

Genetic segregation analysis of familial mitral valve prolapse shows no linkage to fibrillar collagen genes

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SUMMARY Three pedigrees were identified in which mitral valve prolapse seemed to be inherited as a mendelian autosomal dominant trait. The segregation of the genes encoding the major fibrillar collagens present in valve tissue, collagens I and III, was analysed by use of restriction enzyme site variants as genetic markers. In one pedigree there was discordance between the segregation of the disease and markers for all three collagen genes. In another, there was discordance between the disease and markers for both collagen I loci. This is evidence against the disease being generally the result of mutations of the genes encoding the major fibrillar collagens.

Mitral valve prolapse is one of the commonest human cardiac abnormalities, with a prevalence of about 5%. However, it has also been recognised, through improvements in echocardiography, that there is considerable variation in mitral valve mobility in the healthy population. Nevertheless, mitral valve prolapse can be identified as a distinct entity by applying strict diagnostic criteria relating to the degree of redundancy of the valve and the presence of mitral regurgitation. Most affected individuals are symptom free but a proportion suffer from atypical chest pain. Infective endocarditis, spontaneous cordal rupture, progressive mitral regurgitation, and sudden death are rare complications. The earliest histological change in mitral valve prolapse is disruption of the pars fibrosa with fragmentation of collagen bundles and an associated myxomatous degeneration. Subsequent changes include thickening of the endocardial surfaces and dilatation of the mitral annulus, both consequences of the stretching and elongation process initiated by the primary structural degeneration. The subject has been well reviewed.¹⁻⁷

Two arguments have persuaded investigators that the primary change that initiates the pathological sequence might be a collagen abnormality. Firstly, collagen is the major stress bearing component of the pars fibrosa. Secondly, mitral valve prolapse is a complication of some inherited connective tissue disorders in which there is evidence of a systemic

collagen abnormality. These include the Marfan syndrome,^{8,9} Ehlers-Danlos syndrome,¹⁰ and osteogenesis imperfecta¹¹ though whether the prevalence of mitral valve prolapse is any higher in osteogenesis imperfecta than in the normal population has recently been questioned.¹² Mitral valve prolapse has also been associated with less well defined connective tissue disorders such as the joint hypermobility syndrome.¹³

Primary mitral valve prolapse, uncomplicated by other symptoms of connective tissue disease, can appear as an inherited trait. The familial nature was first detected by the finding of systolic clicks and murmurs in two or three generations of several pedigrees.^{14,15} As echocardiography improved the diagnostic sensitivity, the number of affected first degree relatives rose towards 50%—the value expected for an autosomal dominant trait with full penetrance.¹⁶⁻¹⁸ When, in some series, lower values were reported,¹⁹ this was attributed to technical differences in recognition or reduced penetrance. There was complete concordance in six pairs of monozygotic twins.^{15,20,21} Most series have noted that the expression is affected by sex; the incidence among male first degree relatives is about half that in females. There also seems to be an age dependent expression with a significant increase in adults compared with children. The net result of these studies is the recognition of primary mitral valve prolapse as an autosomal dominant trait with incomplete penetrance and variable expression.

As is often the case, precise defects in collagen have been difficult to pinpoint. The loose arrangement of

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collagen bundles seen in the pars fibrosa with broken and coiled fibres undoubtedly has mechanical consequences for the leaflets. Analytical studies have shown a higher rate of collagen synthesis in cultured affected valves than in controls but they did not identify a specific cause.²²

The predominant collagens in the mitral valve are collagens I and III, though there are also appreciable amounts of collagen V.²³ Fibrillar collagens are triple helical trimers that form stress resisting fibres via a system of intermolecular covalent bonds. Collagen I, the major species in the valve and chordae, has two distinct subunits, called the alpha-1 and alpha-2 chains, each molecule being a heterotrimer of two alpha-1 and an alpha-2 chain. Collagen III, on the other hand, is a homotrimer of three identical chains. The collagen I subunits are encoded at the COL1A1 (alpha-1 chains) and COL1A2 (alpha-2 chains) structural genes that in human beings have been localised to the non-syntenic loci on 17q and 7q respectively. The collagen III subunit is encoded at COL3A1 on 2q.

