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The William Allan Memorial Award Lecture:

Genetic Nosology: Three Approaches

VICTOR A. MCKUSICK[1]

I am deeply grateful for being selected William A. Allan Awardee. It is a nice feeling to be appreciated by one's colleagues. I am particularly pleased to receive the Allan Award from this year's president. Twenty years ago on the shores of Puget Sound and on the banks of the Chesapeake, a parallel development of novel type took place—a division of medical genetics in a department of medicine. Because of this and other parallelisms, Arno and I have always felt a strong brotherhood, with only a little sibling rivalry!

Receiving this award reminds me that I have been very lucky, in many ways. I shall mention only three ways without amplifying on any of them. I have been lucky in time and place. I have been lucky in my colleagues, including many able junior colleagues. I have been lucky in having a rich variety of fascinating topics available to me for study.

This reference to topics for study leads me directly to the subject of this discourse. At the risk of spreading myself too thin, I have chosen to speak to you on three topics that have absorbed my attention for the last 15 to 25 years: heritable disorders of connective tissue (work initiated more than 25 years ago), the gene map of the X and other human chromosomes (an interest for about 20 years), and the clinical population genetics of the Old Order Amish (an ongoing study of 15 years' standing). A scarlet thread running through the three is genetic nosology, which I prefer to define as the delineation of genetic diseases.

HERITABLE DISORDERS OF CONNECTIVE TISSUE

The concept of generalized Mendelian defects of connective tissue has, it seems, been a useful one, and the term for them "heritable disorders of connective tissue" has proved durable. The growth in the field is reflected by the steadily increasing size of successive editions of *Heritable Disorders of Connective Tissue* [1], beginning with the first in 1956. In part, this has been due to the addition of new chapters prompted by nosologic advance (e.g., the addition of a chapter on homocystinuria beginning with the 1966 edition) but in larger part to the burgeoning of nosologic information in each of the originally discussed areas.

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[1] Department of Medicine, Johns Hopkins University School of Medicine and Johns Hopkins Hospital, Baltimore, Maryland 21205.

Heritable disorders of connective tissue can be divided into those that affect primarily the fibrous elements (collagen and elastin) and those involving the ground substance, specifically mucopolysaccharide. Delineation of heterogeneity in the Marfan syndrome, osteogenesis imperfecta, and pseudoxanthoma elasticum is progressing slowly [2]. Success has been somewhat greater in the case of the Ehlers-Danlos syndrome because biochemical characterization has come to the aid of the clinical approach; eight forms have been tentatively identified [3, 4].

As reflected in the chart of relative growth rate (fig. 1), nosologic progress has been greatest in the mucopolysaccharidoses, especially since 1960. Tracing these advances illustrates the intimate interdependence of clinical, genetic, and biochemical studies in genetic nosology [5].

In the 1956 and 1960 editions of *Heritable Disorders of Connective Tissue,* the chapter on the mucopolysaccharidoses was entitled "The Hurler Syndrome." Already in 1956, however, we recognized that two different forms existed: a severe disorder that appeared to be autosomal recessive and a second clinically milder form that appeared to be X-linked. The 1956 edition presented a table contrasting the two forms in regard to corneal clouding and other features, and further suggested that "one might, with historic justification, refer to the disorder inherited as an autosomal recessive as the Hurler syndrome, and to that inherited as a sex-linked recessive as the Hunter syndrome . . ." (p. 176).

In discussing "the future in the study of heritable disorders of connective tissue" the 1956 edition stated (p. 210):

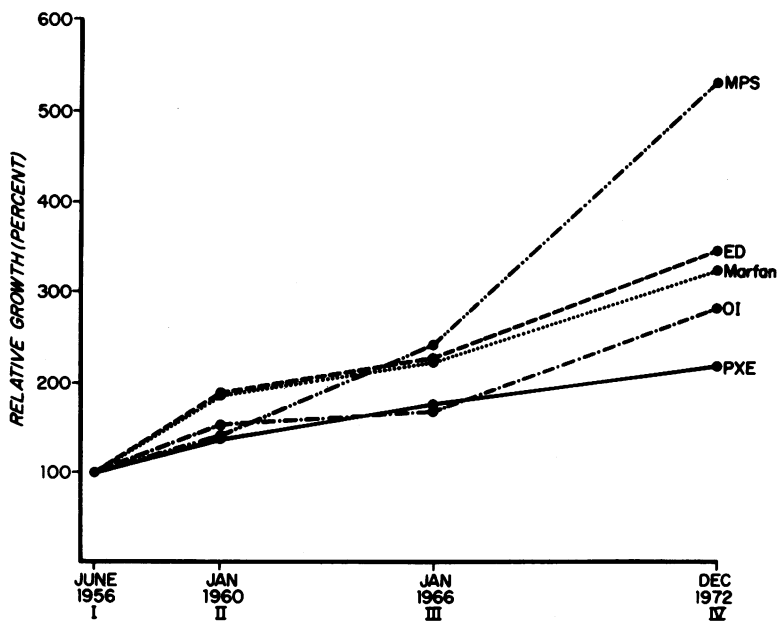

FIG. 1.—Relative growth of *Heritable Disorders of Connective Tissue* (in percent of page numbers in 1956 edition).

> Tissue culture of fibroblasts is possibly one of the more promising, although as yet unexplored, techniques for the study of heritable disorders of connective tissue. (In general, tissue culture has been too little used in physiological genetics.) . . . the first objective of tissue culture studies should be in the *in vitro* replication of the morphologic abnormalities. . . . in the Hurler syndrome one can with justification anticipate success. . . . i.e., the "gargoyle cell" may be demonstrable in culture.

Nine years later, Danes and Bearn [6] confirmed the prediction. Although the metachromasia they used as the cellulo-phenotypic equivalent of the disease has been superceded by cellular characteristics more specific because they are closer to the primary action of the mutant gene, Danes and Bearn gave a "shot in the arm" to the field. But I am getting ahead of the story.

Between the 1956 and 1960 editions, Dorfman and Meyer independently discovered mucopolysaccharidurıa. It was in the period between the 1960 and the 1966 editions that the pattern of specific mucopolysaccharides in the urine was exploited, in combination with analysis of phenotype and family patterns, to classify the mucopolysaccharidoses into six separate entities, each designated by a Roman numeral and alternatively by an eponym (fig.2).

