Medical Genetics for Internists

Homocystinuria & Marfan Syndrome

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HOMOCYSTINURIA

INTRODUCTION

Many patients with inherited disease now live into adult life and are cared for by internists. Most of these inherited diseases are primarily in the realm of the pediatrician. However, there are several inherited diseases where the diagnosis is frequently first made on an adult medical service. I have selected two of these diseases to discuss. One is an inherited metabolic disease - homocystinuria and the other is an inherited dysmorphology of connective tissue - Marfan syndrome. I will emphasize the clinical aspects of these two diseases that share a similar phenotype, since these two enities should be familar to internists.

I will begin the discussion by focusing first on homcystinuria and describe two patients where the diagnosis was made on the internal medicine service.

CASE PRESENTATIONS

A 33-year-old photographer was admitted to University Hospital of Cleveland with the sudden onset of crushing precordial pain with radiation to the left arm and to the left mandible. The patient was diaphoretic and tachycardic with a pulse rate of 130. EKG demonstrated Q waves in I, AVL and the lateral chest leads with concave ST segment elevations. The CPK was 1,350 with 66% MB isozyme. Past medical history revealed recurrent episodes of thrombophlebitis since the age of 17 years. The patient also had a life long history of a personality disorder characterized by abusive behavior. Ten years prior to admission a spontaneous right femoral artery thrombosis occurred requiring illiac-popliteal bypass surgery. At the age of 25 years bilateral ocular surgery was carried out at New York Eye and Ear Infirmary.

Urinary homocystine was 480u Mole/day, plasma homocystine was 0.14 u Mole/ml, and plasma methionine was 0.32u Mole/ml. Skin fibroblast cultures were obtained and sent to S. Harvey Mudd at the N.I.H. for cystathionine synthase assay. The patient's physician requested that the patient be treated with 500mg of pyridoxine, and large doses of vitamin B_{12} and folic acid. The patient responded to this regimen with normalization of serum homocystine and methionine levels. Several months later the results of assays on fibroblast cultures indicated that the patient had a pyridoxine responsive form of cystathionine synthase deficiency and the B_{12} and folic acid supplements were discontinued.

A 30-year-old white housewife presented to University Hospital at Cleveland for evaluation of dyspnea, chronic cough and sputum production, lethargy, low back and knee pain. PMH included a diagnosis of asthma, mitral valve prolapse, DJD of knees, severe myopia and a Mafanoid habitus. At age 23, while on oral contraceptives, she was hospitalized at another hospital where pulmonary embolus was documented by V/Q scan and head CT demonstrated an old left occipitoparietal infarct.

PE revealed a tall, thin, anxious woman with a barking cough and stutter. BP 130/80, P 100 and irregular, RR 17. She had a malar flush. Slit lamp revealed downward ectopia lentis. ENT exam demonstrated friable turbinates. Chest was without a pectus abnormality. Cardiac exam was notable for an early systolic click and mid-systolic murmur. Back showed moderate scoliosis. Fingers had arachnodactyly but were not hypermobile and had a negative thumb sign. Arm span was four inches greater than height.

Labs revealed normal CBC and chemistries. EKG showed PACs and RVH. CXR and spine films revealed scoliosis, biconcave vertebral bodies and severe osteopenia. Echocardiogram demonstrated normal chamber size, a pansystolic MVP and a slightly sausage-shaped ascending aorta of normal dimension. PFTs revealed mild restriction values and a negative methacholine challenge. Vocal cords and sinus films were normal. Homocystinuria urine screen was positive and plasma homocystine was elevated [1.2mg/dl (normal 0)] as was methionine [6.8 mg/dl (normal 0.6-1.0)].

Treatment with pyridoxine, vitamin B_6 and folate and calcium was begun. She has been advised that pregnancy, oral contraceptives and surgical procedures place her at increased thomboembolic risk. One month after treatment plasma homocysteine was undectable.

OVERVIEW

Homocystinuria is caused by at least five different genetic defects (1). The classical and most common form of the disease is a recessively inherited deficiency of the enzyme cystathionine synthase that condenses homocysteine with serine to form cystathionine. There are two forms of cystathionine synthase deficiency one that responds to vitamin B_6 or pyridoxine. The other is a non-responder. Homocystinuria that responds to pyridoxine is a milder form of the disease and frequently escapes detection until adult life (2). We will examine the metabolically important methionine to homocysteine pathway or cycle later.

CLINICAL PHENOTYPE

Although there is variability in the clinical expression of classical homocystinuria in part due to genetic heterogenity of the disease the clinical phenotype is sufficiently characteristic that it should be recognized by internists. Four organ systems exhibit the major involvement in most patients. These are: 1) the eye, 2) the musculoskeletal system, 3) CNS, and 4) the vascular system. There is some evidence that the SH group of homocysteine may cause certain of the phenotypic effects. The feeding of methionine or homocysteine to experimental animals does not reproduce the phenotypic effects of homocystinuria (3,4). However, SH groups have been shown to affect the crosslinking of certain fibrillar proteins (1,5,6). In support of the importance of these sulfhydryl groups in causing connective tissue abnormalities is an experimental model in which penicillamine, a dimethyl cysteine, induces abnormalities in rats similar to that seen in homocystinuria (7). Let us examine in some details each of the phenotypic effects or abnormalities and determine how homocysteine may contribute to these.

A. Effects on the Eye: Table 1 describes the eye findings, the most frequent being dislocated or ectopic lens.

TABLE 1

HOMOCYSTINURIA - OCULAR FINDINGS

A. Very Frequent
1. **Ectopia Lentis**
2. **Iridodonesis**
3. **Myopia**

B. Less Frequent
1. **Glaucoma**
2. **Optic Atrophy**
3. **Retinal Degeneration**
4. **Retinal Detachment**
5. **Cataracts**

This abnormality may occur as early as 2 or 3 years of life in non-pyridoxine responsive patients (2). As shown in Figure 1, a time to event graph, a dislocated lens is present in 80% of pyridoxine-responsive patients by 20 years of life (1,2).

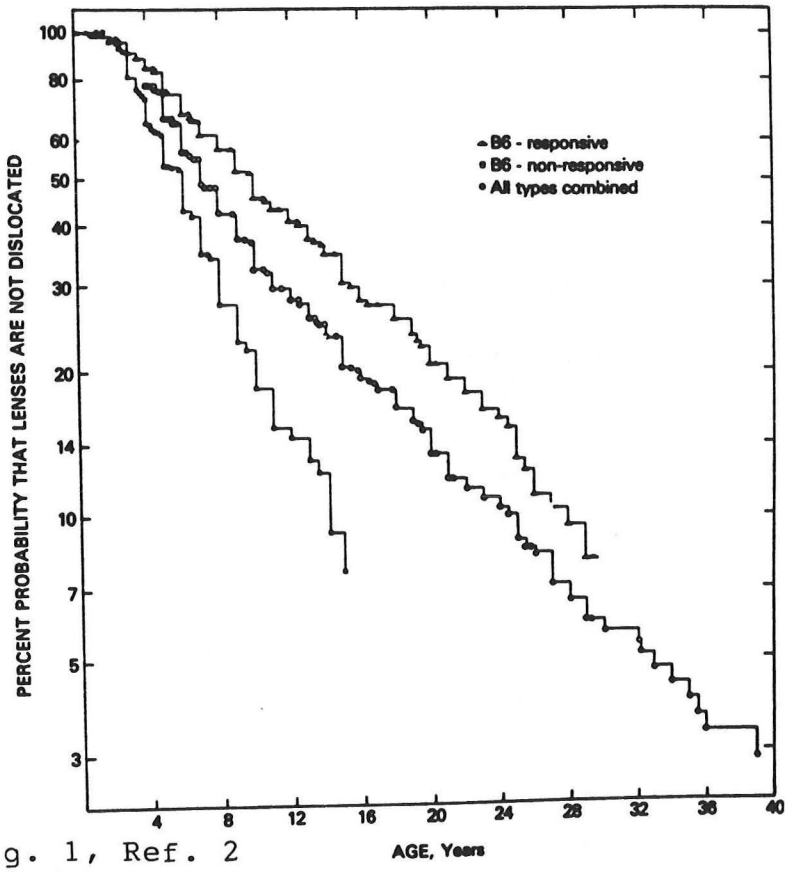

Fig. 1, Ref. 2

The lens normally is held in place in the eye by suspensory ligaments which are rich in cysteine residues and disulfide bonds. These disulfide bridges are important in maintaining the integrity of the fibers (8). Homocysteine is postulated to interfere with this cross-linking and to disrupt the fibers, leading to a dislocation of the lens downward (9). Ninety percent of individuals with both types of homocystinuria have the ectopic lens by the age of 20 (2). Ectopic lenses are also seen in other connective tissue disease, for example Marfan's syndrome where there is an abnormality in the structure of fibrous proteins. However, in this case the suspensory ligaments are not disrupted, but are merely stretched and the lens tends to dislocate in an upward direction. On physical examination one can detect dislocated lens by watching the iris as the eye moves rapidly. The iris will quiver because it is no longer supported by the lens and this quivering is called iridodonesis. With dislocation of the lens vision becomes myopic. If the dislocated lens is displaced anteriorly it can obstruct the pupil and produce acute glaucoma (10). These are the major eye findings in patients with homocystinuria.

B. Effect on Bone: Let us now examine the putative effects of elevated homocysteine on the development of the skeletal system. Many patients with homocystinuria have a marfanoid appearance with long thin extremities, hypermobile joints, and scoliosis (Table 2) (4,2).

TABLE 2

HOMOCYSTINURIA - SKELETAL FINDINGS

A. Very Frequent
   1. Osteoporosis
   2. Biconcave Vertebrae
   3. Scoliosis
   4. Dolichostenomelia (Long thin Extremities)
   5. Genu Valgum
   6. Pes Cavus

B. Less Frequent
   1. Arachnodactyly
   2. Pectus Excavatum or Carinatum

The skeletal abnormalities in homocystinuria are frequently distinguished from those of Marfan's syndrome by the presence of osteoporosis in homocystinuria. The osteoporosis is striking and hypomineralization of bone in a adolescent or young adult should alert the physician to the possible diagnosis of homocystinuria. As shown in Figure 2, 50% of patients with homocystinuria will have spinal osteoporosis by 20 years (2).

