Marfan syndrome in South Africa – a molecular genetic approach to diagnosis

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To the Editor: Marfan syndrome (MFS) (MIM 154700) is a heritable disorder of connective tissue characterised by excessive height and limb length disproportion, together with ocular, cardiovascular and skeletal manifestations. The condition is an autosomal dominant trait, and can be transmitted from generation to generation with variable severity. The determinant gene FBN1 is situated at the chromosomal locus 15q21.1 and codes for the protein fibrillin-1.[1]

Since 1972 more than 160 persons with a provisional diagnosis of MFS have been referred to the genetic clinic at Groote Schuur Hospital, University of Cape Town. The condition is present in all population groups and there is no obvious predilection for any particular community.[2] The frequency of MFS in the USA is quoted as 1 in 5 000. If this figure is extrapolated to South Africa, it can be estimated that there could be 10 000 affected persons in this country.

Genetic counselling and appropriate medical management of MFS are dependent on diagnostic precision, which can be a difficult matter. Indeed, many persons with a marfanoid habitus have the less severe MVP (mitral valve prolapse)[3] or MASS (mitral valve, aortic root dilation, skeletal, skin) syndromes.[4] Moreover, it is uncertain whether or not the accepted diagnostic criteria for MFS are applicable to all populations in South Africa. Molecular investigations would go a long way towards resolving these problems, but in terms of local health priorities and economic constraints, it is not possible to offer routine molecular diagnostic laboratory services. In a pilot study to address this issue we investigated DNA specimens from 9 South Africans with a clinical diagnosis of possible MFS. In order to confirm the validity of the molecular screening programme in the local context, some of these persons met the full diagnostic criteria for MFS, while others represented the MVP-MASS spectrum.

Methods

Mutation analysis was undertaken at St George's Hospital Medical School, London, on DNA specimens from 9 South Africans with a provisional diagnosis of MFS, using conventional techniques.[5] The polymerase chain reaction (PCR), denaturing high-performance liquid chromatography (dHPLC) analysis and direct sequencing were carried out in order to perform mutation screening involving 65 exons in the FBN1 gene.

Results

Both positive and negative results were obtained; these have important implications for the affected South Africans and their families. Brief case reports are provided in order to give perspective on the practical issues involved, to demonstrate the diagnostic value of this test, and to explore the feasibility of molecular testing for MFS in South Africa.

Case reports

Patient 1

Patient 1 was a member of a large South African family of Indian stock. Clinically, she had severe manifestations of MFS – she was tall, with limb length disproportion and marked arachnodactyly. Her eyes were normal. Dilatation of the root of the aorta and incompetence of the aortic valve led to her final hospital admission. She died suddenly in 1989 at the age of 42 years, following cardiac surgery. Her eldest son was found to have the characteristic Marfanoid habitus, and a slightly increased aortic root diameter of 3.9 cm (upper normal limit 3.8 cm) at the age of 20 years; a total of 12 family members in 3 generations had been previously examined clinically and deemed to be affected.

A heterozygote substitution of nucleotide 7713 from T to A in exon 62 of the FBN1 gene was identified. This previously unreported mutation introduces a premature termination codon in place of a cysteine (C2571X) and it could lead to a truncated protein encoded by the gene, with disruptive consequences for the protein structure.

The family members, who are widely dispersed throughout South Africa, will be contacted and genetic counselling will be offered. The cascade screening technique will be employed and close co-operation with cardiological colleagues will be maintained. Thereafter, following this feasibility study, diagnostic confirmation will be undertaken using a molecular probe for the specific mutation, using buccal cheek swabs, sputum, or DNA obtained from venous blood specimens.

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Patient 2

The second patient, a boy of mixed Cape ancestry, was fostered at an early age and no family history was available. He had a marfanoid habitus, bilateral dislocated lenses and mitral valve prolapse. DNA investigations revealed a heterozygous substitution of nucleotide 6749 from A to G in exon 55 which is predicted to affect the cbEGF-like domain number 35 of the fibrillin-1 protein. This previously unreported mutation affects one of the amino acids that are invariable in this type of domain throughout the protein (E2250G). The finding is consistent with a causative mutation that could result in disruption of the fibrillin-1 protein structure.

The mutation confirms the diagnosis of MFS, and provides a basis for rational management and for prenatal or postnatal diagnosis of any of his future offspring who have a 50:50 chance of inheriting the mutation.