Between the 1966 and the 1972 edition, Dr. Elizabeth Neufeld and her colleagues appeared on the scene. In experiments, now classic, using cultured fibroblasts, they demonstrated deficiency of so-called corrective factors in individual mucopolysaccharidoses. The six-way classification was corroborated, with two notable exceptions—two separate defects were demonstrated as leading to the same phenotype, the Sanfilippo syndrome (MPS III); the defect in MPS I and V (Hurler and Scheie syndromes) involved the same corrective factor or enzyme (subsequently shown to be α-L-iduronidase), despite the widely different phenotype. This suggested that the Hurler and Scheie syndromes were the homozygotes for two different alleles. The Hurler syndrome was compared to SS disease among the hemoglobinopathies and the Scheie syndrome to CC disease. By further analogy, a genetic compound comparable to SC disease was predicted and tentatively identified with a characteristic phenotype. These patients long outlive the Hurler patients but are shorter of stature and much more severely handicapped than are the Scheie patients. Like SC disease, the presumed Hurler-Scheie compound not only has a phenotype of severity intermediate between that of the two homozygotes, but also has some unique features, particularly a receding jaw and generally characteristic facies.

That the Hurler and Scheie syndromes are determined by allelic genes is supported by failure of complementation in cell-fusion studies by H. Galjaard (personal communication, 1975). Whether the so-called Hurler-Scheie compound is that and not a homozygote for another allele at the iduronidase locus we cannot say. None of the parents of suspected genetic compound cases are consanguineous as might be the case if the patients are in fact homozygotes. The 1972 classification (fig. 3) postulated allelic forms of MPS II and MPS VI as well, as the basis for phenotypic diversity observed with deficiency of iduronate sulfatase and arylsulfatase B, respectively.

In 1965, Hers [7] defined the following five characteristics of lysosomal diseases: (1) intracellular storage of material; (2) storage material is heterogeneous; (3) deposition is

1966
THE GENETIC MUCOPOLYSACCHARIDOSES

MPS I	Hurler	Early clouding of cornea, grave manifestations	Autosomal recessive	Dermatan sulfate Heparan sulfate	
MPS II	Hunter	No clouding of cornea, milder course	X-linked recessive	Dermatan sulfate Heparan sulfate	
MPS III	Sanfilippo	Mild somatic, severe central nervous system effects	Autosomal recessive	Heparan sulfate	
MPS VI	Morquio	Severe, distinctive bone changes, cloudy cornea, aortic regurgitation	Autosomal recessive	Keratan sulfate	
MPS V	Scheie	Stiff joints, cloudy cornea, aortic valve disease, normal intelligence, and (?) life-span	Autosomal recessive	Dermatan sulfate Heparan sulfate	
MPS IV	Maroteaux–Lamy	Severe osseous and corneal change, normal intellect	Autosomal recessive	Dermatan sulfate	

FIG. 2

vacuolar (i.e., membrane bound); (4) several tissues and organs are involved; and (5) the disorders are progressive. A sixth, the potential for enzyme replacement, was added later. Two other striking features of lysosomal diseases might be added. (1) Allelic mutations lead to widely diverse phenotypes [8]. This is the Hurler-Scheie phenomenon, which occurs also in the various forms of Gaucher disease, Niemann-Pick disease, G_{M1}-gangliosidosis, metachromatic leukodystrophy, fucosidosis, and Tay-Sachs disease—indeed probably in most lysosomal diseases. (2) The same phenotype may be produced by any one of several different enzyme deficiencies. This

1972

THE GENETIC MUCOPOLYSACCHARIDOSES

			GENETICS		
MPS I H	Hurler	Clouding of cornea, grave manifestations, death usually before age 10	Homozygous for MPS I H gene	Dermatan sulfate Heparan sulfate	α-L-iduronidase
MPS I S	Scheie	Stiff joints, cloudy cornea, aortic valve disease, normal intelligence and (?) life-span	Homozygosity for MPS I S gene	Dermatan sulfate Heparan sulfate	α-L-iduronidase
MPS I H/S	Hurler-Scheie	Intermediate phenotype	Genetic compound of MPS I H and MPS I S genes	Dermatan sulfate Heparan sulfate	α-L-iduronidase
MPS II, severe	Hunter, severe	No corneal clouding, milder course than in MPS I H, death before 15 years	Hemizygous for X-linked gene	Dermatan sulfate Heparan sulfate	Hunter correction factor
MPS II, mild	Hunter mild	Survival to 30s to 60s, fair intelligence	Hemizygous for X-linked allele	Dermatan sulfate Heparan sulfate	Hunter correction factor
MPS III A	Sanfilippo A	Indistinguishable phenotype: Mild somatic, severe central nervous system effects	Homozygous for Sanfilippo A gene	Heparan sulfate	Heparan N-sulfatase
MPS III B	Sanfilippo B		Homozygous for Sanfilippo B gene	Heparan sulfate	N-acetyl-α-D-glucosaminidase
MPS IV	Morquio	Severe, distinctive bone changes, cloudy cornea, aortic regurgitation	Homozygous for Morquio gene	Keratan sulfate	
MPS V	VACANT				
MPS VI, severe	Maroteaux-Lamy, classic severe	Severe osseous and corneal change; valvular heart disease; striking WBC inclusions; normal intellect; survival to 20s	Homozygous for Maroteaux-Lamy (M-L) gene	Dermatan sulfate	Arylsulfatase B (N-acetylgalactosamine 4-sulfatase)
MPS VI, mild	Maroteaux-Lamy, mild	Mild osseous and corneal change, normal intellect, aortic stenosis	Homozygous for allele at M-L locus	Dermatan sulfate	Arylsulfatase B (N-acetylgalactosamine 4-sulfatase)
MPS VII	β-Glucuronidase deficiency	Hepatosplenomegaly, dysostosis multiplex, mental retardation variable; WBC inclusions	Homozygous for mutant gene at β-glucuronidase locus	Dermatan sulfate Heparan sulfate	β-Glucuronidase

FIG. 3

is the Sanfilippo phenomenon. Another example: angiokeratoma is produced not only by the α-galactosidase deficiency of classic Fabry disease, but also by one form of α-fucosidase deficiency [9]. Because of the heterogeneity (multiplicity) of substrates on which the lysosomal enzymes can operate, it is perhaps not unexpected that different mutant enzymes might have different substrate repertories with different phenotypic consequences. (See O'Brien [10, 11] for biochemical confirmation of this expectation.) Furthermore, since multiple enzymes are required for the stepwise degra-

dation of many macromolecules (e.g., heparan sulfate), phenotypically similar or identical diseases might be expected from any one of several enzyme deficiencies. These two characteristics hold for most categories of Mendelian disease, but they seem to be exaggerated in the lysosomal diseases.

