and bone mass in postmenopausal women: the OFELY study. *J Bone Miner Res* 1996;**11**:827–34.
17 Ross Eccleshall T, Garnero P, Gross C, *et al*. Lack of correlation between start codon polymorphism of the vitamin D receptor gene and bone mineral density in premenopausal French women: the OFELY study. *J Bone Miner Res* 1998; **13**:31–5.
18 Kung AWC, Yeung SSC, Lau KS. Vitamin D receptor gene polymorphisms and peak bone mass in southern Chinese women. *Bone* 1998;**22**:389–93.
19 Hansen TS, Abrahamsen B, Henriksen FL, *et al*. Vitamin D receptor alleles do not predict bone mineral density or bone loss in Danish perimenopausal women. *Bone* 1998;**22**:571–5.

20 Gong G, Stern HS, Cheng SC, *et al*. The association of bone mineral density with vitamin D receptor gene polymorphisms. *Osteoporos Int* 1999;**9**:55–64.
21 Anon. Freely association [editorial]. *Nat Genet* 1999;**22**:1–2.
22 Siffert W, Rosskopf D, Siffert G, *et al*. Association of human G-protein β3 subunit variant with hypertension. *Nat Gen* 1998;**18**:45–48.

23 Iacoviello L, Di Castelnuovo A, De Knijff P, *et al*. Polymorphisms in the coagulation factor VII gene and the risk of myocardial infarction. *N Engl J Med* 1998;**338**:79–85.
24 Hubacek JA, Pit'ha J, Skodova Z, *et al*. C(-260)→T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. *Circulation* 1999;**99**:3218–20.

IMAGES IN CARDIOLOGY

Magnetic resonance of vertebral steal syndrome

A 63 year old man presented with exertional dyspnoea and fatigue. Twelve years previously he had undergone quadruple coronary artery bypass surgery. On clinical examination there were no palpable pulses in the left arm. Cardiac catheterisation performed via the right femoral artery revealed occluded native vessels and three patent saphenous vein grafts. It was not possible to identify the left internal mammary artery (LIMA) graft as the left subclavian artery was occluded.

A gadolinium contrast enhanced magnetic resonance angiogram (MRA) was performed to identify the anatomy. Maximum intensity projection reformatting of the MRA demonstrated occlusion of the left subclavian artery (below, arrow). A phase contrast velocity map was performed after handgrip exercise, at the level of the vertebral arteries, to assess for the possibility of vertebral steal syndrome. The velocity encoded image (top right) acquired in systole, with cranial blood flow displayed as black and caudal flow white, demonstrated retrograde flow down the left vertebral artery during systole (white arrow) with normal cranial flow up the left internal carotid (black arrow) and right sided vessels. This was confirmed by the mean velocity profile over time for each artery (bottom right). Positive velocity indicates cranial flow in the right internal carotid artery (RICA), the left internal
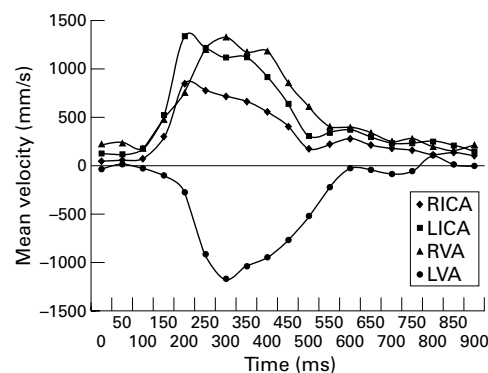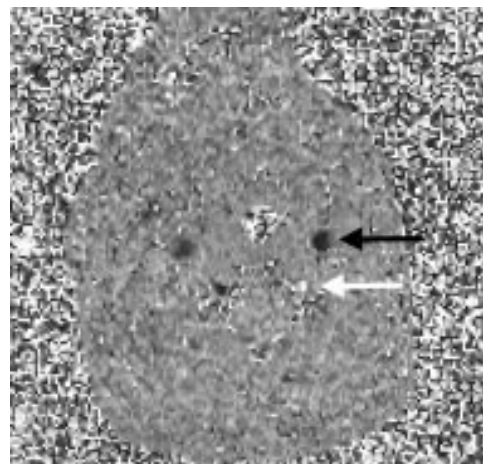
carotid artery (LICA) and the right vertebral artery (RVA). Negative velocity indicates caudal flow in the left vertebral artery (LVA). MRA also demonstrated patency of the LIMA graft (not shown).

An exercise myocardial SPECT perfusion scan documented no reversible myocardial ischaemia and the patient is currently asymptomatic with medical management. Contrast enhanced MRA with velocity mapping is a robust minimally invasive technique that should be used when subclavian artery steal syndrome is suspected.

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