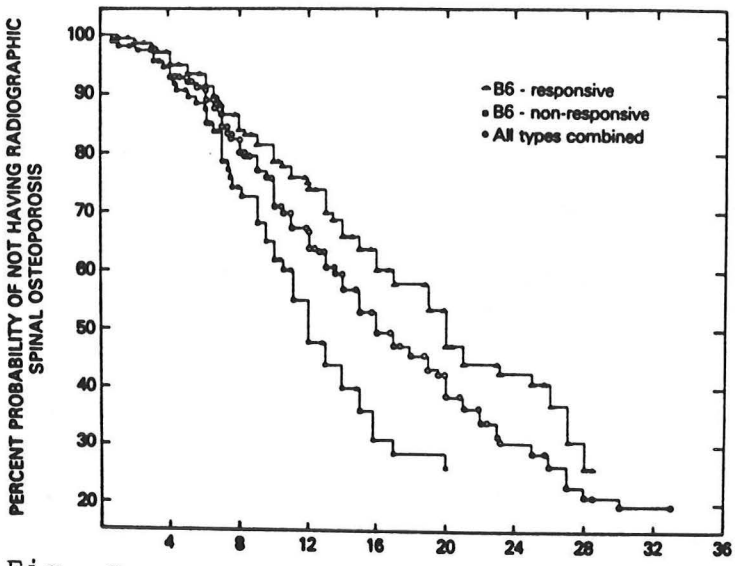

Fig. 2, Ref 2

Biconcave vertebrae, the so called cod fish vertebrae, are due to severe osteoporosis of the spine. The biconcavity of the vertebrae is caused by erosion of the nucleus pulposa into the body of the vertebrae (11,12). Homocystinuria apparently interferes with the production of osteoid perhaps by inhibiting the formation of disulfide bridges necessary to strengthen the osteoid. Osteoid deficiency causes reduced mineralization of the bone or osteoporosis. Scoliosis is also present, since the supporting ligaments of the spine are affected by the disease.

C. Central Nervous System: Mental retardation and developmental delay are the most frequent CNS findings (Table 3).

TABLE 3

HOMOCYSTINURIA - CNS FINDINGS

A. Frequent
   1. Mental Retardation
B. Less Frequent
   1. Psychiatric Disturbances
   2. Seizures
   3. Abnormal ECG
   4. Spasticity
   5. Focal Neurologic Signs

The incidence of these abnormalities depend on whether the homocystinuria is pyridoxine responsive and whether the disease is diagnosed early in life. In the untreated non-vitamin responsive form the IQ is in general less than in the

pyridoxine responders (2). However, when pyridoxine-responsive homocystinuria individuals' intelligence is compared to unaffected sibs 63 percent score significantly lower in standard tests.

Approximately 20 percent of untreated patients with cystathionine synthase deficiency have seizures (2). Focal findings, for example neurologic signs or EEG abnormalities are usually associated with vascular events, thromboembolism. In seven untreated homocystineine patients ages 10 to 30 extrapyramidal features and impaired proprioception were found on clinical examination (15). MR imaging showed focal areas of gliosis in the white matter of two of the patients. There were not abnormalities in the basal ganglia. Psychiatric abnormalities as observed in our first patient appear to be relatively common. A study of 63 patients with homocystinuria found clinically significant psychiatric illness in 51 percent (14). Personality disorders were most common (19 percent) with behavioral abnormalities (17 percent) a close second. Depression (10 percent) and obsessive compulsive behaviour (5 percent) were less frequent.

The mechanisms responsible for the CNS abnormalities are not known. Cystathionine the product of cystathionine synthase is present in high concentrations in normal brains. It is not clear that a deficincy of this product causes abnormalities. A neurotransmitter role for cystathionine is controversial (17). In animal experiments high doses of methionine and homocysteine are associated with seizures (18,19). Homocysteine appears to be the predominant factor in inducing seizures (20). However, these effects are noted at very high concentrations of the amino acids where interference with amino acid transport in the brain would be expected (21). It has been suggested that increased levels of S-adenosylmethione and S-adenosylhomocysteine, two substances that are proximal to the cystathionine synthase deficiency, might affect biochemical activity in the brain (22). However, none of the proposed chemical mediators has been shown to play a substantial role in the dysfunction of the CNS and their importance is under judgement.

Another view is that the vascular abnormalities that we will discuss next may be a major factor in the CNS dysfunction. With respect to focal neurological defects, there is little question regarding the importance of cerebral thromboembolism. However, retardation without focal signs is the usual presentation of patients with homocystinuria.

D. Effect on Vascular System: The vascular abnormalities are the usual cause for a patient to seek medical care and are also the usual cause of death (24). Both arterial and venous thrombosis are prominent presentations. Approximately half are venous and half are arterial (2) (Table 4).

Table 4.

HOMOCYSTINURIA - VASCULAR FINDINGS

A. Very Frequent
   1. Arterial and Venous Thromboemboli
   2. Malar Flush
   3. Livedo Reticularis

An arteriogram on an adult who had a clot in his popliteal artery shows ridged and beaded irregular pattern of the lumen in the iliac and fermoral vessels. This appearance is the typical radiographic finding in homocystinuria (24). These changes are due to marked thickening and fibrosis of the intima. I warn you that if you suspect homocystinuria you should not do an arteriogram because it will induce the development of further clotting. Peripheral arterial and cerebral arterial thrombosis are the most common form of arterial involvment. In a study, 109 of 253 documented thromboembolic events in patients with homocystinuria involved the peripheral and cerebral arteries (2). Only 10 of 253 were associated with myocardial infarction.

With respect to venous thrombosis 130 (51 percent) involved the peripheral veins and 32 resulted in pulmonary emboli (2). Serious complications of thromboembolism include central retinal artery occlusion (25,26), cor pulmonale secondary to pulmonary artery thrombosis (27) and severe hypertension caused by renal artery thrombosis (28,29). Figure 3 shows the time to event graphs for thromboemboli in untreated patients with homocystinuria (2).

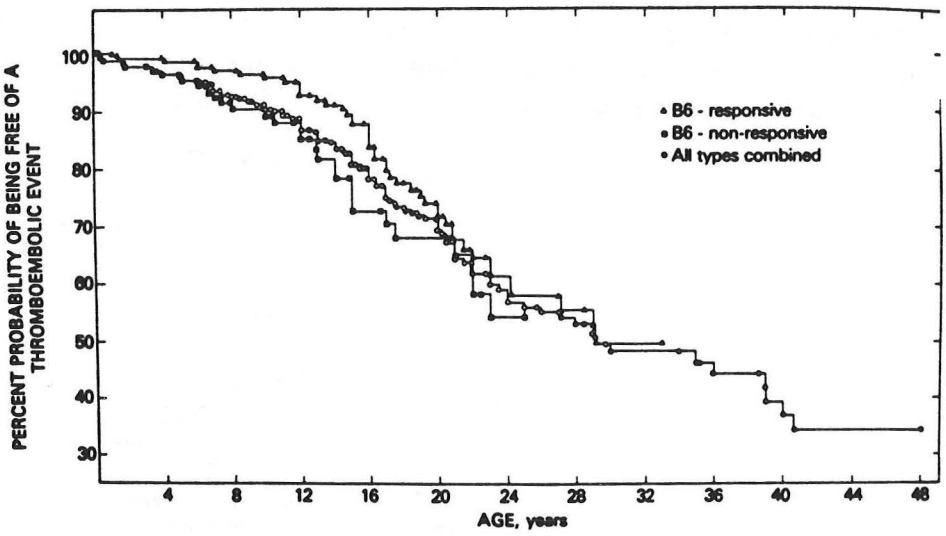

Fig. 23-6 Time-to-event graphs for first clinically detected thromboembolic event in untreated patients. *(From Mudd et al.,* [199] *by permission.)* (2)

Fig. 3

By the age of 30 approximately half the patients will have major thromboembolism. Interestingly the incidence of thrombi post-operatively in homocystinuria patients was only 14 among 241 major surgical procedures (2). Cutaneous vascular abnormalities, for example a malar flushing of the cheeks is often present with a superficial vascular pattern called livedo reticularis.

The pathogenesis of the vascular lesions and the thromboembolic events that are the leading cause of death in homocystinuria is controversial. Increased platelet adhesiveness has been observed in homocystinuric subjects (30). Although these results have been replicated their significance is controversial (31). Homocysteine may also alter prostaglandin synthesis in platelets by promoting increased thromboxane A_2 that may contribute to thrombogenesis (32).

Abnormalities of elastic tissue and smooth muscles in the media of arteries are prominent findings in homocystinuria (24,33). The fibrills tend to be more losely organized, frayed and dispersed than observed in normal arteries. The fibrills appear disrupted and the elastic and muscular coats impinge on the lumen of the vessel. The subintimal tissue proliferates and is markedly thicker. It has been demonstrated that homocysteine is toxic to endothelial cells leading to patchy endothelial denuding that is followed by platelet mediated aggregation and the proliferation of smooth muscle cells and fibroblasts (34-37). The development of arteriosclerotic plaques is also initiated by this process.