Since 1972 (fig. 4), the precise enzymatic nature of the corrective factors deficient in the Hunter and Maroteaux-Lamy syndromes has been established; the enzymatic

October, 1977

THE GENETIC MUCOPOLYSACCHARIDOSES

MPS I H	Hurler	Clouding of cornea, grave manifestations, death usually before age 10	Homozygous for MPS I H gene	Dermatan sulfate Heparan sulfate	α-L-iduronidase
MPS I S	Scheie	Stiff joints, cloudy cornea, aortic valve disease, normal intelligence and (?) life-span	Homozygosity for MPS I S gene	Dermatan sulfate Heparan sulfate	α-L-iduronidase
MPS I H/S	Hurler-Scheie	Intermediate phenotype	Genetic compound of MPS I H and MPS I S genes	Dermatan sulfate Heparan sulfate	α-L-iduronidase
MPS II-XR, severe	Hunter, severe	No corneal clouding, milder course than in MPS I H, death before 15 years	Hemizygous for X-linked gene	Dermatan sulfate Heparan sulfate	Iduronate sulfatase
MPS II-XR, mild	Hunter mild	Survival to 30s to 60s, fair intelligence	Hemizygous for X-linked allele	Dermatan sulfate Heparan sulfate	Iduronate sulfatase
? MPS II-AR	? Autosomal Hunter	Same as mild or severe MPS II-XR	Homozygous for autosomal gene	Dermatan sulfate Heparan sulfate	Iduronate sulfatase
MPS III A	Sanfilippo A	Indistinguishable phenotype: Mild somatic, severe central nervous system effects	Homozygous for Sanfilippo A gene	Heparan sulfate	Heparan N-sulfatase
MPS III B	Sanfilippo B		Homozygous for Sanfilippo B gene	Heparan sulfate	N-acetyl-α-D-glucosaminidase
MPS III C	Sanfilippo C		Homozygous for Sanfilippo C gene	Heparan sulfate	α-Glucosaminidase
MPS IV	Morquio	Severe, distinctive bone changes, cloudy cornea, aortic regurgitation	Homozygous for Morquio gene	Keratan sulfate	Galactosamine-6-sulfate sulfatase
MPS V	VACANT				
MPS VI, severe	Maroteaux-Lamy, classic severe	Severe osseous and corneal change; valvular heart disease; striking WBC inclusions; normal intellect; survival to 20s	Homozygous for Maroteaux-Lamy (M-L) gene	Dermatan sulfate	Arylsulfatase B (N-acetylgalactosamine 4-sulfatase)
MPS VI, intermediate	Maroteaux-Lamy, intermediate	Moderately severe changes	Homozygous for allele at M-L locus or genetic compound	Dermatan sulfate	Arylsulfatase B (N-acetylgalactosamine 4-sulfatase)
MPS VI, mild	Maroteaux-Lamy, mild	Mild osseous and corneal change, normal intellect, aortic stenosis	Homozygous for allele at M-L locus	Dermatan sulfate	Arylsulfatase B (N-acetylgalactosamine 4-sulfatase)
MPS VII	β-Glucuronidase deficiency	Hepatosplenomegaly, dysostosis multiplex, mental retardation variable; WBC inclusions	Homozygous for mutant gene at β-glucuronidase locus	Dermatan sulfate Heparan sulfate	β-Glucuronidase
MPS VIII	Glucosamine-6-sulfate sulfatase deficiency	Short stature, mild dysostosis multiplex, ring-shaped metachromasia of lymphocytes	Homozygous for MPS VIII gene	Keratan sulfate Heparan sulfate	Glucosamine-6-sulfate sulfatase

FIG. 4

deficiency in the Morquio syndrome has been identified; two further mucopolysaccharidoses (MPS VII* and MPS VIII) have been added, an autosomal recessive form of iduronate sulfatase deficiency (i.e., an autosomal recessive form of the Hunter syndrome) has been suggested, and a third phenotypically indistinguishable but enzymatically distinct form of the Sanfilippo syndrome has come to light.

In the future, yet further mucopolysaccharidoses will almost certainly be discovered. This follows from the fact that the deficiency state of some enzymes involved in degradation of heparan sulfate and dermatan sulfate have not yet been found. Other enzymatic bases of previously described phenotypes such as the Morquio syndrome are likely to be found, as well as further allelic varieties of some of the other presently known mucopolysaccharidoses.

THE GENE MAP OF THE HUMAN CHROMOSOMES

As represented in figure 5, at least one gene locus has been assigned with confidence to each of man's 24 chromosomes [12]. In all, over 240 gene loci have been assigned; over 100 have been assigned to the X and about 140 to specific autosomes. Some of the chromosomes are becoming rather crowded, and regional localization on particular chromosomes has been achieved for many loci.

It is a matter of intellectual satisfaction that the colorblindness and classic hemophilia loci, two of the genes longest recognized in man, are known to be situated at the distal end of the long arm of the X; that the Rh locus is toward the end of the short arm of chromosome 1; that the ABO blood group locus is near the end of the long arm of chromosome 9; and that the major histocompatibility complex is determined by genes on the short arm of chromosome 6.

This is all the more remarkable when it is remembered that 10 years ago not a single valid autosomal gene assignment had been made in man. The first linkage of autosomal loci, secretor/Lutheran, was discovered in 1951 by Jan Mohr using the family method [13]. In the next 17 years, arduous study, mainly by the family method, uncovered in all five pairs of linked loci, one trio of linked loci and two tight linkage groups, but it was not until 1968 that a specific gene was assigned to a specific autosome. In several ways 1968 was a watershed year. That year Donahue et al. [14] assigned the Duffy blood group locus to chromosome 1 (by the family method). About the same time, Weiss and Green [15] assigned the thymidine kinase locus to a specific autosome (later shown to be 17) by study of clones derived from interspecies hybrid cells, and soon after Caspersson's group [16], as well as others, introduced chromosome banding methods for unique identification of chromosomes and parts of chromosomes. Thus, the two techniques that have done most for recent progress in mapping, cell hybridization and chromosome banding, became available.

In both mouse and man the situation that at least one gene had been assigned to each chromosome was reached in the spring of 1976 (T. H. Roderick, personal communication). It is interesting to compare the earlier progress of mapping in man and mouse. The first autosomal linkage in the mouse, *albinism* and *pinkeye*, indeed the first

* In fact, MPS VII, β-glucuronidase deficiency, was discovered in time for the 1972 edition, as indicated by the table shown as figure 3.