For the following reasons we set out to analyse the segregation of these three loci in pedigrees of dominantly inherited mitral valve prolapse. Firstly, if the primary defect in primary mitral valve prolapse is indeed a collagen defect then one possibility is that it is caused by a mutation in one of the structural genes. Mutations in these genes are known to cause some, but not all, of the inherited connective tissue disorders with which mitral valve prolapse can be associated. Secondly, although several other genes direct collagen synthesis, notably those encoding the

eight enzymes that modify the protein after translation, the dominant inheritance of mitral valve prolapse suggests that the defect is likely to be in a structural protein rather than an enzyme. Enzyme deficiencies are usually inherited as recessive traits. Thirdly, segregation analysis is a rigorous method for testing candidate genes where, as in the collagen genes, very close markers are available, because any examples of discordance between the candidate gene and the disease gene in a pedigree immediately excludes it as the mutant locus.

Patients and methods

Three pedigrees were ascertained through proposita attending cardiac clinics in London and Oxford.

ASSIGNMENT OF PHENOTYPE

Patients and their relatives were examined clinically and by auscultation (table 1). We took special care to examine for signs of mild systemic connective tissue disease such as joint laxity, skin hyperextensibility, increased span/height ratios, and unusual scleral hue. None was found. Mitral valve prolapse was detected or confirmed by both M mode and cross sectional echocardiography by a Hewlett Packard phased array ultrasound system with 3.5 MHz transducer. M mode echocardiography was recorded with the transducer placed perpendicular to the chest wall. The beam was aimed towards the atrioventricular groove or proximal free wall of the left atrium from the parasternal window while both leaflets of the mitral valve were imaged simultaneously. Cross sectional

Table 1 Phenotype of affected family members

| Pedigree | Clinical | | | | Auscultation | | Other |
|-------------|----------|-------------|----------|---------------------|----------------|-----------------|-----------------------|
| | Age | Palpitation | Dyspnoea | Atypical chest pain | Systolic click | Systolic murmur | |
| Pedigree 1: | | | | | | | |
| I.2 | 65 | 1 | 1 | 0 | 1 | 1 | Mitral valve replaced |
| II.2 | 39 | 1 | 1 | 1 | 0 | 2 | |
| II.4 | 38 | 1 | 0 | 0 | 1 | 1 | |
| Pedigree 2: | | | | | | | |
| II.5 | 48 | 1 | 0 | 0 | 2 | 0 | |
| III.2 | 27 | 0 | 0 | 0 | 0 | 1 | |
| III.3 | 26 | 0 | 0 | 0 | 1 | 1 | |
| III.5 | 18 | 1 | 0 | 1 | 1 | 1 | |
| III.6 | 16 | 0 | 0 | 0 | 2 | 0 | |
| Pedigree 3: | | | | | | | |
| II.1 | 53 | 1 | 1 | 1 | 1 | 0 | |
| II.5 | 44 | 0 | 0 | 0 | 1 | 0 | |
| III.2 | 29 | 0 | 0 | 1 | 1 | 1 | |
| III.3 | 27 | 0 | 0 | 0 | 1 | 0 | |
| III.5 | 20 | 0 | 0 | 0 | 2 | 0 | |
| III.7 | 16 | 0 | 0 | 0 | 1 | 0 | |
| III.8 | 12 | 0 | 0 | 0 | 2 | 0 | |
| IV.2 | 4 | 0 | 0 | 0 | 1 | 0 | |

0 = feature absent; 1 = feature present except for systolic click where 1 = single click, 2 = double click, and systolic murmur where 1 = late systolic, 2 = pansystolic. Mitral valve prolapse was confirmed by echocardiography in all family members scored as affected.