TRANS-SULFURATION PATHWAY

The heritable metabolic defects that cause homocystinuria are enzymatic deficiencies in the trans-sulfuration pathway (1). This pathway converts the sulfur atom of methionine into the sulfur atom of cysteine. Figure 4 in your protocol briefly summarizes this pathway. As demonstrated in the diagram, the pathway is a major route for disposing of methionine and explains why cysteine is a non-essential amino acid. This pathway is of major importance for

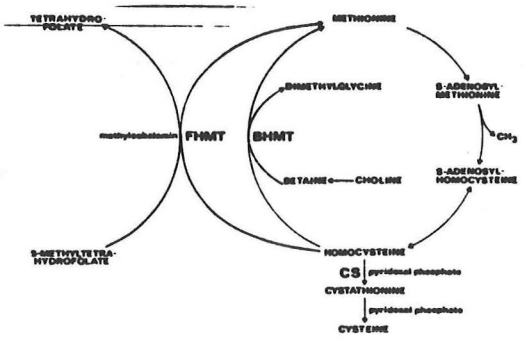

Figure 4. The Trans-sulfuration Pathway.
In the metabolism of the essential amino acid methionine, the step from homocysteine to cystathionine is mediated by cystathionine β-synthase (CS), for which pyridoxal phosphate is a cofactor. Approximately 50 per cent of the homocysteine that is formed is remethylated to methionine with either 5-methyltetrahydrofolate or betaine (trimethylglycine) providing the methyl groups; methylcobalamin is a cofactor for the former reaction. The two enzymes involved are 5-methyltetrahydrofolate:homocysteine methyltransferase (FHMT) and betaine:homocysteine methyltransferase (BHMT). Accumulation of homocysteine results from reduced cystathionine β-synthase activity or reduced remethylation. (43)

two other metabolic reasons: 1) The transmethylation reaction - the methyl group of methionine

is ultimately transferred to many important methylated compounds (38). These include: nucleic acids, amino acids, certain proteins, and vitamins. Because of its sulfonium bond S-adenosylmethioinine may be regarded as a high energy compound and therefore capable energetically of transferring its methyl group (39). 2) The second major feature of the pathway is the remethylation of homocysteine to reform methionine. Approximately 50% of the homocystine formed in this pathway is normally remethylated to methionine (40).

The trans-sulfuration cycle or pathway is initiated when methionine is adenylated by the transfer of an adenosyl moiety of ATP. The S-adenosylmethionine is a high energy compound and is capable of participating in a number of extremely important methyl transfer reactions. Following transfer of the methyl group S-adenosylhomocysteine, a thioether is formed. This then is split to form homocysteine and adenosine. Homocysteine is a key intermediate and a branch point. It can be converted to cystathionine by condensing with serine. This is catalyzed by the enzyme cystathionine *B* synthase which is the step that is deficient in most patients with homocystinuria. Pyridoxine is a co-factor for this enzyme and certain patients with a mutation that affects the affinity of the enzyme for pyridoxine, are greatly improved by large doses of this vitamin. Alternatively, homocysteine can be remethylated to methionine, thus completing the sulfur conservation cycle.

There are at least two alternative mechanisms for homocysteine methylation. In one reaction 5-methyl tetrahydrofolate is the donor and the reaction is catalyzed by a vitamin B^{12} containing enzyme. The enzyme is 5-methyl tetrahydrofolate-homocysteine methyl transferase (41). It is this enzyme which is the major pathway for remethylation of homocysteine and its conversion back to methionine. There are rare forms of homocystinuria that are caused by an inability to form methylcobalamin, the form of vitamin B^{12}, that is needed for the transferase reaction. Other patients are unable to form the 5-methyl tetrahydrofolate because they lack the reductase enzyme necessary to form the methyl donor.

The second mechanism for methylation of homocysteine is through betaine, a choline derived methyl transferase (42). One strategy for treating patients with homocystinuria that does not respond to pyridoxine is to give those patients betaine at a dose of 3 grams twice a day (43-45). The objective is to lower the homocysteine plasma concentration since this is believed to be responsible for the phenotypic effects of this disease. It is of significance that our understanding of the transulfuration pathway has lead to a rational and apparently effective method of treating patients with homocystinuria.

Cystathionine Synthase (CS)

Cystathionine synthase (CS) the enzyme defective in the common form of homocystinuria, plays a central role in sulfur metabolism by condensing serine with homocysteine to form cystathionine (1,2). Both the pyridoxine responsive and the nonresponsive form of the enzyme have been mapped to the long arm of chromosome 21 near the telomere (46a). The genomic structure of the gene has recently been elucidated by Jan Kraus at Colorado (46b). The gene is large, 40 kpb, and has seventeen exons. This gene transcribes three different mRNA's as detected by cDNA cloning. Type I contains the open reading frame for the enzyme and a short

5' untranslated region. It is the functional mRNA for the CS protein. Type II has a 121 bp insertion within the coding region of type I including a 90 bp unique open reading frame and a stop codon in frame. Type III mRNA has a 42 pair base deletion in the COOH-terminus when compared to type I. It would appear that type II and III mRNA represent frequent splicing errors in the transcription of the gene. In fact, type III mRNA appear to be the predominant species in human liver as detected by PCR (46b).

The translational product of the CS gene determined by type I mRNA is a 63,000 dalton polypeptide that associates into a tetramern (46c). This tetrameric form of CS constitutes 95% of the enzyme content in human liver and is almost completely inactive enzymatically. The 63,000 dalton peptide undergoes proteolysis in the hepatocyte to yeild a 48,000 dalton protein (46d). This polypeptide forms a dimer that exhibits a 60 fold increase in specific enzyme activity. The 48,000 dalton species represents only about 5% of the CS protein in the liver but is the active enzyme. Thus multiple mRNA species and post-translational modification of the primary translational product to produce the active enzyme are of great interest.

Mutations in the CS gene are understudy in Jan Kraus's laboratory. The mutations apparently are diverse and differ from one family to another. There is no DNA based test for homocystinuria as yet. The impairment of CS activity as seen in homocystinuria causes homocysteine to accumulate. The increased homocysteine levels accelerate the remethylation pathway and elevate methionine levels. Both pyridoxine responsive and non-responder CS deficiency have hypermethionemia as well as hyperhomocysteinemia. The elevated methionine confirms the deficiency of the CS enzyme. S-adenosylhomocysteine also accumulates and inhibits methyl transfer reactions from S-adenosylmethionine. When methylation is inhibited synthesis of creatine, sarcosine, lecithin and methylation of proteins and nucleic acids are affected (1). The effects of elevated homocysteine levels on fibrillar proteins that contribute to the phenotype were previously described.

Classification of Homocystinuria

Homocystinuria can be cause by several different enzyme deficiencies. By far the most common disorder-causing homocystinuria is a cystathionine synthase deficiency. This is the most frequent inborn error of amino acid metabolism second to PKU or phenylketonuria. This enzyme deficiency is characterized by elevated levels of plasma methionine. There are two forms of

Table 5.

CLASSIFICATION OF HOMOCYSTINURIAS

I. Elevated Blood Methionine
   - A. Cystathioinine Synthase Deficiency
     1. Pyridoxine Responsive
     2. Apoenzyme Defect

II. Low or Normal Methionine Levels and Cystathionineuria
   - A. Methyl Colbalamin Deficiency
   - B. Methyl and Adenosyl Colbalamin Deficiency
   - C. N^5 Methyltetrahydrofolate Methyl Transferase Deficiency
   - D. $N^{5,10}$ Methylenetetrahydrofolate Reductase Deficiency

cystathionine synthase deficiency (2,46). One form is a pyridoxine-responsive enzyme deficiency. Pyridoxine is the cofactor for the enzyme and a partially defective cofactor binding site on the enzyme can be compensated for by increasing pyridoxine availability. With large doses of pyridoxine (100mg-1000mg per day) one can partially restore the enzyme activity and relieve the elevated homocysteine levels. In some patients pyridoxine response can be magnified by adding folic acid (47,48). The second type of cystathionine synthase deficiency is that in which there is a defective catalytic site on the enzyme and that does not respond to pyridoxine (49). It is therefore termed the non-pyridoxine responsibe form of homocystinuria. Because cystathionine synthase is required for the condensation of homocyteine with serine to produce cystathionine the levels of homocysteine are increased. This results in a compensatory enhancement of the methylation of homocysteine with elevation of the methionine levels in plasma. There are rarer forms of homocystinuria due to inherited defects in homocysteine remethylation to form methionine. Individuals with these defects have a low or normal methionine. Therefore the level of methionine is a key to classifying the disease homocystinuria into either that with an elevated methioinine level, which is due to cystathioinine synthase deficiency or with normal or low plasma methionine which is due to a remethylation deficiency.

Remethylation Deficiencies

Homocysteinine methylation defects can be classified into defects of B_{12} metabolism or defects of folic acid metabolism. We will first consider abnormalities in B_{12} metabolism (50). There are two forms of homocystinuria associated with abnormal B_{12} metabolism. The first is an inability of the body to reduce cobalamin (vitamin B_{12}). The deficiency of cobalamin reductase blocks the conversion of B_{12} to its active cofactor forms - methylcobalamin and adenosyl-cobalamin. A defect in B_{12} reduction leads not only to homocystinuria becasue of the importance of methyl cobalamin as a co-factor for the remethylation of homocysteine, but also to methylmalonic aciduria, since adenosyl-cobalamin is a co-factor in the propionic acid pathway (51). This produces a combined metabolic defect resulting in homocystinuria and methyl malonic aciduria. Infants with this defect are affected early in life. The clinical picture is dominated by the presence of methyl malonic aciduria and severe acidosis. If they survive, an anemia characterized by macrocytosis and a megaloblastic bone marrow develops. Folic acid and vitamin B_{12} are essential for nucleic acid synthesis and the deficiency of the active forms of vitamin B_{12} impairs DNA synthesis leading to enlarged cells.

Another mutation which can produce homocystinuria is a defect in the enzyme that converts reduced cobalamin to methyl cobalamin the co-factor needed for the methyl tetrahydrofolate transferase reaction that remethylates homocysteine. Infants with this abnormality show severe mental retardation, homocystinuria with low methioinine levels cystathionineuria and megaloblastic anemia (1).

Inborn errors of folic acid metabolism also cause a defective remethylation of homocysteine and result in homocystinuria. Two different mechanism or enzymes are responsible. One is a mutation in the homocysteine N5 methyl tetrahydrofolate methyl

transferase, the enzyme that transfers the methyl group from the methyl tetrahydrofolate to homocysteine to form methionine (52). The second enzyme defect is in the methyl tetrahydrofolate reductase. This is an enzyme required to make the N5-methyl tetrahydrofolate. The clinical picture in these two deficiencies in folic acid metabolism are severe mental retardation, homocystinuria with low methionine levels and if the child survives, the development of a megaloblastic anemia. It is of interest that infants with these disorders show the vascular lesions, that is the tendency to develop thrombi in arteries and veins, that the more common form of homocystinuria exhibit (52).