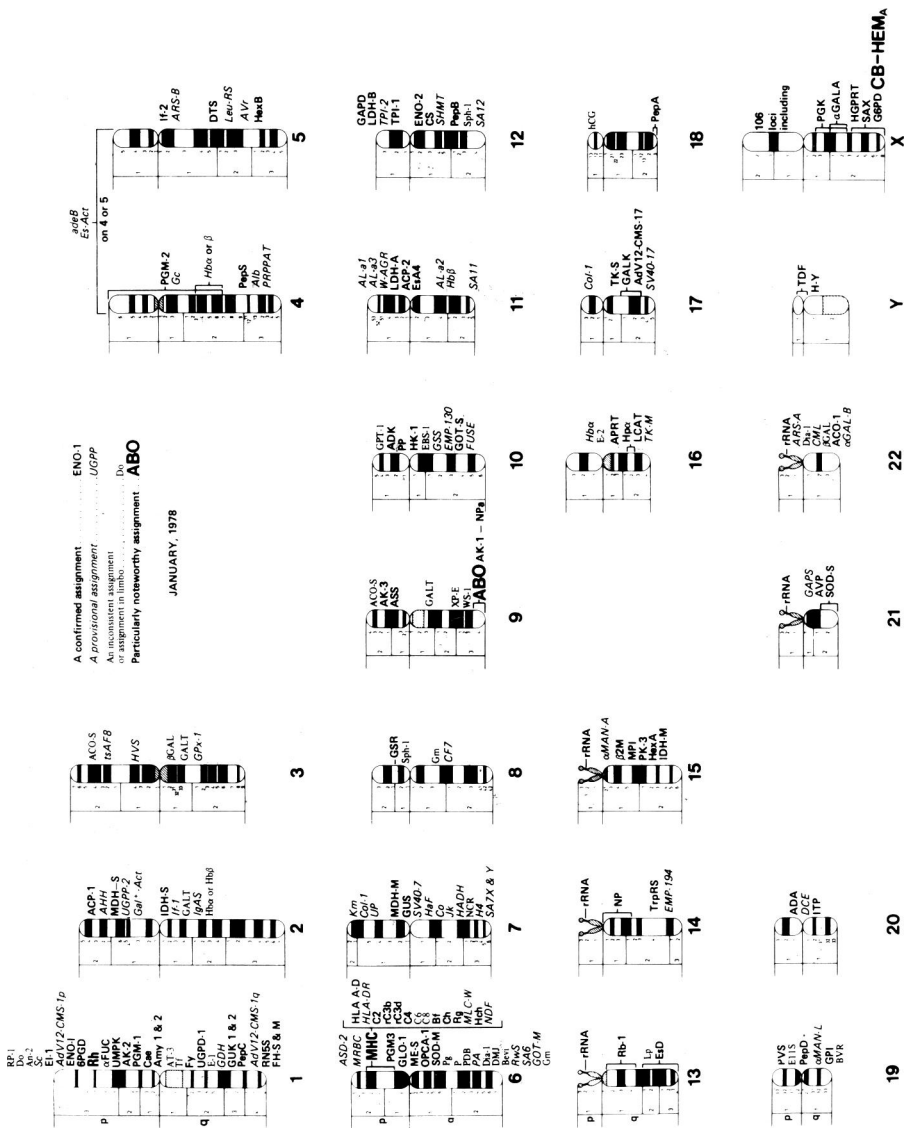

FIG. 5.—A gene map of the human chromosome, January 1, 1978.

autosomal linkage in any mammal, was established in 1915 by Haldane et al. [17]. The first X-linkage in the mouse was not found, however, until 1953 by which time 12 autosomal linkage groups (two were later shown to be on the same chromosome) were known in that species [18]. The situation is precisely the opposite in man: by the time the first autosomal linkage group was discovered in 1951 [13], about 36 X-linkages were known.

A brief review of the mapping of one part of the X chromosome may illustrate the development of human chromosome mapping in general. A characteristic pedigree pattern of colorblindness was known at least since Horner [19] in the last century and had been adumbrated in the observations of Dalton [20] in his own family a century before that. The first gene to be assigned to a specific chromosome in man, perhaps in any organism, was colorblindness assigned to the X chromosome by Wilson in 1911 [21]. The first genetic interval in man, that between the hemophilia and colorblindness loci, was estimated by Haldane et al. [22, 23]. His estimate was confounded by genetic heterogeneity (i.e., existence of two X-linked hemophilias—one tightly linked to colorblindness and one unlinked—a fact that C.A.B. Smith (personal communication, 1960) demonstrated on re-examination of the original data. In the early 1960s, three groups [24–26] showed close linkage of the colorblindness loci and the G6PD locus, and in 1965 Boyer and Graham [27] reported the close linkage of G6PD and hemophilia A. Assignment of the colorblindness cluster to the long arm of the X was achieved by Ricciuti and Ruddle [28] using the KOP X/14 translocation in the mouse-human hybrid cell system and using G6PD as the member of the cluster that could be studied in cultured cells. Several workers [29], studying other aberrant X chromosomes in the hybrid cell system, narrowed the assignment of G6PD (and indirectly the closely linked hemophilia and colorblindness loci) to the distal third of the long arm.

New methods, some presented at this meeting, can be expected to contribute further to filling up the map. Although a lion's share of the chromosomal assignments have been achieved by the method of somatic cell hybridization, the family method has not been unproductive; there is mutual potentiation of the two approaches. I count about 40 linkages found since 1968 by the family method.

THE CLINICAL POPULATION GENETICS OF AN INBRED GROUP

The Old Order Amish [30] represent an almost strictly endogamous religious sect. Although descended from a limited number of founders, many of whom immigrated to the United States before the American Revolution, the Amish now number over 75,000 persons. The group is made up of a number of moderately distinct demes. The coefficient of inbreeding for these populations is high; for example, in the Lancaster County (Pennsylvania) Amish community, the coefficient is .026 (a minimal estimate). This is the equivalent of all couples being related slightly less closely than first cousins once removed. Only two of 1,849 married couples (in the Lancaster community in 1973) were not demonstrably consanguineous (D. Bolling, personal communication, 1977), at least remotely.