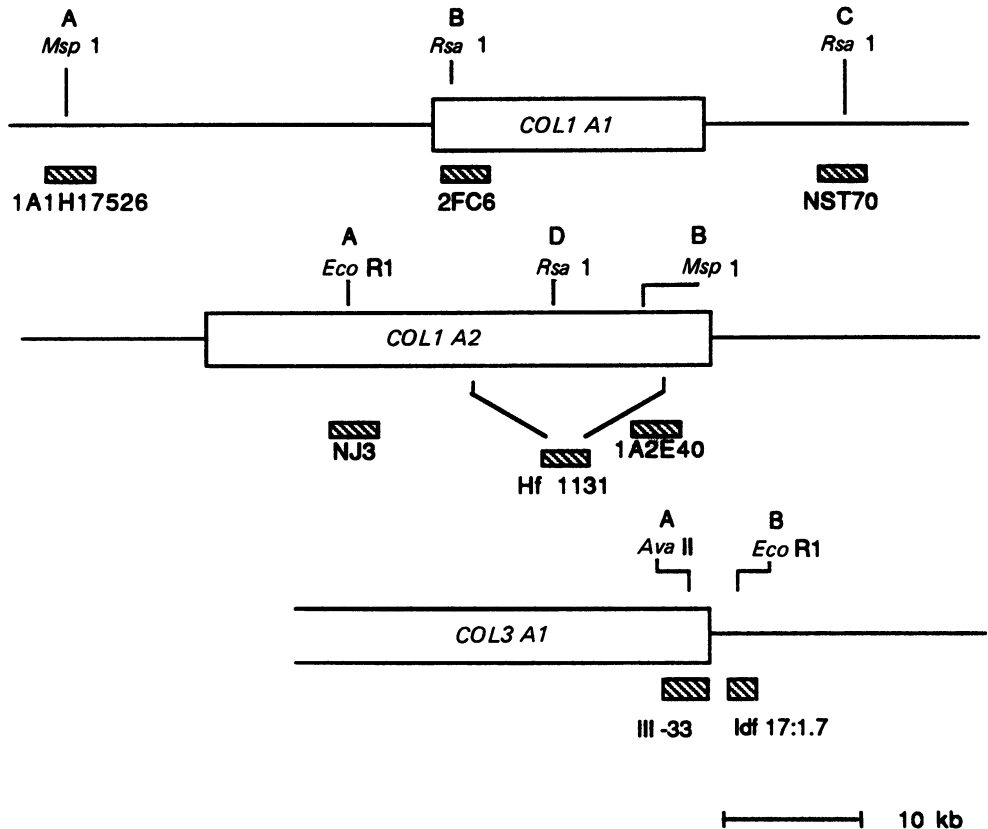


Fig 1 Map of the segregation marker systems at COL1A1, COL1A2, and COL3A1.

echocardiography recordings were made from the parasternal long axis and the apical four chamber views.

Pedigree 1

Propositus II.2—A 39 year old man with a known family history of mitral valve prolapse underwent emergency mitral valve replacement because of worsening congestive heart failure in the presence of a recent pansystolic murmur. Although he had had a heart murmur since birth he had been symptom free until a few years ago when he first experienced recurrent episodes of palpitation and mild chest pain unrelated to exercise. He made a good recovery.

Pedigree 2

Propositus III.5—A 19 year old man presented with a history of recurrent episodes of palpitation. The physical examination was entirely normal except for a late systolic click followed by a murmur heard over the mitral area. Echocardiographic examination showed considerable elongation of the anterior mitral valve leaflet and a mobile cystic mass that seemed to

be continuous with its tip. The findings in this case have been described elsewhere.²⁴

Pedigree 3

Propositus III.2—A 29 year old man presented with a history of atypical chest pain and palpitation. A late systolic click and murmur were heard over the apex. The physical examination was otherwise unremarkable. The echocardiographic examination showed similar appearances to those seen in pedigree 2.

Table 1 summarises the clinical, auscultatory, and echocardiographic findings in the affected family members. Relatives scored as unaffected were normal for all variables tested.

ASSIGNMENT OF GENOTYPE

DNA was prepared from peripheral blood taken into edetic acid. Restriction enzyme digestion and agarose electrophoresis blotting on to nitrocellulose filters followed our modifications of standard protocols.²⁵ Hybridisation probes (fig 1) were labelled with ³²P by either nick translation or random primer directed

Table 2 Haplotype notations at COL1A1, COL1A2, and COL3A1

| | | COL1A1 | | |
|-----------|-----------|---------------|---------------|--------------|
| Marker | | A | B | C |
| Enzyme | | <i>Msp</i> 1 | <i>Rsa</i> 1 | <i>Rsa</i> 1 |
| Probe | | 1A1H17526 | 2FC6 | NST70 |
| Reference | | 26 | 26 | 27 |
| | Haplotype | | | |
| | 1 | + | + | + |
| | 2 | + | + | - |
| | 3 | + | - | + |
| | 4 | + | - | - |
| | 5 | - | + | + |
| | 6 | - | + | - |
| | 7 | - | - | + |
| | 8 | - | - | - |
| | | COL1A2 | | |
| Marker | | A | B | D |
| Enzyme | | <i>Eco</i> R1 | <i>Msp</i> 1 | <i>Rsa</i> 1 |
| Probe | | NJ3 | 1A2E40 | Hf1131 |
| Reference | | 28 | 29 | 30 |
| | 1 | + | + | + |
| | 2 | + | + | - |
| | 3 | + | - | + |
| | 4 | + | - | - |
| | 5 | - | + | - |
| | 6 | - | + | + |
| | 7 | - | - | + |
| | 8 | - | - | - |
| | | COL3A1 | | |
| Marker | | A | B | |
| Enzyme | | <i>Ava</i> II | <i>Eco</i> R1 | |
| Probe | | pIII-33 | IdF17:1.7 | |
| Reference | | 31 | 32 | |
| | 1 | + | + | |
| | 2 | + | - | |
| | 3 | - | + | |
| | 4 | - | - | |

synthesis. Hybridisations were carried out overnight at 42°C in a mixture containing 50% v/v formamide, 5% w/v dextran sulphate, and 200 µg/ml heparin after prehybridisation of the filter overnight with the same mixture without dextran sulphate.