Heterozygotes for Homocystinuria

Although the homozygous affected individuals, that is individuals that have two mutant genes for cystathionine synthase and have homocystinuria, are relatively rare, occurring about 1 in 150,000 to 200,000 live births, the heterozygous carriers of the mutant genes are estimated to be between 1 in 70 and 1 in 200 individuals (1). An intriging question is: does the carrier who appears to be phenotypically normal have an increased risk for premature vascular occlusive disease such as clotting in the arteries or veins? This was studied by Boers and his associates who examined 75 patients who had occlusive arterial disease, demonstrated by angiography (53). These patients were under the age of 50 and did not have diabetes, elevated plasma lipids or hypertension that would predispose them to arterial disease. Of these patients, 25 had peripheral arterial disease, 25 had had a stroke due to thrombosis in a cerebral artery and 25 had a myocardial infarction (Figure 5).

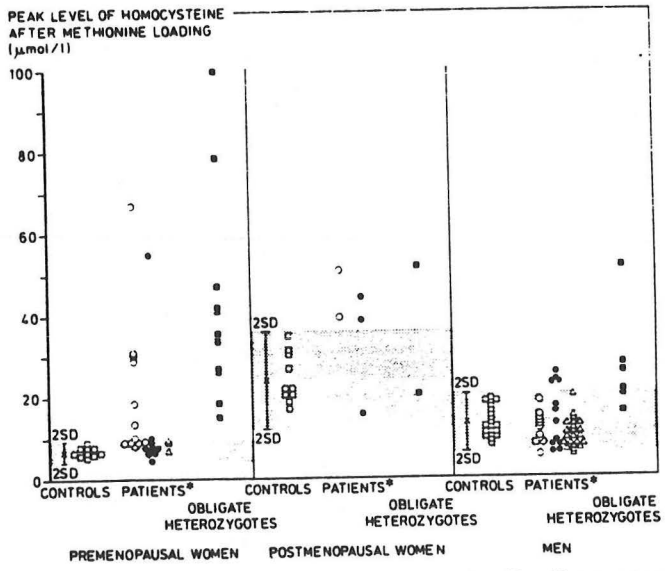

Figure 5. Peak Serum Levels of Homocysteine after Methionine Loading in 75 Patients with Premature Occlusive Arterial Disease and in Comparison Groups, According to Sex and Menopausal Status.

The patients are denoted by an asterisk; 25 patients had occlusive peripheral arterial disease (○), 25 had occlusive cerebrovascular disease (●), and 25 had had a myocardial infarction (△). The comparison groups consisted of 40 control subjects (□) and 20 obligate heterozygotes for homocystinuria (■). (53)

They examined the frequency of elevated plasma homocysteine after loading the patient with methioinine. The patients were sub-divided into premenopausal women because methionine metabolism is more active in these women and they tend to have lower homocysteine levels normally than do either post-menopausal women or men (54). It has been proposed that one of the reasons that premenopausal women have less vascular disease than post-menopausal women and men is that they have lower homocysteine levels in their plasma (54). In this figure the shaded area shows the normal range of homocysteine after a standard methionine load for the three groups of patients. The error bars are plus or minus 2 standard deviations. Patients were considered to be heterozygous for a mutant allele at the cystathionine synthase locus if the peak level of homocysteine exceeded the mean level by 2 standard deviations. The open circles are patients with occlusive peripheral arterial disease, the closed circles are occlusive cerebrovascular disease and the triangles represent coronary artery thrombosis. The controls are 40 patients with no history of arterial occlusive disease (they are shown by the open squares) and 20 obligatory heterozygotes, individuals who have had a child with homocystinuria and must carry one mutant gene. They are represented by the closed squares. The probability of detecting a heterozygote by chance in a normal population is approximately 1 in 70 and one subject in the control group of 40 appeared to be a carrier. There were no carriers detected in the myocardial infarction group represented by the open triangles. Boer related this finding to the relative rarity of myocardial infarcion in homozygotes for homocystinuria. Fourteen patients in the remaining 50 had levels of homocysteine after methionine load that exceeded the normal plasma levels by more than 2 standard deviations: seven of the 25 with premature peripheral arterial disease and 7 of the 25 with premature cerebrovascular thrombosis. All but 2 of the obligatory heterozygotes exceeded the upper limits of the plasma homocysteine levels selected. Confirmation of the heterozygous status of the subjects with elevated homocysteine after methionine load were confirmed by enzyme assays on skin fibroblasts cultures (53).

The conclusions from this study are of importance. Thirty percent of patients with premature peripheral vascular disease and cerebrovascular disease appear to be heterozygotes for cystathionine synthase deficiency. Thereofre, peripheral vascular disease or stroke due to arterial thrombosis in an individual under the age of 50 who has no risk factors for arterial disease should alert the physician to the possibility that this individual is a carrier of a mutant gene for homocystinuria. Boers' group has shown in a preliminary study that pyridoxine normalizes the homocysteine level after methionine load in 6 of 11 patients who appeared to heterozygotes (53).

There is considerable controversy regarding the role of homocysteinemia in heterozygotes and the incidence of coronary artery disease. Mudd was unable to document by questionnaire to obligatory heterozygotes whether the incidence of myocardial infarction or stroke was increased (54). On the other hand, Swift and Morrell dispute Mudd's data and claim that deaths from cardiovascular disease is increased in obligatory heterozygotes (55). In a recent NEJM paper Clarke and his associated have studied peak homocysteine levels after a methionine load in 27 normal subjects, 25 obligatory heterozygotes and 113 patients with vascular disease under the age of 55 (Figure 6) (56).

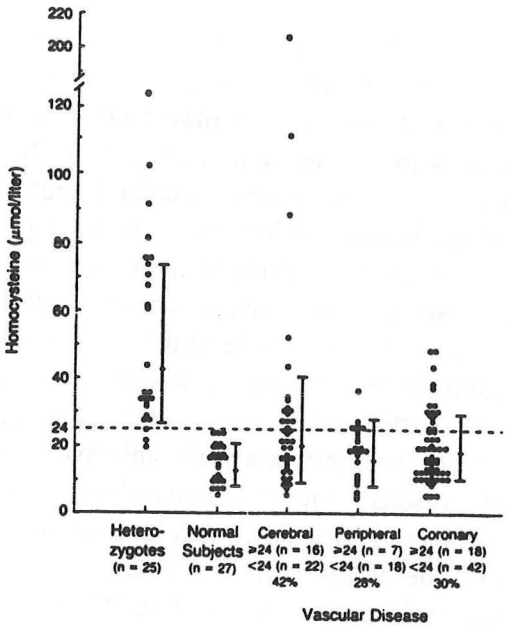

Figure 6 Serum Homocysteine Levels in Obligate Heterozygotes for Cystathionine β-Synthase Deficiency, Normal Subjects, and Patients with Cerebral, Peripheral, or Coronary Vascular Disease.

The values shown are the peak post–methionine-loading serum level of non–protein-bound homocysteine in each subject. The dashed line at 24 μmol per liter represents the level above which patients were considered to have hyperhomocysteinemia. The I bars denote the means ±1 SD of the log values. The values below the three groups with vascular disease indicate the percentage of patients with hyperhomocysteinemia. (56)

Thirty-eight had cerebral disease, 15 had peripheral vascular disease and 60 had myocardial infarctions or angiographic evidence of severe coronary disease. As shown in Figure 7, all but three of the obligatory heterozygotes had serum levels of nonprotein homocysteine greater than 24 u moles/liter. None of the normal subject exceeded this level. Sixteen of the 38 patients or 42% with cerebrovascular disease, 7 of 25 patients or 28% with peripheral vascular disease and 18 of 60 or 30% patients with coronary artery disease exceeded the cutoff level of 24 u molar for homocysteine (56). Confirmation of heterzygosity was carried out in 23 patients by measurement of cystathionine synthase activity in skin fibroblast cultures (Figure 7).

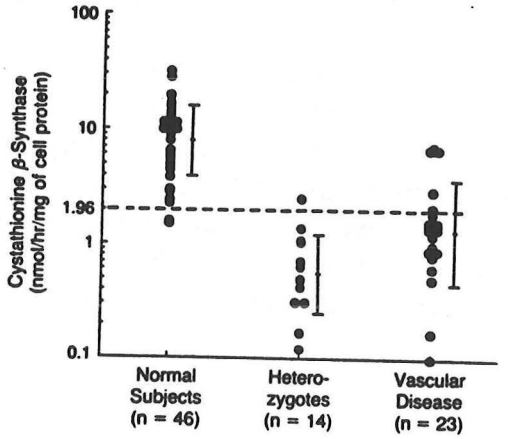

Figure 7 Cystathionine β-Synthase Activity in Cultured Skin Fibroblasts from 23 Patients with Vascular Disease and Hyperhomocysteinemia and in Two Comparison Groups.

The normal range of activity was defined in a separate, unpublished study of 46 normal subjects and 14 obligate heterozygotes for cystathionine β-synthase deficiency. The I bars denote the means ±1 SD of the log values. The dashed line at 1.96 nmol per hour per milligram of cell protein represents the lower limit of normal (mean −1.96 SD of the log mean value in the normal subjects). (56)

Patients are often reluctant to have skin biopsy so all putative heterozygotes were not assayed. On the other hand blood is easily obtained and Dr. Goldstein has shown that PHA stimulated lymphocytes from heterozygotes have reduced cystathionine synthase activity in 14 of 17 subjects (57).

Other studies have also shown an association of hyper-homocysteinemia after methionine loading and premature coronary artery disease (58). The somewhat inconsistent data on the role of homocysteine in coronary artery disease may be due to small numbers of patients in earlier studies, using only acid soluble homocysteine levels and neglecting the larger protein bound component (this is true of both Boers and Clarkes studies 53,56) and failure to separate women into premenopausal and post-menopausal groups (true of Clarkes study 56). The data on peripheral vascular disease and stroke in hyperhomocysteinemia after a methionine load is convincing.

A medical resident, Jim Trotter, and a medical student who has just complete the first year, are carrying out a summer research study of venous thormbosis in young patients without any obvious etiology. They conducted methionine loads on Parkland volunteers with deep venous thrombosis of unknown cause. They will determine the proportion of individuals who are heterozygous carriers of a mutant gene for homocystinuria.