In each deme, the influence of founder effect is evident from the distinctive distribution of family names; for example, seven family names, each of them

originating from a unique immigrant founder, account for over 78% of Lancaster County Amish, total population about 13,000 (table 1), and a different set of seven names account for about an equal portion of the Holmes County (Ohio) Amish. Founder effect is analogous to cloning. It is as though the Amish immigrant founders were "streaked out" like bacteria across the belt of America east of the Mississippi, as though, when colonies sprang up—not, to be sure, from single individuals, yet from a small number of persons—an assay was performed on the genome of the founders. Each Amish deme tends to have its characteristic collection of recessive disorders. Random genetic drift can contribute further to the enrichment of specific genes in each deme.

In addition to high consanguinity, founder effect, and drift, large family size increases the "visibility" of recessives by increasing the probability of more than merely one sib being affected. Furthermore, the sociologic distinctiveness of the Amish, a marker for the extended family they essentially represent, serves to highlight any anomalous phenotype that occurs among them. "Groupness" as a factor in increased visibility of recessives may be illustrated by thalassemia. This condition (or these conditions), although frequent around the Mediterranean Sea, which subsequently gave the now generally used name to the disease, was first clearly described, not in the Mediterranean littoral, but in Detroit, Michigan, by pediatrician Cooley [31]. (We tend to forget that thalassemia was called Cooley's anemia for many years, although I note that the eponym is being substituted for the tongue-twister professional designation in such uses as federal legislation.) Cooley could not but be impressed with the uniqueness of the severe anemic disorder, in considerable part because it occurred in children of a particular ethnic group. It is true that although in his case reports he noted Mediterranean origin, Cooley [31] did not list ethnic extraction as one of the six "reasons for putting the cases in one group."

Capitalizing on the factors for increased visibility of recessives among the Amish, our group and others have uncovered 12 or more new recessive disorders. Cartilage-hair hypoplasia is a paradigm of the usefulness of inbred groups for the description of "new" recessives. Almost 80 cases were found among the Amish in the initial study published in 1965 [32], and probably the number now approaches 100. In addition to the defect in cartilage and hair, there are three features highly variable in expression: an

TABLE 1

FREQUENCY AND ORIGIN OF SURNAMES OF THE 1,849 MARRIED MEN IN THE LANCASTER COUNTY AMISH SETTLEMENT IN 1973

FAMILY NAME	FREQUENCY		FOUNDING IMMIGRANT	DATE OF IMMIGRATION
	No.	%		
Stoltzfus	475	25.7	Nicholas Stoltzfus	1766
King	233	12.6	Samuel King	1744
Fisher	203	11.1	Christian Fisher	1750
Beiler	198	10.7	Jacob Beiler	1737
Esch, Esh	121	6.5	Jacob Esch	1751
Lapp	119	6.4	John Lapp	1773
Zook	100	5.4	John Zug	1742

immune deficiency (leading to lymphopenia and severe, even fatal varicella), a nonproliferative disorder of myeloid cells (leading to anemia and neutropenia), and an intestinal problem manifest as malabsorption and megacolon. The basic defect, presumably an enzyme deficiency, has thus far eluded identification.

As with the other disorders discovered among the Amish, cartilage-hair hypoplasia, once delineated among them, has been found in non-Amish in various ethnic groups around the world. The second largest collection of cases of cartilage-hair hypoplasia is in Finland where about 30 cases are now known (H. R. Nevanlinna, personal communication, 1977). A common ancestry of the Finns and the Amish as a basis of cartilage-hair hypoplasia in the two groups is highly unlikely. However, the genetic structure of the Finnish population and the way in which that structure evolved to its present state show parallels to the Amish [33]. Thus, we are probably dealing with independent but possibly identical mutations that acquired a relatively high gene frequency in each population because of similar founder-drift factors.

Both previously known and "new" disorders can be studied to advantage among the Amish because of the reasonable confidence that one is dealing, in the sizeable collection of cases one may find, with one and the same gene in each case. The Ellis-van Creveld syndrome (six-fingered dwarfism) illustrates this point. Since work on this disorder in the Amish was first published in 1964 [34], additional infants with the Ellis-van Creveld syndrome have been born in the Lancaster County settlement, bringing the total to 40 affected sibships and 82 affected individuals. All 80 parents of these 40 sibships trace their ancestry to one Samuel King and his wife. The frequency of the Ellis-van Creveld syndrome in the Lancaster County Amish is not less than 50 per 10,000 births. When a coefficient of consanguinity (namely, $\alpha = .011$) relevant to the common ancestral couple (Samuel King and wife) is used, the frequency of the gene is estimated to be .066. The frequency of heterozygous carriers is estimated to be about 12.3%. Recall (table 1) that 12.6% of Lancaster County Amish carry the surname (King) of the couple from whom the EvC gene was presumably derived.

Why is the EvC gene so frequent in the Lancaster County Amish settlement? Selective advantage of heterozygotes (who, incidentally show no abnormality) is unlikely. The environmental circumstances under which the Amish live are not greatly different from those of many other populations, and although EvC has been observed in many different ethnic groups and in all parts of the world, it is everywhere, except in the single Amish deme, rare.

Founder effect, perhaps aided and abetted by random genetic drift, appears to be the main factor in the high frequency of the EvC gene in the Lancaster County Amish. The proportion of homozygotes attributable separately to consanguinity and to the high gene frequency (those cases that would occur without any consanguinity) can be derived from another form of the formula used in estimating gene frequency. The cases attributable to consanguinity (αq) have a frequency of seven per 10,000 or 14% of the whole.

Thus, founder effect/drift is a more important factor than consanguinity in determining the high frequency of EvC cases. It will be seen that at gene frequencies in excess of .06, for example, consanguinity is a minor contributor to the homozygote group.

The population genetics of rare recessive diseases has been studied in several other populations which bear similarities in genetic structure to the Old Order Amish. Among the French Canadians [35], moderately distinct demes are identifiable, descended from a limited number of founders, and showing an unusual frequency of diseases such as tyrosinemia, the Morquio syndrome, and agenesis of the corpus callosum. As alluded to earlier, the Finns [33, 36] represent another parallel to the Amish on a larger scale in terms of time and numbers. After going through a population bottleneck in the early stages of populating present-day Finland, during which time a small founder population was spread out ("streaked out" if you will) over an extensive area, the Finns remained separate, mainly by reason of their distinctive Finnish-Ugrian language, but also because of geographic barriers and distance, from both Slavic neighbors to the one side and Germanic neighbors to the other. The cloning phenomenon occurred here.* Today, a considerable number of rare recessives are unusually frequent in Finns (H. R. Nevanlinna, personal communication, 1977) [36] and in some cases have been found only in Finns. Conversely, other recessives (e.g., phenylketonuria) are unusually rare in Finns. Mapping of the place of residence of the grandparents of cases of high frequency disorders suggests the existence of moderately distinct demes based on founder effect, as in the Amish.

