

Reply to "The question of heterogeneity in Marfan syndrome"

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In Reply – Dietz and colleagues raise several issues in relation to our article on the assignment of a second locus for MFS: the legitimacy of the MFS diagnosis the reliability of the clinical investigations and hence the validity of our linkage analyses, and so question our conclusion of genetic heterogeneity in MFS.

The diagnosis of MFS was recognized in our family repeatedly and independently by several clinicians before 1986, when the « Berlin nosology[2] » was proposed. After 1986, this diagnosis remained unchanged, notably in subject III-38, when using these recognized criteria. This patient died of an acute aortic dissection and his necropsy showed skeletal abnormalities (height 186 cm, arachnodactyly, pectus excavatum), a past history of surgery for inguinal hernia, and dilation of the ascending aorta[4]. Contrary to the authors'claim, there is no reference in the « Berlin nosology » to the lack of diagnostic significance of a specific physical feature that is not observed in the rest of the family. Eleven other family members (III-4, III-32, III-36, III-37, III-38, III-40, III-42, IV-6, IV-10, IV-49, IV-51) are also considered affected using the same criteria. Furthermore, it cannot be ignored that IV-6 died suddenly at nine years of age after complaining of chest pains, III-32, III-38 and III-42 died of ascertained aortic dissection (a major unquestionable diagnostic criteria for MFS) and III-49 was operated upon for aortic dissection (histologic studies, compatible with the diagnosis and performed in independent institutions from ours, are available for subjects III-32, III-38 and III-49). In addition II-3, II-7 and III-32 are obligate carriers. Because of the absence of ocular involvement, we were compelled to re-name the syndrome «Marfan-like» or even « a new connective-tissue disorder ». However, prominent figures in the field[10] (and the reviewers of our paper[1]) did not endorse this volte-face and considered MFS as the diagnosis, based on the original clinical features of the family published in 1993 [4].

In contrast, Dietz et al . disprove the diagnosis. This is their right. However, we cannot accept their biased reinterpretation of our data which is performed with a mind set on demonstrating the existence of two independent genetic traits in this family and in which each clinical feature is weighed one by one regardless of the overall clinical presentation of a given individual. Despite their claim that the normalization methods they used are « the same[3]» as comparable to those we reported, this is not the case. In effect, with their methods, stature is abnormal in only 11 subjects (versus 18 as compared to the French normal values (see ref. [4]) and the upper segment-to-lower segment ratio is aberrant in 11 patients, none of which have short limbed dwarfism.

Similarly, the criteria they used to standardize our aortic diameter measurements were obtained with a different technique applied to a different level of the aorta[6]. In effect, we used M-mode guided by two dimensional (2-D) echocardiography (and not 2-D echocardiography alone) and measured the aortic diameter always at the same level, namely when 2 aortic cusps were visualized[12] (and not the widest diameter of the sinuses of Valsalva). As reported by Roman et al [6], the M-mode diameter is smaller than the diameter measured at the same level by 2-D echocardiography (30.9 ± 4.2 vs 29.8 ± 3.9 ; $p < 0.05$) and of course smaller than the widest diameter of the aorta at the level termed «sinuses of Valsalva» by these authors (31.7 ± 3.9 vs 29.8 ± 3.9 ; $p < 0.01$)[6]. As a particularly striking example, individual III-52, who has an aortic M-Mode diameter of 39 mm ($>$ mean +2 SD with the proper normalization reported in 1993), has an aortic diameter of 48 mm when measured with 2-D echocardiography according to the Roman et al. [6] (also $>$ mean + 2 SD). Consequently, it is not surprising that Dietz et al , find aortic dilation in only 4 individuals. This is in sharp contrast to our data, derived from the appropriate normalizing procedure[12], which revealed aortic dilation in 13 family members. Finally, contrary to what Dietz and colleagues claim, this normalizing procedure[12] does take into account age as well as body surface area and is widely accepted.

Disregarding the manipulation of our published data, the dispute is clearly based upon the examination of the family at large, which lacks one of the major criteria for the diagnosis of MFS (such as eye involvement). But a family of this size is exceptional. We believe that the issue is not whether this is a genuine MFS but is: which diagnosis could unambiguously be made in similar patients in the absence of sufficient family data for linkage analysis or discriminating biological markers?

Our family has been under investigation for many years. Since several clinicians had independently evaluated family members (phenotypic status as given in ref. [5]), all members were re-assessed using more stringent criteria[4]. This led to a change in phenotypic status of some individuals as Dietz et al. correctly note.

Correct discrimination between affected and unaffected individuals is critical for linkage analysis. However, no absolute and definitive rule could be used in the family: the international criteria[2] were clearly inadequate since at least one member (III-49), operated upon because aortic dissection, would not have been recognized by these criteria. Consequently, we developed a pragmatic « case by case » approach[4], which in effect refers to the actual clinical follow-up of these patients. With this classification, we confirmed the exclusion data previously reported with the collagen genes[5], excluded linkage to the fibrillin genes[4], constructed an exclusion map, and established conclusive linkage with 3p markers[1]. In this family, an autosomal dominant disease gene segregates which can be mapped by exclusion mapping[13]. The most likely location is where the highest lod score is obtained. Our lod score is high and remains significant even if we take into account the large number of tests that were performed. Indeed, the 144 markers we studied correspond approximately to 70 independent tests. Since the probability that one of them reaches the 4.89 value is only 1 per 1,000 [14], linkage is established in this family. Furthermore, all the two point, lod scores were computed with complete penetrance. Changing the penetrance at that stage of the analysis would have substantially raised our highest lod score value but was not done. However, the haplotype analysis that was done in the family (after linkage was established) clearly showed that the penetrance was not complete with the clinical criteria we had used to classify family members. Therefore, it was solely to refine the map position of MFS2 with the LINKMAP program that we changed the value of this parameter since the misspecification of penetrance has a strong effect on θ [15].

Finally, the last issue raised by Dietz et al. is that of genetic heterogeneity. Our belief in genetic heterogeneity in MFS stems not only from the linkage data in our family, but also from published data showing that it has been impossible to identify mutations in the fibrillin gene in a number of patients and that in some individuals with « definite Marfan syndrome » no anomaly was observed in fibrillin metabolism. Although it is likely that normal fibrillin metabolism does not exclude the presence of an abnormal fibrillin associated with perturbation of microfibrillar structure and function, microfibrils contain not only fibrillin but also other components. Therefore, it is also possible that these MFS patients have abnormal microfibrillar structure or function due to anomalies carried by another component or by proteins associated with microfibrils. This interpretation has never been seriously considered and no effort has been undertaken to investigate fully the question of genetic heterogeneity despite knowledge, as early as 1991 (refs [16],[17]), that in the French family the mutation locus was not MFS1 (fibrillin). Consequently, it is not surprising that in a family in which fibrillin haplotypes did not segregate in all affected members, heterogeneity was not evoked. The absence of cosegregation was explained by the simultaneous existence of two different connective-tissue disorders (MFS and another still unknown)[18], a very unlikely situation except if one is willing to consider that connective-tissue disorders are frequent. More alarmingly, under the cover of homogeneity, haplotype analyses with fibrillin polymorphic markers were used for diagnosis in an at-risk individual, in a small family in which the presence of a fibrillin defect had not been conclusively demonstrated[18].

Indeed, genetic heterogeneity for MFS is a major issue to which our results make a significant contribution and which now requires a large objective and open-minded collaborative effort, in the interest of patients and their families.

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