Summary

Homocystinuria and its variants are experiments of nature that emphasize the importance of the methionine pathway to normal development and health. An understanding of the methionine pathway and its regulation at the biochemical level has led to rational therapy for this group of diseases. Moreover, the role of excess SH groups from homocysteine in producing the phenotype suggest that cross-linking of fibrillar proteins is of utmost importance for their stability and integrity. Homocystinuria exemplifies the interplay between clinical observations and biochemical research that has provided new insight into the role of the trans-sulfuration pathway in metabolic regulation and has lead to the development of rational and innovative treatments of diseases involving this pathway.

MARFAN SYNDROME

INTRODUCTION

The similarities in phenotype between Marfan syndrome and homocystinuria provide a rationale for combining them their discussion. Marfan syndrome has excited medical interest out of proportion to its frequency. In part this is the result of: 1) Its dramatic presentation with aortic dissection or rupture, 2) The protean manifestations of this mutant gene that brings these patients to the attention of a variety of medical specialists. These include internists, pediatricians, ophthalmologists, orthopedists, cardiologists and family physicians. 3) The enhanced medical interest in Marfan syndrome is further promoted because the marfan phenotype overlaps with similar phenotypes due to genetic heterogenity that exists in the normal population. Therefore, Marfan syndrome is often entertained in differential diagnosis of patients with a marfanoid habitus. The current lack of a specific biochemical test for the marfan gene makes the diagnosis difficult. One must rely on clinical criteria and a family history to determine if an individual

with a marfanoid appearance is in fact afflicted with Marfan syndrome or has a genetic constitution that mimics the phenotype. 4) The possible affliction of a venerated American president, Abraham Lincoln, adds to the interest in this syndrome.

Case Presentation: KD is a 54 year old woman who was first diagnosed as Marfan syndrome at the age of 48 when she developed glaucoma secondary to a dislocated lense. Noted at that time to have arachnodactyly and was tall with an unusually long lower body segment. Family history revealed that her father died of a dissecting aneurysm of the aorta at age of 46. A sister had bilateral lense dislocations and a brother had a dissecting aneurysm of aorta repaired several years ago. She has seven children. One who was unusually tall died of an aortic dissection. Patient was in good health until she awoke the night prior to admission to UH of Cleveland with intense back pain radiating anteriorly to the substernal area. BP RA 170/85; LA 170/85; RL absent pulse and pressure; LL 160/80. Heart was without murmurs or gallop. Abdominal examination showed a pulsating mass to right of umbilicus with a bruit. The right leg was cooler than the left. An aortogram showed a type 3 dissection of the aorta extending to the femorals. The right renal artery did not visualize but there was flow to the right kidney. The patient was treated medically with nitroprusside, propranolol and aldomet to maintain systolic BP at approximately 100. The patient's course was complicated by difficulty in controlling BP which required frequent adjustments in medications. However she gradually stabilized without evidence of further dissection and 20 days after admission was transferred to Houston for evaluation by Dr. Denton Cooley for surgery.

CLINICAL FEATURES

Marfan syndrome is a dominantly inherited mesenchymal dysplasia. On the basis of recent experience the prevalance of classic Marfan syndrome is 4-6 per 100,000 people without racial or ethnic predilections (59,60). However, since the manifestations may extend from the limits of the normal to the most characteristic and "classic" case in which the diagnosis is unquestionable the actual prevalence of this syndrome may be considerably greater than the above figures suggest. Moreover, because of the frequency of the marfanoid habitus in adolescents and young adults, the disease is frequently entertained in a differential diagnosis on an adult medical service.

In the classic form of Marfan syndrome it is associated with abnormalities of the eye, the aorta, the skeleton, the chest wall, and joints. We will examine the most prevalent clinical features observed in this disease.

Ocular Manifestations - Table 6

Table 6.

OCULAR FINDINGS - MARFAN SYNDROME

I.	Upward subluxation of lens - 50-80%
II.	Myopia
III.	Flat cornea

Subluxation of the lenses occurs mainly with an upward displacement. The reason for the upward displacement of the lenses is that the suspensory of the lens are stretched but the zonular fibers remain intact permitting normal accomodation. You will recall that in homocystinuria, a disorder which is commonly confused with Marfan syndrome, the lens is displace in a downward position because the fibers disintegrate and are disrupted. Under these circumstances the eye cannot accomodate. In Marfan syndrome the dislocation of the lens occurs

in approximately 50-80% of patients (61). Dislocation of the lens is present at the first detailed ophthalmological examination suggesting that the redundancy of the suspensory fibers of the lens may be present *in utero*. In contrast to homocystinuria the displacement of the lens is rarely progressive. On physical examination one can suspect the presence of a dislocated lens by having the patient move the eye and noting the quivering of the iris in the inferior pole. Alternatively when doing a fundoscopic examination on the patient one may note that the inferior portion of the retina has a different focal plane than the central and superior portions of the retina.

In approximately 30% of patients with Marfan syndrome myopia is severe and there is a tendency for retinal detachment. Myopia is a relatively common finding in the normal population so that this sign is less specific than a displacement of the lens.

Cardiovascular Manifestations - Table 7

Table 7.

Cardiovascular Findings - Marfan Syndrome

I.	Valvular insufficiency AI, MR, MVP - 60-80%
II.	Aortic root dilatation
III.	Aortic dissecting aneurysm

Approximately 60% of Marfan patients have a murmur detected by auscultating the heart. Either mitral or aortic regurgitation or mitral valve prolapse are the commonest findings. Aortic insufficiency which is present in approximately 10% of patients is the most specific finding. In adult patients with Marfan syndrome mitral regurgitation or mitral valve prolapse of a clinically significant degree is present in approximately 65% (62).

Aortic root enlargement is usually not detected by standard chest x-rays until the dilitation is pronounced and until aortic regurgitation or dissection has occurred. The root of the aorta is first to dilate and is not well appreciated on a chest x-ray since it lies within the cardiovascular silhouette. Echocardiography has greatly altered the sensitivity of detecting early aortic root enlargement and has improved both the diagnosis and management of patients (63,64a,64b). In children and adults the diameter of the aortic root measured at the level of the aortic valve cusp is frequently greater than the upper limit of normal on the basis of body surface area. The most characteristic finding is to see an aorta dilated several centimeters above the valve giving it a "sausage" configuration. Moderate dilitation of the aorta is accompanied by a paradoxical movement of the posterior aortic wall (65). This may impede closure during early systole - isometric ventricular contraction. Two dimensional and Doppler echocardiography has also contributed greatly to the detection and measurement of mitral valve prolapse and mitral regurgitation (64b).

Skeletal Manifestations - Table 8

Table 8.

Skeletal Findings - Marfan Syndrome

I.	Scoliosis - multiple segments
II.	Pectus abnormalities
III.	Dolichostenomelia
IV.	Arachnodactyly

V. Joint laxity or contractures

The marfan habitus is characterized by an increased length of the limbs when compared to the trunk (dolichostenomelia). The lower segment length, that is from the pubis to the floor when divided into the upper segment length, that is the height minus the lower segment length provides a ratio that is usually 2 standard deviations less than normal (59). One must be cautious because race, age, and sex influence the standard measurements. Therefore, an individual patient suspected of Marfan syndrome must have his measurements compared to tables that account for differences between races, ages, and sex.

Scoliosis is a common finding in Marfan syndrome. The scoliosis most characteristic of Marfan's is one that involves multiple sites or segments along the lumbar spine (60). The scoliosis generally worsens as the patient gets older. Clinically the significance of multiple segment scoliosis can best be appreciated by observing the erect patient from the rear as they bend forward at the hips. Deformities of the anterior thorax are also common and include pectus excavatum which is more common than the keel shaped pectus carinatum.

Arachnodactyly or long tapered fingers is a subjective feature and one that is not terribly specific for Marfan syndrome. Simple maneuvers for example, the thumb sign in which the thumb projects beyond the ulnar border of the hand when the hand is clenched over the thumb is not infrequently observed in normal individuals. A second sign is the wrist sign which is positive if the distal phalanges of the thumb and the little finger of one hand overlap when wrapped around the opposite wrist. Both of these signs are helpful when positive but are subject to interpreter variation and are more associated with laxity of the joints of the hand than they are with the length of the fingers.

Joint laxity is of little help in making the diagnosis of Marfan syndrome. In fact there are two clinical subgroups of Marfan syndrome. In one the joints are hypermobile and the laxity approaches that seen in type I Ehlers-Danlos syndrome (66). A second form of Marfans syndrome has contractures and stiffness of the joints (67). These two variations of joint mobility appeared to occur in different families.

Pulmonary Manifestations - Table 9

Table 9.

Pulmonary Findings - Marfan Syndrome

I.	Blebs
II.	Reduced total lung capacity
III.	Reduced residual volume
IV.	Restrictive chest wall disease

Spontaneous pneumothorax and congenital lung blebs have been reported in patients with classic Marfan syndrome. The presence of lung blebs and the deforming kyphoscoliosis or pectus excavatum may impair pulmonary function (68). The most common abnormalities are a reduction in total lung capacity and a reduced residual volume (60).

EVALUATION OF SUSPECTED MARFANS SYNDROME

Table 10.

Evaluation of Suspected Marfan Syndrome (60)

I. Family history - dominant
II. Examine all family members
III. Examination must include:
  A. Body proportions
  B. Opthalmological - slit lamp
  C. Echocardiogram - aorta & valves

A detailed family history is required and the physician should make an effort to examine other family members and close relatives for the signs of Marfan syndrome. The variation in clinical presentation makes mandatory the careful evaluation of all family members and relatives. This is true for most dominantly inherited diseases because a grandfather and parent may show minor manifestations of a disease in which the child exhibits a full blown and devastating clinical picture. In order to ensure that the child is not a new mutation an examination of all close relatives is required. The heterogenity in Marfan syndrome is characterized by its variable expression and the interfamilial differences in onset of clinical findings. The penetrance of the gene is apparently complete and skipped generations do not occur. Approximately 85% of Marfan syndrome patients have a family history. The 15% of patients who are new mutations tend to exhibit a paternal age affect (69). That is, they are the progeny of older fathers, age 55 years or more. The mutation rate per genetic locus is dependent on the number of replications and it is reasonable that new mutations may occur in older fathers because of the extended period of spermatogenesis. In counseling families in which there is a sporadic mutation particularly if the father is older, the physician should be cautious about quoting a risk of recurrance. The risk may not be the mutation rate which is 1 in a million or more. A mutation may occur in a progenitor of a sperm and the father may carry a clone of sperms all with the same mutation. Therefore, the risk to subsequent pregnancies may be as high as 1 in 100. Paternal age effects have been demonstrated for other dominantly inherited mutations including neurofibromatosis and achondroplasia.