The founder-drift hypothesis for the high frequency of Tay-Sachs disease [37] and certain other recessive disorders among the Ashkenazim appear particularly attractive because of parallels in demographic history between the Ashkenazi Jews [38] on the one hand and the Amish, French Canadians, and Finns on the other. This hypothesis has obtained some theoretical support from an analysis by Rao and Morton [39]. The ancestry of several recessive disorders can be shown to be concentrated in different sections of the so-called Jewish Pale [40], again suggesting separate demes.

The usefulness of Amish populations both in the detection of "new" recessives and in their subsequent delineation is illustrated by the Kaufman syndrome: hydrometrocolpos, postaxial polydactyly and congenital heart disease. In 1964, we reported two Amish sibships, each with two females with hydrometrocolpos (i.e., transverse vaginal septum) [41]. All four cases traced their ancestry to a man named Christopher Beiler and his wife. In 1968, we reported a third sibship with one case of hydrometrocolpos, again tracing back through both parents to the same Beiler couple [42].

In 1972, Kaufman et al. [43] suggested that in fact hydrometrocolpos is part of a syndrome that embraces also postaxial polydactyly and congenital heart disease. This suggestion, based on a single case, has been amply confirmed by restudy of the three previously known Amish sibships and of three additional recently identified sibships (fig. 6).

These six sibships contain 42 sibs, 15 of whom are affected, affection being defined as presence of at least one of the three features. Only one of the 15 affected persons is known to have all three manifestations (table 2). Independent variation of the several

* Hybrid and hybridization are terms used in at least four senses in genetics: populational, organismal, cellular, and molecular. In human genetics, the term clonal is used on at least three of these levels. Although it is doubtful that we need the term in population genetics, the analogy between founder effect and cloning may be a useful one in teaching.

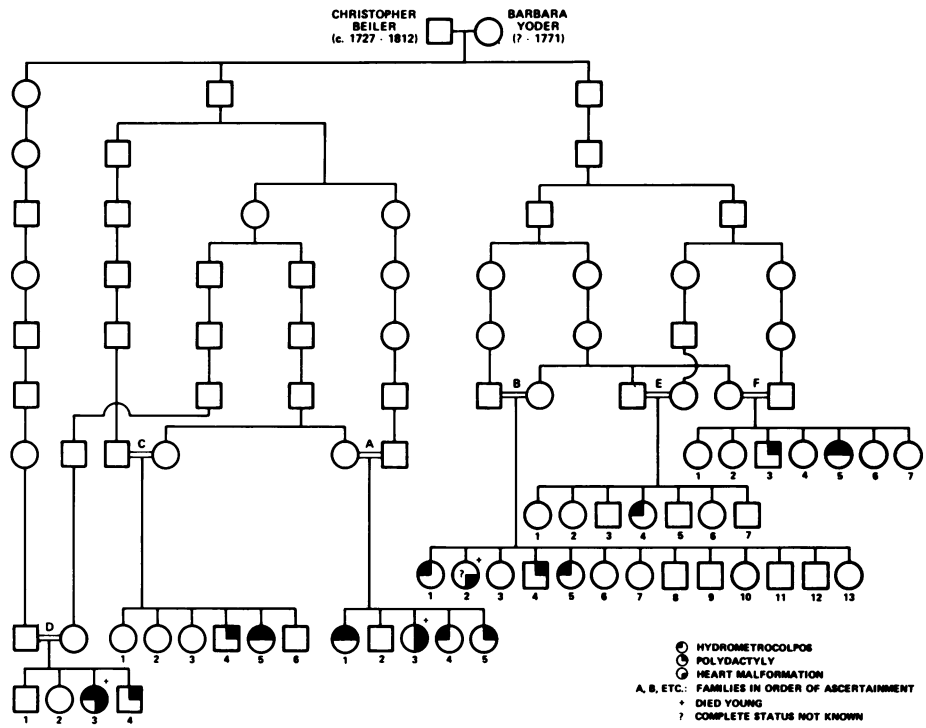

FIG 6.—Pedigree of Kaufman syndrome. Information concerning sibship D was provided by Drs. Patricia Christensen, Stephen J. Shochat, and Victor Whitman of the Milton S. Hershey Medical Center, Hershey, Pennsylvania.

components of a syndrome is well illustrated. The polydactyly varies from four limb postaxial hexadactyly through postaxial hexadactyly of a single limb to polydactyly of the postminimi type. We are in the process of tracing the Kaufman gene further by pursuing cases of postaxial polydactyly previously known to exist in the Amish (independently of the Ellis-van Creveld syndrome), but never looked at from the point of view of this syndrome.

One sibship of the six (E in fig. 6) contains only a single affected member out of seven children, and in that case of the Kaufman syndrome hydrometrocolpos is the only manifestation. Thus far, polydactyly has been the only manifestation in males.* The cardiovascular abnormalities (in three individuals) have been atrial septal defect, ventricular septal defect, and pulmonary hypertension. One person with surgically corrected hydrometrocolpos is married but thus far childless.

Can one seriously doubt that the three features are pleiotropic manifestations of a single recessive gene? I think not. On the model of single ascertainment, the data fit the recessive hypothesis precisely (E. A. Murphy, personal communication, 1977). There

* I have recently seen a 30-year-old man with four limb postaxial hexadactyly and atrial septal defect of ostium primum type. I suppose this represents the Kaufman syndrome, but no relatives show any of the features, and the parents are not related.

TABLE 2

THE KAUFMAN SYNDROME

Characteristics	Female (No. = 28)	Male (No. = 14)
Polydactyly alone	1	4
Hydrometrocolpos alone	4	...
Polydactyly + hydrometrocolpos	3	...
Polydactyly + cardiac malformation	2	...
Polydactyly + cardiac malformation and hydrometrocolpos	1	...
Total	11	4

would, however, be considerable question about both the validity of the triad as a syndrome and the recessive inheritance were it not for the opportunity to observe the large number of cases in the extended Amish family.