Patient 3

This boy of mixed Cape ancestry, born in 2002, had severe manifestations of MFS including classic skeletal features, gross myopia, and mitral regurgitation that necessitated repair by the age of 3 years. Joint contractures present at birth improved with time and had resolved at the age of 3. The differential diagnosis was either severe neonatal MFS, or Beals syndrome.

A mutation found in exon 32 consisted of a heterozygote substitution from G to T at position –1 in the flanking region of exon 32, occurring at a site classically involved in exon splicing. This mutation has not been identified previously, and it is believed to be causative since it could produce an altered protein by changing the reading frame and resulting in a truncated protein. This finding confirms the diagnosis of MFS and facilitates prognostication and management decisions.

Patients 4 - 9

In one patient, DNA extracted from a histological specimen in a paraffin block from a deceased relative proved to be technically inadequate. No mutation in the fibrillin-1 gene was detected in the DNA of 5 other patients. One of these individuals had the classical features of MFS and a positive family history; further molecular studies will be undertaken. The remaining 4 all had the MVP or MASS phenotype rather than the classic MFS; the negative results provided further reassurance concerning the long-term prognosis and appropriate medical management.

Discussion

In the MFS screening clinic at St George's Hospital, London, only 4 of 10 referred patients actually have MFS. The others have an uncomplicated non-syndromic marfanoid habitus, or 'overlap' conditions in the MVP/MASS spectrum. A very small minority have specific genetic disorders such as the Beals syndrome (congenital contractural arachnodactyly (MIM 121050)) or the Shprintzen-Goldberg syndrome (MIM 182212).

The experience at the Genetic Clinic at Groote Schuur Hospital, Cape Town is very similar.[2]

A firm diagnosis of MFS has important implications for management and prognosis. These can include lifelong antihypertensive therapy to reduce the force of the cardiac impulse, and regular surveillance of the aortic root, heart, eyes and skeleton. Major surgery may be necessary for aortic or valvular incompetence, and there is a significant risk of potentially lethal dissection of the aorta in adulthood. Genetically, an affected person has an even chance of transmitting the disorder to each of his or her offspring.

In contrast to MFS, the marfanoid habitus-MASS-MVP group of conditions are comparatively benign, and compatible with a normal lifestyle. It therefore follows that diagnostic precision is crucial and that both positive and negative molecular findings are of considerable benefit to affected persons and their families.

In Europe, a full-scale genetic and molecular service for individuals and families with MFS is now available.[6] The cost of the initial mutation analysis for each affected individual is approximately R10 000 in South African currency. Once the specific mutation has been identified, focused testing in other family members is substantially cheaper (R1 500). Tracing family members and providing appropriate genetic counselling and medical management can be a major undertaking; in Europe these activities are carried out by networked medical genetic facilities, and all costs are met from national governmental resources. In view of the serious complications that can occur in MFS this expensive service is cost-effective.

In South Africa it is predicted that there are more than 10 000 people with MFS, and many more with the marfanoid habitus-MVP-MASS spectrum of disorders. These individuals and their families would benefit from molecular screening and as demonstrated in this pilot project, this approach is feasible. The technology is available or could be developed in this country and the existing personnel and infrastructure could be expanded in order to deliver a molecular testing service of this type. In the first instance, cascade screening, genetic counselling and molecular testing will be undertaken in the large South African family in which a unique mutation has been detected.

The fact that the MFS gene is large and that most affected families are unique with regard to their own mutation has been used as an argument against routine molecular genetic screening. It has been suggested that simple techniques such as clinical examination, ophthalmological assessment and echocardiography would be adequate for the diagnostic categorisation of potentially affected individuals in a family with MFS. This approach has largely been superseded by molecular technology in Europe and the USA, but it may still be appropriate in a developing country such as South Africa. It is relevant, however, that screening of this type would

cost about R1 500 and would have to be repeated at regular intervals. Conversely, molecular diagnosis, with an initial cost of about R10 000 would be definitive in a family where the mutation was detected, and would not need to be repeated in the affected persons. In this way savings would be made since annual echocardiograms would not be necessary for those shown to be unaffected.

In the final analysis, the diagnostic screening procedures undertaken will probably depend on personal and national priorities for the allocation of available resources.

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