A thorough physical examination looking for the findings of Marfan syndrome that have previously been described is essential. The examination should include measurement of body proportions. A detailed ophthalmological evaluation including slit lamp examination with pupils fully dilated is mandatory. An echocardiogram with measurements of the aorta root and evaluation of the mitral, tricuspid, and aortic valves is essential. With these procedures the diagnosis should be made in approximately 90% of patients.

There are four major criteria for the diagnosis (Table 11).

Table 11.

Criteria for Diagnosis of Marfan Syndrome (60)

I. Positive family history
II. Ectopic lens
III. Dilated aortic root or AI
IV. Severe scoliosis
* Need two of four criteria
OR "Soft" findings - marfanoid habitus, MVP, myopia

These include: 1) A positive and documented family history. 2) The presence of a displaced lens

in which the zonular or suspensory fibers are stretched and redundant but not ruptured. 3) Cardiovascular findings, the most diagnostic of which are dilated aortic root, and aortic insufficiency. 4) Skeletal manifestations, the most characteristic being a severe scoliosis involving multiple segments of the spine and deformity of the anterior thorax. Pyeritz and McKussick recommend that at least two of these "hard" criteria be present before a diagnosis of Marfan syndrome is established (60). Soft features of a marfanoid syndrome including a body habitus with relatively long arms and legs and long fingers; myopia, mitral valve prolapse are "soft" features that are frequently found in the population as part of the genetic heterogenity observed normally. Using these soft criteria to diagnose the so called "forme fruste" is inappropriate and a confusing diagnosis.

MANAGEMENT

Because of the complexity of Marfan's syndrome patients should be cared for in a multidisciplinary setting where various specialists can handle those aspects of the disease that require a sophisticated and complex treatment.

Cardiovascular Complications

Life expectancy in patients with Marfan's syndrome is reduced by 50-60% (70). The most frequent cause of death approximating 95% of all deaths in patients with Marfan syndrome are the cardiovascular complications. These require the closest follow-up and expectant therapy. The major life threatening complication involves the affects of the disease on the aorta. Severe aortic regurgitation, dissection and rupture occuring in the ascending aorta are the most feared. To anticipate the threat of these complications an echocardiogram should be obtained annually. If the diameter of the aorta exceeds the upper limit of normal as corrected for body surface area by 50% or more, evaluations must be performed every six months. In adults rupture and dissection occur when the diameter of the aorta exceeds 6cm. A cardiologist and cardio thoracic surgeon should plan the optimal time to replace the aortic valve and the ascending aorta. Elective prophylactic repair of the ascending aorta once marked dilitation occurs prevents the catastrophic dissection or aortic rupture (71). An aortic valve prosthesis sewn to one end of a woven synthetic aortic tube is inserted and the coronary arteries are anastamosed. The mortality for this operation in the best hands is well below 10%. Table 12 summarizes Svensson results in 151 Marfan patients (71).

Table 12.

Cardiovascular Surgery for Marfan Syndrome (71)

I.	280 operations on 151 patients
II.	Mitral valve replacement in 13
III.	aortic valve replacement in 135
IV.	Aorta segment replacement in 141
V.	Total aorta replacement in 10
VI.	Thoracic aorta replacement in 7
VII.	Descending and abdominal aorta in 20
*	Survival 30d - 97%, 5 yr - 75%, 10 yr - 56%

Propanolol is recommended prophylactically in all patients with echocardiographic evidence of any enlargement of the aorta root. Propanolol is used to retard aortic dilatation

through its negative ionotrophic action. Although used for many years the evidence that propanolol delays the aortic complication of Marfan syndrome is currently unclear (60).

Patients should have prophylaxis for endocarditis when undergoing dental or surgical procedures. The activity of patients with Marfan syndrome should be restricted. Contact sports, lifting weight, isometric exercises and participating in strenuous physical activity should be discouraged.

Ocular Complications

An experience ophthalmologist who is familiar with the treatment of dislocated lenses should manage the ocular complications in Marfan patients. Since subluxation of the lens is almost always present during the first ophthalmological examination it is imperative that any inqualities of refraction between the two eyes be corrected at the earliest opportunity. If correction is delayed amblyopia to the direction and dislocation of the lens is likely to occur. Recognition delays can lead to this complication.

The myopia which many of these patients have, predisposes them to retinal detachments and patients should be followed for this complication. Unlike homocystinuria the subluxed lens rarely requires removal, however occassionally it will sublux and produce an acute glaucoma.

Skeletal Complications

Scoliosis is probably the most severe of the skeletal lesions associated with Marfan syndrome and is a progressive. Adolescent growth spurts can exacerbate the tendency for scoliosis. Estrogen administration to retard growth has been used in female patients but there is no conclusive evidence to show whether this therapy mitigates the effects of the disease (72).

The joint instability which follows the laxity of ligaments and hypermobile joints in Marfan syndrome may require orthopedic correction. If cardiopulmonary compromise occurs because of anterior thorax abnormalities surgical correction of this condition may be required.

Counseling

Patients with Marfan syndrome are at risk of having 50% of their children afflicted with this disease. They should be informed and educated as to the complications and severity of the disease. Any woman who has echocardiographic evidence of aortic dilitation should be advised against pregnancy. There is a well documented increased risk of vascular rupture during or shortly after pregnancy in women with Marfan syndrome. The final decision obviously rests with the family based on their Mores, religion, and their perception of the risks and rewards.

Gene Localization and Biochemical Defect in Marfan Syndrome

The clinical heterogenity of phenotypes in Marfan syndrome suggest that more than one genetic locus may be involved in producing the disease. Biochemical findings have clearly shown an increase in extractable collagen and elastic fibers from tissues of Marfan syndrome and putative defects in the crosslinks of collagen and elastic fibers (73,74). However the structure and metabolism of collagen type I and III was found to be normal in 25 unrelated patients, 23

with typical Marfan syndrome and 2 infants with a severe form of the disease (75). Moreover, genetic studies have excluded various types of fibrillar and non-fibrillar collagens as candidate molecules (75-79). There are two notable exceptions. Byers et al (80) and Phillips et al (81) reported a glutamine to arginine substitution in residue 618 of the alpha 2(1) collagen chain of several patients with Marfan syndrome. Pulkkinen et al (82) reported a marked decrease in the mRNA of decorin an abundant dermato proteoglycan in skin cultures of an infant with the severe form of Marfan syndrome. Nevertheless, it appears as if a mutation in the collagen genes or in the elastin gene is unlikely to explain the biochemical abnormality in most patients with "classic" Marfan syndrome.

Using reverse genetics, that is using linkage analysis with DNA polymorphic markers in selected chromosomes of the human genome Kainulainen et al (83) mapped the "Marfan locus" in five Finnish families to the long arm of chromosome 15. Recently Dretz et al (84) confirmed the assignment of Marfan gene to chromosome 15 and suggested its location near the centromere at 15q15-a21.3. This latter study was carried out on four large American families with classic Marfan syndrome. A second study on different American families suggests that the locus may be more distal on the long arm of chromosome 15(85). The chromosomal localization of a putative Marfan gene should facilitate identification of the gene product. An immediate benefit is the potential for diagnosis of equivocal cases in selected families and perhaps prenatal diagnosis.

Recently Hollister and his associates have reported that Marfan syndrome cosegregrates with an abnormal elastin-associated microfibrillar structure called fibrillin (86). The abnormal pattern and distribution of these micrifribils was detected using a monocloncal antibody to fibrillin. Microfibrillar fibers are widely distributed in human tissue and occur as pleiomorphic linear bundles containing many individual microfibrils. It appears that the fibers serve as scaffolding for the deposition of elastin during elastogenesis (87). In many tissues the fibers become incorporated in whole or in part into elastic structures. Fibrillin is a 350kd glycoprotein which is only partially characterized but specific antibodies have been raised to this antigen (88). The cloning of a cDNA to fibrillin has not been reported to my knowledge. In a blinded study of skin biopsies and/or skin fibroblast cultures from 27 patients with Marfan syndrome 24 were correctly identified by the decreased content of microfibrillar fibers (Figure 8) (89).

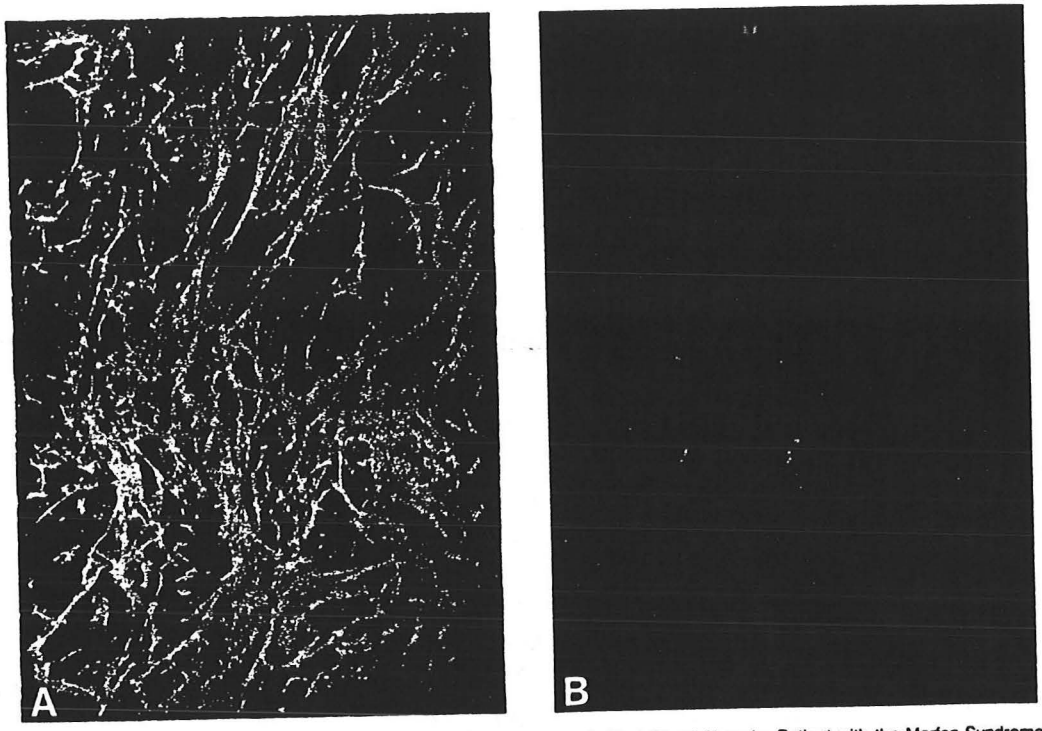

Figure 8 Hyperconfluent Multilayers of Dermal Fibroblast from a Normal Subject (Panel A) and a Patient with the Marfan Syndrome (Panel B) (×300).