GENETIC NOSOLOGY

Etymologically, nosology means "the study of disease." (Clearly, "nosology of genetic disease" contains a tautology, hence, "genetic nosology.") It is usually defined as "the classification of disease." If classification of cases means the pigeonholing of similar cases that presumably represent an etiologically homogeneous entity, then it agrees with my view of nosology. Genetic nosology to me means delineation of specific genetic diseases. Classification of disease can also mean, not the classifying of cases (i.e., delineation of entities) but the classifying of entities into a hierarchical taxonomy. Before one knows the basic nature of each entity, such a taxonomy is difficult. A systematics based only on phenotype is shaky; on the other hand, the taxonomy follows a full delineation effortlessly. This is illustrated by figure 7, a taxonomy for the relatively well-understood α-L-iduronidase deficiencies.

The scientific and practical value of delineation needs no discussion; the scientific and practical value of taxonomy may be questioned. Perhaps its greatest value is mnemonic and therefore lies in the organization it gives to the growing body of otherwise seemingly unrelated information on genetic disease. Stanbury, Wyngaarden, and Fredrickson [5] engage in taxonomy of a highly useful nature when they divide their well-known book into sections on disorders of lipids, carbohydrates, amino acids, and so on, and further divide the sections into chapters dealing with related entities within the larger classes. A taxonomy may help the clinical geneticist maintain some order in his mind concerning the disorders he deals with, since entities of the same taxonomic class tend to behave similarly. Perhaps even phenotypically based taxonomies, such as Pinsky's "phenotypic communities of human malformation syndromes" [44, 45] have usefulness in pointing to common developmental (i.e., pathoembryologic) mechanisms, even though the entities clustered together may be etiologically diverse.

A focus of what I have told you about heritable disorders of connective tissue and the Amish is delineation of genetic diseases, and in a sense gene mapping serves that function also. Genetics is really gene delineation. It is fascinating to witness the

TABLE 3

MENDELIAN PHENOTYPES IN MAN (PRESUMED NO. OF LOCI)

MODE OF INHERITANCE	VERSCHUER 1958	*Mendelian Inheritance in Man* (McKUSICK)				
		1966	1968	1971	1975	Oct. 1977
Autosomal dominant	285	269 (+568)	344 (+449)	415 (+528)	583 (+635)	696 (+770)
Autosomal recessive	89	237 (+294)	280 (+349)	365 (+418)	466 (+481)	512 (+561)
X-linked	38	68 (+51)	68 (+55)	86 (+64)	93 (+78)	106 (+90)
Total	412	574 (+913)	692 (+853)	866 (+1,010)	1,142 (+1,194)	1,314 (+1,421)
Grand total	. . .	1,487	1,545	1,876	2,336	2,735

NOTE. — Nos. in parentheses relate to entries for which the particular mode of inheritance is not firmly established or the status as indicating a locus separate from another entry is not certain.

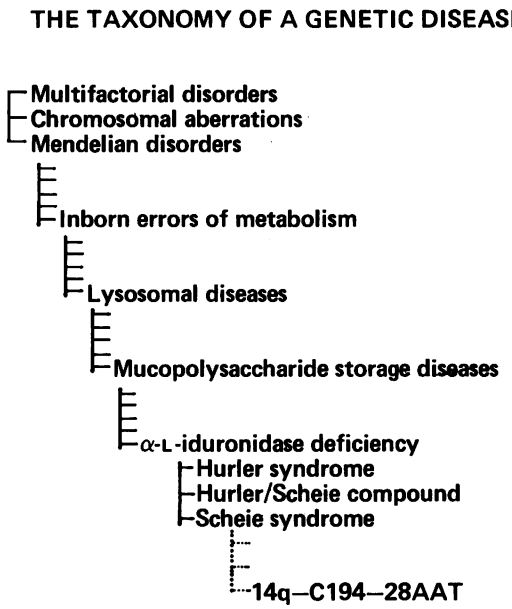

FIG. 7. — A taxonomy of one form of α-L-iduronidase deficiency. A fantasy is indicated by dotted lines: an ultimate delineation based on chromosomal localization and precise codon altered in the mutation. The fantasized designation means the α-L-iduronidase structural gene is cistron 194 from the centromere on 14q and that the Scheie syndrome is caused by mutation in its 28th codon, which reads AAT rather than the wild type.

delineation, by methods Pontecorvo called parasexual, of genes that were previously not susceptible to study by Mendelian methods because no allelic variation was known. These include genes that determine the human vulnerability to diphtheria toxin and polio virus (assigned by somatic cell hybridization to chromosomes 5 and 19, respectively). Included is also the gene (or genes) for histone IV (assigned to chromosome 7 by in situ DNA-RNA hybridization), which judging from its strong evolutionary conservatism must be of vital importance to chromosome structure and function.

Progress in genetic nosology and in delineation of genes in general (i.e., genetics) is reflected by the numbers of entries in *Mendelian Inheritance in Man* [46]. The number of reasonably established loci (see table 3) is now over 1,300 (240 of which, or almost a fifth, are assigned to specific chromosomes).

As Dr. Motulsky has told you, today happens to be my birthday. I thank all of you, cherished colleagues, for this birthday present.

REFERENCES

1. McKUSICK VA: *Heritable Disorders of Connective Tissue*, 4th ed. St. Louis, Mo., C. V. Mosby, 1972
2. McKUSICK VA: The classification of heritable disorders of connective tissue. *Birth Defects: Orig Art Ser* 11:1–9, 1975
3. McKUSICK VA, MARTIN GR: Molecular defects in collagen. *Ann Intern Med* 82:585–586, 1975