Three restriction site dimorphisms at both COL1A1,^{26 27} COL1A2²⁸⁻³⁰ and two at COL3A1^{31 32} were used as genetic linkage markers. Figure 1 shows the positions of the markers on the physical gene maps. Use of these three markers distinguished eight haplotypes at both COL1A1 and COL1A2 with four possible combinations allowed at COL3A1. Table 2 shows the notations of markers at the three loci. Where it was relevant to the analysis we confirmed paternity or maternity by means of the minisatellite probe 15.1.11.4.³³

Results

Figure 2 shows the genotypes at the three loci and table 3 summarises the results. Discordance between markers and mitral valve prolapse was seen in the pedigrees as follows.

Pedigree 1

COL1A2—Both II.2 and II.4 have inherited the gene for mitral valve prolapse from their mother I.2 but different COL1A2 alleles.

COL1A1 and COL3A1 markers at both loci were both non-informative because I.2 was homozygous in each case so the alleles could not be distinguished.

Pedigree 2

COL1A1—III.2 and III.3 both inherited the gene for mitral valve prolapse from their mother II.5 but different COL1A1 alleles.

COL1A2—Both II.4 and III.2 are double heterozygotes for COL1A2 markers. This means that they could have one of two possible genotypes 1-4 or 2-3 though examination of the pedigree shows that both individuals must have the same genotype. Consider the case where II.4 and III.2 have genotype 1-4 at COL1A2. In this case, III.2 must have inherited allele 4 from her father and allele 1 from her mother. However, III.3 must have inherited allele 1 from her father and allele 2 from her mother. So the two daughters have received different COL1A2 alleles from their mother but both have inherited the gene for mitral valve prolapse. Much the same argument applies if both II.4 and III.2 have genotypes 2-3. In this case II.5 has passed allele 2 to her daughter III.2 and allele 1 to III.3 but the gene for mitral valve prolapse to both.

COL3A1—II.5 is homozygous for COL3A1 markers so the pedigree is not informative.

Individual II.1, who was not examined, was reported to have been turned down for army selection because of a heart murmur. This raises the possibility that the gene for mitral valve prolapse is present in his sibs, one of whom is II.4, the father of the affected individuals in generation III. II.4 was thoroughly examined and no signs of mitral valve prolapse were seen by careful auscultation or echocardiography.

Pedigree 3

COL1A1—III.5 carries the gene for mitral valve prolapse but has no COL1A1 allele in common with either his two brothers III.2 and III.3 or his niece IV.2, all of whom also carry the gene for mitral valve prolapse.

COL1A2—III.2 and III.3 are both affected but have no COL1A2 alleles in common.

COL3A1—Consider III.7 and III.8. Both are affected but have inherited different COL3A1 alleles from their father.

Discussion

Several factors complicate linkage analysis in disorders such as mitral valve prolapse. First, it is a