The subject and patient are those described in Figure 1. After acetone fixation, the cells were stained as described in Figure 1. The normal fibroblasts show a prominent meshwork of fibrous materials in the 48-hour assay. In contrast, the fibroblasts of the patient with the Marfan syndrome show a sharply decreased amount of immunostainable fibrous meshwork. (89)

Of twenty-five patients with other heritable connective tissue diseases, for example Ehler-Danlos syndrome, pseudoxonthoma elasticum, annuloaortic ectasia, mandibulosacral dysplasia and the Stickler syndrome, 19 were correctly identified as not having Marfan syndrome. Six patients had immunofluorescence assay results that overlapped with Marfan syndrome and they were misclassified. In a Conference Report of a workshop on Marfan syndrome Petros Tsiporiros mentioned that biopsy of skin from patients with homocystinuria also showed reduced microfibrillar flourescence (90). Whether a mutation in the putative fibrillin gene is responsible for many instances of Marfan syndrome or whether it is an epiphenomenon remains to be established. However, recent preogress indicates that there may be a "light at the end of the tunnel" for Marfan syndrome.

REFERENCES

1. Mudd SH, Levy HL, Skovly F. Disorders of trans-sulfuration in Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The Metabolic Basis of Inherited Disease 1989, 693-734.
2. Mudd SH, Skovy F, Levy HL, et al. The natural history of homocystinuria due to cystathionine synthase deficiency. Am J Hum Genet 37:1, 1985.
3. Brown JH, Allison JB. Effects of excess dietary d*l*-methionine and/or *l*-arginine on rats. Proc Soc Exp Biol Med 69:196,1948.
4. Cohen HP, Choitz HC, Berg CP. Response of rats to diets high in methionine and related compounds. J Nutr 64:555, 1958.
5. Siegel RC. Lysyl oxidase. Int Rev Connect Tissue Res 8:73, 1979.
6. Harris ED Jr, Sjoerdsma A. Collagen profile in various clinical conditions. Lancet 2:707, 1966.
7. Harris ED Jr, Sjoerdsma A. Effect of penicillamine on human collagen and its possible application to treatment of scleroderma. Lancet 2:996, 1966.
8. Mudd SH, Levy HL. Disorders of transsulfuration, in Stanbury JB, Wyngaarden JB, Frederickson DS (eds). The Metabolic Basis of Inherited Disease, 4th ed. New York, McGraw-Hill, 1978, p 458.
9. Hayasaka S, Asano Y, Tateda H, Hoshi K, Koga Y. Lens subluxation in homocystinuria. Acta Ophthalmol (Copenh) 62:425, 1984.
10. Elkington AR, Freedman SS, Jay B, Wright P. Anterior dislocation of the lens in homocystinuria. Br J Ophthalmol 57:235, 1973.
11. Morreels CL Jr, Fletcher BD, Weilbaecher RG, Dorst JP. The roentgenographic features of homocystinuria. Radiology 90:1150, 1968.
12. Schedewie H, Willich E, Grobe H, Schmidt H, Muller KM. Skeletal findings in homocystinuria: A collaborative study. Pediatr Radiol 1:12, 1973.
13. Cochran FB, Sweetman L, Schmidt K, et al. Pyridoxine-unresponsive homocystinuria with an unusual clinical course. Am J Med Genet 35:519, 1990.
14. Abbott MH, Folstein SE, Abbey H, Pyeritz RE. Psychiatric manifestations of homocystinuria due to cystathionine *B*-synthase deficiency. Am J Med Genet 26:959, 1987.
15. Ludolphy AC, Ullrich K, Bick U, Fahrendorf G, Przyrembel H. Functional and morphological deficits in late-treated patients with homocystinuria: a clinical , electrophysiologic and MRI study. Acta Neurologica Scandinavica 83:161, 1991.
16. Tallan HH, Moore S, Stein WH. L-cystathionine in human brain. J Biol Chem 230:707, 1958.
17. Tudball N, Beaumont A. Studies on the neurochemical properties of cystathionine. Biochem Biophys Acta 588:285, 1979.
18. Blennow G, Folbergrova J, Nilsson B, Siesjo BK. Cerebral metabolic and circulatory changes in the rat during sustained seizures induced by DL-homocysteine. Brain Res 179:129, 1979.
19. Wuerthele SE, Yasuda RP, Freed WJ, Hoffer BJ. The effect of local application of homocysteine on neuronal activity in the central nervous system of the rat. Life Sci 31:2683, 1982.
20. Freed WJ, Taylor SP, Luchins DJ, Wyatt RJ, Gillin JC. Production of convulsions in mice by the combination of methionine and homocysteine. Psychopharmacology 69:275, 1980.
21. Tudball N, Griffiths R. Biochemical changes in the brain of experimental animals in

response to elevated plasma homocystine and methionine. J Neurochem 26:1149, 1976.
22. Cantoni GL. The centrality of S-adenosylhomocysteinase in the regulation of the biological utilization of S-adenosylmethionine, in Borchardt RT, Creveling CR, Ueland PM (eds). Biological Methylation and Drug Design. Bethesda, MD. The Human Press, 1986, p 227.
23. Monreal M, Callejas JM, Martorell A, Silveira P, Gallego M, Lafoz E, Casals A. Occlusive arterial disease as a form of presentation of homocystinuria. J Cardiovascular Surg 32:137, 1991.
24. Schimke RN, McKusick VA, Huang T, Pollack AD. Homocystinuria. JAMA 193:711, 1965
25. V.D. Berg W, Verbraak FD, Bos PJ. Homocystinuria presenting as central retinal artery occlusion and longstanding thromboembolic disease. Brit J Ophthalmol 74:696, 1990.
26. Wilson RS, Ruiz RS. Bilateral central retinal artery occlusion in homocystinuria. Arch Ophthalmol 82:267, 1969.
27. Brandstetter Y, Weinhouse E, Splaingard ML, Tang TT. Cor pulmonale as a complication of methylmalonic acidemia and homocystinuria (Cbl-C type). Am J Med Genet 36:167, 1990.
28. Pousse H, Ayachi R, Essoussi AS, el May A, al Amri H. Two familial cases of homocystinuria one of which revealed by fatal hypertensive encephalopathy [Fre]. Annal Pediatr 37:189, 1990.
29. Cusworth DC, Dent CE. Homocystinuria. Br Med Bull 25:42, 1969.
30. McDonald L, Bray C, Field C, Love F, Davies B. Homocystinuria, thrombosis, and the blood-platelts. Lancet 1:745, 1964.
31. Hilden M, Brandt NJ, Nilsson IM, Schonheyder F. Investigations of coagulation and fibrinolysis in homocystinuria. Acta Med Scand 195:533, 1974.
32. Graeber JE, Slott JH, Ulane RE, Schulman JD, Stuart MJ. Effect of homocysteine and homocystine on platelet and vascular arachidonic acid metabolism. Pediatr Res 16:490, 1982.
33. McKusick VA. Heritable Disorders of Connective Tissue, 4th ed. St. Louis, CV Mosby, 1972, p 224.
34. Harker LA, Ross R, Slichter SJ, Scott CR. Homocystine induced arteriosclerosis: The role of endothelial cell injury and platelet response in its genesis. J Clin Invest 58:731, 1976.
35. Wall RT, Harlan JM, Harker LA, Striker GE. Homocysteine induced endothelial cell injury in vitro: A model for the study of vascular injury. Thromb Res 18:113, 1980.
36. Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. J Clin Invest 77;1370, 1986.
37. Rodgers GM, Kane WH. Activation of endogenous factor V by a homocysteine-induced vascular endothelial cell activator. J Clin Invest 77:1909, 1986.
38. Mudd SH. Biochemical mechanisms in methyl group transfer, in Fishman WH (ed): Metabolic Conjugation and Metaoblic Hydrolysis. New York, Academic, vol 3, 1973, p 297.
39. Mudd SH, Klee WA, Ross PD. Enthalpy changes accompanying the transfer of a methyl group from S-adenosylmethionine and other sulfonium compounds to homocysteine. Biochemistry 5:1653, 1966.
40. Klee WA, Richards HH, Cantoni GL. The synthesis of methionine by enzymic transmethylation, VII. Existence of two separate homocysteine methylpherases in