4. STEWART RE, HOLLISTER DW, RIMOIN DL: A new variant of Ehlers-Danlos syndrome: an autosomal dominant disorder of fragile skin, abnormal scarring, and generalized periodontosis. *Birth Defects: Orig Art Ser* 13 (3B):85–93, 1977
5. MCKUSICK VA, NEUFELD EF, KELLY TE: The mucopolysaccharide storage diseases, in *The Metabolic Basis of Inherited Disease*, 4th ed., edited by STANBURY JB, WYNGAARDEN JB, FREDRICKSON DS, New York, McGraw-Hill, 1978, pp 1282–1307
6. DANES BS, BEARN AG: Hurler's syndrome; demonstration of an inherited disorder of connective tissue in cell culture. *Science* 149:987–989, 1965
7. HERS HG: Inborn lysosomal diseases. *Gastroenterology* 48:625–633, 1965
8. MCKUSICK VA: Phenotypic diversity of human diseases resulting from allelic series. *Am J Hum Genet* 25:446–456, 1973
9. EPINETTE WW, NORINS AL, DREW AL, ZEMAN W, PATEL V: Angiokeratoma corporis diffusum with α-L-fucosidase deficiency. *Arch Dermatol* 107:754–757, 1973
10. O'BRIEN JS: Molecular genetics of GM1-β-galactosidase. *Clin Genet* 8:303–313, 1975
11. O'BRIEN JS, NORDEN AGW: Nature of the mutation in adult β-galactosidase deficient patients. *Am J Hum Genet* 29:184–190, 1977
12. MCKUSICK VA, RUDDLE FH: The status of the gene map of the human chromosomes. *Science* 196:390–405, 1977
13. MOHR J: A search for linkage between the Lutheran blood group and other hereditary characters. *Acta Pathol Microbiol Scand* 28:207–210, 1951
14. DONAHUE RP, BIAS WB, RENWICK JH, MCKUSICK VA: Probable assignment of the Duffy blood group locus to chromosome 1 in man. *Proc Natl Acad Sci USA* 61:949–955, 1968
15. WEISS MC, GREEN H: Human-mouse hybrid cell lines containing partial complements of human chromosomes and functioning human genes. *Proc Natl Acad Sci USA* 58:1104–1111, 1967
16. CASPERSSON T, LOMAKKA G, ZECH L: The 24 fluorescence patterns of the human metaphase chromosomes—distinguishing characters and variability. *Hereditas* 67:89–102, 1971
17. HALDANE JBS, SPRUNT AD, HALDANE NM: Reduplication in mice. (Preliminary communication.) *J Genet* 5:132–135, 1915
18. DICKIE MM: The expanding knowledge of the genome of the mouse. *J Natl Cancer Inst* 15:607–684, 1954
19. HORNER F: Die Erblichkeit des Daltonismus. Ein Beitrag zum Vererburgsgesetz. *Amtl Ber Verwaltung d Medizinalwesens Kanten Zurich,* 1876, pp 208–211
20. DALTON J: Extraordinary facts relating to the vision of colours: with observations (read in Oct. 1794). *Mem Philos Soc Manchester* 5:28–45, 1798
21. WILSON EB: The sex chromosomes. *Arch Mikrosk Anat Entwicklungsmech* 77:249–271, 1911
22. BELL J, HALDANE JBS: The linkage between the genes for colour-blindness and haemophilia in man. *Proc R Soc Med* 123:199–250, 1937
23. HALDANE JBS, SMITH CAB: A new estimate of the linkage between the genes for colour-blindness and haemophilia in man. *Ann Eugen (Lond)* 14:10–31, 1947
24. SINISCALCO M, MOTULSKY AG, LATTE B, BERNINI L: Indagini genetiche pulla predisposizione al favismo. II. Dati familiari associazione geneica con il daltonismo. *Atti Accad Maz Lincei Rc* 28:1–7, 1960
25. ADAM A: Linkage between deficiency of glucose-6-phosphate dehydrogenase and colour blindness. *Nature* 189:636, 1961
26. PORTER IH, SCHULZE J, MCKUSICK VA: Genetical linkage between the loci for glucose-6-phosphate dehydrogenase deficiency and colour-blindness in American Negroes. *Ann Hum Genet* 26:107–122, 1962
27. BOYER SH, GRAHAM JB: Linkage between the X chromosome loci for glucose-6-phosphate dehydrogenase electrophoretic variation and hemophilia A. *Am J Hum Genet* 17:320–324, 1965

28. RICCIUTI F. RUDDLE FH: Assignment of nucleoside phosphorylase to D-14 and localization of X-linked loci in man by somatic cell genetics. *Nature [New Biol]* 241:180–182, 1973
29. BERGSMA D: Human gene mapping 3. *Birth Defects: Orig Art Ser* 12(7):54–59. 1976
30. McKUSICK VA: *Medical Genetic Studies of the Amish. Selected Papers*. Baltimore, Johns Hopkins Univ. Press, 1978
31. COOLEY TB, WITWER ER, LEE P: Anemia in children with splenomegaly and peculiar changes in the bones. *Am J Dis Child* 34:347–363, 1927
32. McKUSICK VA, ELDRIDGE R, HOSTETLER JA, EGELAND JA, RUANGWIT U: Dwarfism in the Amish. II. Cartilage-hair hypoplasia. *Bull Johns Hopkins Hosp* 116:295–326, 1965
33. NEVANLINNA HR: The Finnish population structure: a genetic and genealogical study. *Hereditas* 71:195–236, 1972
34. McKUSICK VA, EGELAND JA, ELDRIDGE R, KRUSEN DE: Dwarfism in the Amish. I. The Ellis-van Creveld syndrome. *Bull Johns Hopkins Hosp* 115:306–336, 1964
35. LABERGE C: Population genetics and health care delivery: the Quebec experience, in *Advances in Human Genetics*, vol. 6, edited by HARRIS H, HIRSCHHORN K, New York, Plenum Press, 1976, pp 323–374
36. NORIO R, NEVANLINNA HR, PERHEENTUPA J: Hereditary disease in Finland: rare flora in rare soil. *Ann Clin Res* 5:109–141, 1973
37. CHASE GA, McKUSICK VA: Founder effect in Tay-Sachs disease. *Am J Hum Genet* 24:339–350, 1972
38. FRAIKOR AL: Tay-Sachs disease: genetic drift among the Ashkenazim Jews. *Soc Biol* 24:117–134, 1977
39. RAO DC, MORTON NE: Large deviations in the distribution of rare genes. *Am J Hum Genet* 25:594–597, 1973
40. GOODMAN RM: *Genetic Disorders among the Jewish People*. Baltimore, Johns Hopkins Univ. Press, 1978
41. McKUSICK VA, BAUER RL, KOOP CE, SCOTT RB: Hydrometrocolpos as a simply inherited malformation. *J Am Med Assoc*. 189:813–816, 1964
42. McKUSICK VA, WEILBAECHER RG, GREGG WG: Recessive inheritance of a congenital malformation syndrome. *J Am Med Assoc* 204:113–118, 1968
43. KAUFMAN RL, HARTMAN AF, McALISTER WH: Family studies in congenital heart disease. II. A syndrome of hydrometrocolpos, postaxial polydactyly. *Birth Defects: Orig Art Ser* 8(5):85–87, 1972
44. PINSKY L: A community of human malformation syndromes involving the Mullerian ducts, distal extremities, urinary tract and ears. *Teratology* 9:65–79, 1974
45. PINSKY L: The community of human malformation syndromes that shares ectodermal dysplasia and deformities of the hands and feet. *Teratology* 11:227–242, 1975
46. McKUSICK VA: *Mendelian Inheritance in Man*, 5th ed. Baltimore, Johns Hopkins Univ. Press, 1978