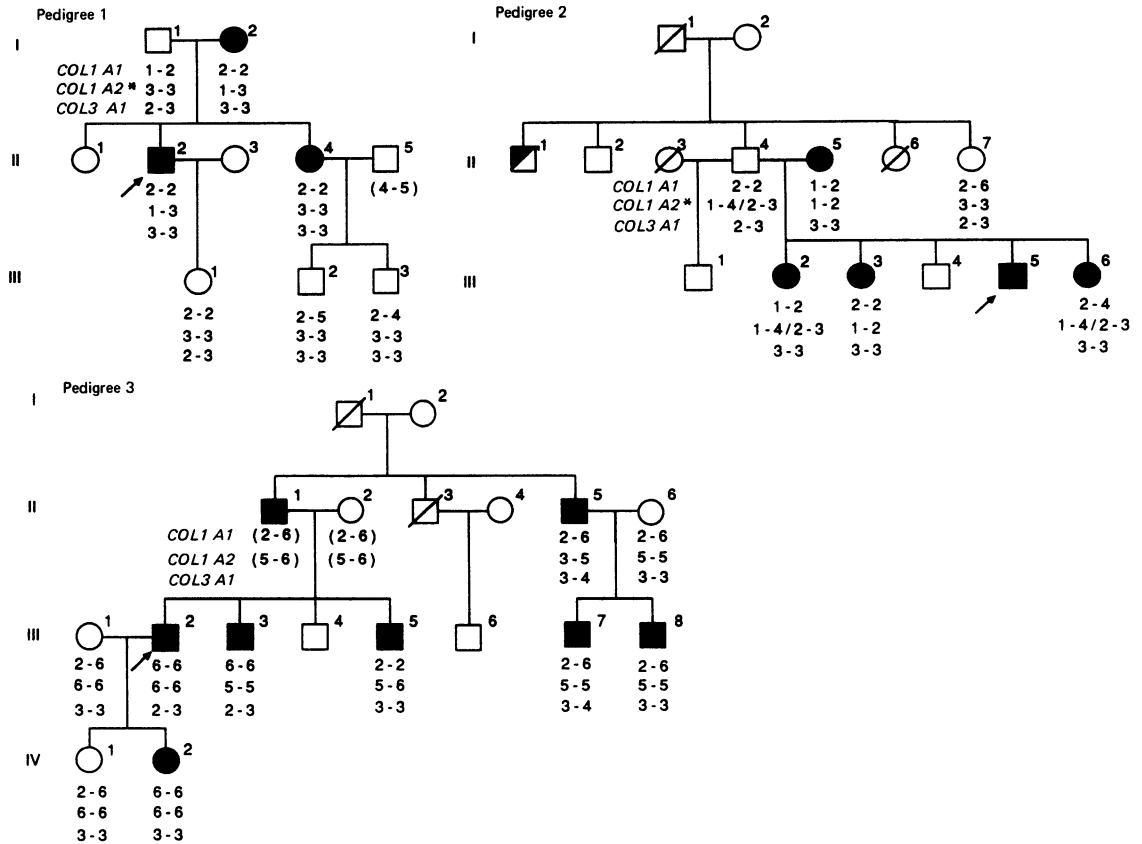


Fig 2 Three pedigrees showing mitral valve prolapse with collagen genotypes are shown in table 1. Black symbols are individuals scored as affected, white symbols are individuals who did not show symptoms of mitral valve prolapse. Propositi are indicated by arrows. The partly shaded symbol indicates an individual II.1 in pedigree 2 who was not examined but was turned down by the army because of a "heart murmur". In pedigrees 2 and 3, the COL1A2* haplotypes are incomplete and are as follows: 1 = A + B +; 2 = A + B -; 3 = A - B +; 4 = A - B -. Deduced genotypes are shown in parentheses.

common disorder so that sibships may receive mitral valve prolapse genes from both parents. Second, the penetrance is incomplete. This makes analysis based on unaffected individuals more uncertain than in fully penetrant disorders because there is a high probability that they may have inherited the gene

without its expression being detected in the phenotype despite very careful examination. Third, it is not clear that mitral valve prolapse is always inherited so that there is a possibility that individuals in a pedigree scored as affected may not have received the mutant gene but are phenocopies for an unrelated reason.

Bearing these important considerations in mind we showed that neither of the two genes encoding the subunits of collagen I segregated with the mitral valve prolapse phenotype in two pedigrees in which the disorder appeared to be behaving as an autosomal dominant mendelian trait. In a third pedigree we excluded one of the genes, COL1A2, but not the other. Largely because of a lack of useful markers at COL3A1, we have only been able to analyse the segregation of this locus in one pedigree and this analysis showed discordance.

Table 3 Segregation of COL1A1, COL1A2, and COL3A1 in three pedigrees showing mitral valve prolapse

| Pedigree | Locus | | |
|----------|--------|--------|--------|
| | COL1A1 | COL1A2 | COL3A1 |
| | NI | DS | NI |
| | DS | DS | DS |
| | DS | DS | DS |

NI, non-informative; DS, discordant.

There are several reasons why we are cautious about interpretation of these results, but we can discount the probability of recombination between the markers at the loci and the physical limits of the gene. The maximum distance between markers is 50 kb which, barring unusually high recombination rates at the loci, translates to a crossover frequency of about 0.0005 per meiosis. This is insignificant compared with the other potential sources of error.

This study can only be regarded as preliminary and needs to be extended to larger pedigrees in which the segregation of the disease as an autosomal dominant trait is clearer and discordance can be demonstrated in several meioses. Nevertheless, this study suggests that the primary gene locus for mitral valve prolapse is not linked to either of the two collagen I loci nor, probably, to the collagen III locus. If this is the case then mutations in these genes cannot be the cause of the disease.

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