mammalian liver. Biochim Biophys Acta 54:157, 1961.
41. Taylor RT, Weissbach H. $N^5$0methyltetrahydrofolate-homocysteine methyltransferases, in Boyer PD (ed): The Enzymes, 3d ed. New York, Academic, 1973, vol 9, part b, p 121.
42. Durell J, Anderson DG, Cantoni GL. The synthesis of methionine by enzymic transmethylation. I. Purification and properties of the tin homocysteine methylpherase. Biochim Biophys Acta 26:270, 1957.
43. Wilcken DEL, Wilcken B, Dudman, NPB, Tyrrell PA. Homocystinuria - the effects of betaine in the treatment of patients not responsive to pyridoxine. N Engl J Med 309:448, 1983.
44. Wilcken DEL, Dudman NPB, Tyrrell PA. Homocystinuria due to cystathionine B-synthase deficiency - the effects of betaine treatment in pyridoxine-responsive patients. Metabolism 34:1115, 1985.
45. Pullon DHH. Aspects of treatment of homocystinuria: An illustrative case report. NZ Med J 101:10, 1988.
46. Mudd SH, Edwards WA, Loeb PM, Brown MS, Laster L. Homocystinuria due to cystathionine synthase deficiency: The effect of pyridoxine. J Clin Invest 49:1762, 1970.
46a. Munke M, Kraus JP, Ohura T, Francke U. The gene for cystathionine *B*-synthase (CBS) maps to the subtelomeric region on human chromosome 17. Am J Hum Genet 42:550, 1988.
46b. Kraus JP. Molecular analysis of cystathionine beta-synthase-- a gene on chromosome 21. Prog Clin & Bio Res 360:201, 1990.
46c. Skovby F, Kraus JP, Rosenberg LE. Biosynthesis of human cystathionine *B*-synthase in cultured fibroblasts. J Biol Chem 259:583, 1984.
46d. Kraus JP, Rosenberg LE. Cystathionine *B*-synthase from human liver: Improved purification scheme and additional characterization of the enzyme in crude and pure form. Arch Biochem Biophys 222:44, 1983.
47. Wilcken B, Turner B. Homocystinuria: Reduced folate levels during pyridoxine treatment. Arch Dis Child 48:58, 1973.
48. Morrow G III, Barness LA. Combined vitamin responsiveness in homocystinuria. J Pediatr 81:946, 1972.
49. Shih VA, Efron ML. Pyridoxine-unresponsive homocystinuria. N Engl J Med 283:1206, 1970.
50. Fenton WA, Rosenberg LE. Inherited disorders of cobalamin transport and metabolism, in Scriver CR, Beaudet AL, Sly WS, Valle D (eds). The Metabolic Basis of Inherited Diseases, 1989, p 2065-2082.
51. Ribes A, Briones P, Vilaseca MA, Lluch M, Rodes M, Maya A, Campistol J, Pascual P, Suormala T, Baumgartner R. Methylmalonic aciduria with homocystinuria: Biochemical studies, treatment and clinical course of a Cbl-C patient. Eur J Pediatr 149:412, 1990.
52. Cooper BA, Rosenblatt DS. Inherited defects of vitamin B_{12}-responsive neonatal megaloblastic anemia and homocystinuria with associated reduced methionine synthase activity. Blood 69:1128, 1987.
53. Boers G, Smals A, Kloppenborg P, Trijbels F, Bakkeren J, Schoonderwaldt H, Fowler B, Kleijer W. Heterzygosity for homocystinuria in premature arterial disease. N Engl J Med 314:850, 1986.
54a. Mudd SH, Havlik R, Levy HL, McKusick VA, Feinleib M. A study of cardiovascular risk in heterozygotes for homocystinuria. Am J Hum Genet 33:883, 1981.
54b. Boers GH, Smals AG, Trijbels FJ, Leermakers AL, Kloppenborg PW. Unique efficiency

of methionine metabolism in premenopausal women may protect against vascular disease in the reproductive years. J Clin Invest 72:1971, 1983.
55. Swift ME, Shultz TD. Relationship of vitamins B-6 and B-12 to homocysteine levels: Risk for coronary artery disease. Nutr Rep Interna 34:1, 1986.
56. Clarke R, Daly L, Robinson K, et al. Hyperhomocysteinemia: An independent risk factor for vascular disease. New Eng J Med 324:1149, 1991.
57. Goldstein JL, Campbell BK, Gartler SM. Homocystinuria: Heterozygote detection using phytohemagglutinin-stimulated lymphocytes. J Clin Invest 52:218, 1973.
58. Murphy-Chutorian DR, Wexman MP, Grieco AJ, Heininger JA, Glassman E, Gaull GE, NG SKC, Feit F, Wexman K, Fox AC. Methionine intolerance: A possible risk factor for coronary artery disease. J Am Coll Cardiol 6:725, 1985.
59. McKusick VA. Heritable disorders of connective tissue. Fourth edition CV Mosby, St. Louis 1972, pp 61-223.
60. Pyeritz RE, McKusick VA. The Marfan syndrome: diagnosis and management. N Engl J Med 300:772, 1979.
61. Cross HE, Jensen AD. Ocular manifestations in the Marfan syndrome and homocystinuria. Am J Ophthalmol 75:405, 1973.
62. McKusick VA. The cardiovascular aspects of Marfan's syndrome: a heritable disorder of connective tissue. Circulation 11:321, 1955.
63. Brown OR, DeMots H, Kloster FE, et al. Aortic root dilatation and mitral valve prolapse in Marfan's syndrome: an echocardiographic study. Circulation 52: 651, 1975.
64a. Come PC, Bulkley BH, McKusick VA, et al. Echocardiographic recognition of silent aortic root dilatation in Marfan's syndrome. CHest 72:789, 1977.
64b. Geva T, Sanders SP, Diogenes MS, Rockenmacher S, Van Praagh R. Two-dimensional and Doppler echocardiographic and pathologic characteristics of the infantile Marfan syndrome. Am J of Cardio 65:1230, 1990.
65. Atsuchi Y, Nagai Y, Komatsu Y, et al. Echocardiographic manifestations of annulo-aortic ectasia: its "paradoxical" motion of the aorta and premature systolic closure of the aortic valve. Am Heart J 93:428, 1977.
66. Walker BA, Beighton PH, Murdoch JL. The marfanoid hypermobility syndrome. Ann Int Med 71:349, 1969.
67. Beals RK, Hecht F. Congenital contractural arachnodactyly: a heritable disorder of connective tissue. J Bone Joint Surg 53:987, 1971.
68. Lipton RA, Greenwald RA, Seriff NS. Pneumothorax and bilateral honeycombed lung in Marfan syndrome: report of a case and review of the pulmonary abnormalities in this disorder. Am Rev Respir Dis 104:924, 1971.
69. Murdoch JL, Walker BA, McKusick VA. Parental age effects on the occurrence of new mutations for the Marfan syndrome. Ann Hum Genet 35:331, 1972.
70. Murdoch JL, Walker BA, Halpern BL, et al. Life expectancy and causes of death in the Marfan syndrome. N Engl J Med 286:804, 1972.
71. Svensson LG, Crawford ES, Coselli JS, Safi HJ, Hess KR. Impact of cardiovascular operation in the Marfan patient. Circulation 80:I233, 1989.
72. Skovby F, McKusick VA. Estrogen treatment of tall stature in girls with the Marfan syndrome. Birth Defects 13(3C):155, 1977.
73. Abraham PA, Perejda AJ, Carnes WH, Uitto J. Marfan syndrome: demonstration of abnormal elastin in the aorta. J Clin Invest 70:1245, 1982.
74. Boucek KJ, Noble NL, Gunja-Smith Z, Butler WT. The Marfan syndrome: a deficiency

in chemically stable collagen cross-links. N Engl J Med 305:988, 1981.
75. Harley VR, Chan D, Rogers JG, Cole WG. Marfan syndrome: absence of type I or III collagen structural defects in 25 patients. J Inherited Metab Dis 13:219, 1990.
76. Dalgleish R, Hawkins JR, Keston M. Exclusion of the alpha 2(I) and alpha 1 (III) collagen genes as the mutant loci in a Marfan syndrome family. J Med Genet 24:148, 1987.
77. Francomano CA, Streeten EA, Meyers DA, Pyeritz RE. Exclusion of fibrillar procollagens as causes of Marfan syndrome. Am J Med Genet 29:457, 1988.
78. Ogilvie DJ, Wordsworth BP, Priestley LM, Dalgleish R, Schmidtke J, Zoll B, Sykes BC. Segregation of all four major fibrillar collagen genes in the Marfan syndrome. Am J Hum Genet 41:1071, 1987.
79. Tsipouras P, Borresen AL, Bamforth S, Harper P$, Berg K. Marfan syndrome: exclusion of genetic linkage to the COL1A2 gene. Clin Genet 30:428, 1986.
80. Byers PH, Siegel RC, Peterson KE, Rowe DW, Holbrooke KA, Smith LT, Chang YH, et al. Marfan syndrome: abnormal a2 chain in type I collagen. Proc Natl Acad Sci USA 78:7745, 1981.
81. Phillips CL, Shrago-Howe AW, Pinnell SR, Wenstrup RJ. A substitution at a non-glycine position in the triple-helical domain of pro-alpha 2(I) collagen chains present in an individual with a variant of the Marfan syndrome. J Clin Invest 86:1723, 1990.
82. Pulkkinen L, Kainulainen K, Krusius T, Makinen P, Schollin J, Gustavsson KH, Peltonen L. Deficient expression of the gene coding for decorin in a lethal form of Marfan syndrome. J Biol Chem 265:17780, 1990.
83. Kainulainen K, Pulkkinen L, Savolainen A, Kaitila I, Peltonen L. Location on chromosome 15 of the gene defect causing Marfan syndrome. N Engl J Med 323:935, 1990.
84. Dietz HC, Pyeritz RE, Hall BD, Cadle RG, Hamosh A, Schwartz J, Meyers DA Francomano CA. The Marfan syndrome locus: confirmation of assignment to chromosome 15 and identification of tightly linked markers at 15q15-q21.3. Genomics 9:355, 1991.
85. Tsipouras P, Sarfarazi M, Devi A, Weiffenbach B, Boxer M. Marfan syndrome is closely linked to a marker on chromosome 15q1.5q2.1. Proc Natl Acad Sci USA 88:4486, 1991.
86. Godfrey M, Menashe Vv, Webber RG, et al. Cosegregation of elastin-associated microfibrillar abnormalities with the Marfan phenotype in families. Am J Hum Genet 46:652, 1990.
87. Cleary EG, Gibson MA. Elastin-associated microfibrils and microfibrillar proteins. Int Rev Connect Tissue Res 10:97, 1983.
88. Sakai LY, Keene DR, Engvall E. Fibrillin, a new 350kd glycoprotein is a component of extracellular microfibrils. J Cell Biol 103:2499, 1986.
89. Hollister DW, Godfrey M, Sakai LY, Pyeritz RE. Immunohistologic abnormalities of the microfibrillar-fiber system in the Marfan syndrome. N Engl J Med 323:152, 1990.
90. Tsipouras PA. A workshop on Marfan syndrome, 10 June 1989, Farmington, CT, USA. J Med Gene 27:139, 1990.