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Observations over 50 years concerning intestinal polyposis, Marfan syndrome and achondroplasia

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This is a personal account of progress in medical genetics over the past half-century. It might be subtitled "The confessions of an opportunist, chauvinist, and dilettante."

Opportunism gets a bum rap from dictionaries, which define the term (in one example) as "the art, policy, or practice of taking advantage of opportunities or circumstances, especially with little regard for principles or consequences." Opportunism in the clinic and in the laboratory has played an important role in the progress of medicine, however. Opportunism and serendipity are related though distinct entities. I am keenly aware of the influence of both in my career. For example, my investigations in medical genetics began in June 1947, near the end of my internship, with a teenage patient, Harold Parker, who had melanin spots and jejunal polyps. The principle of pleiotropism of gene action learned from that syndrome was easily extended to the category subsequently designated "heritable disorders of connective tissue" when patients with the Marfan syndrome turned up in my cardiologic experience. The opportunity to study heart sounds and murmurs by sound spectrography came when, by chance, I heard of that technique developed at the Bell Telephone Laboratories. My studies of the Amish were an outgrowth of reading the manuscript of John Hostetler's "Amish Society" when it came to the Johns Hopkins University Press committee, of which I was a member. A relatively high frequency of dwarfism among the Amish led to the identification of two separate forms of autosomal recessive skeletal dysplasia and a widening interest in skeletal dysplasias in general.

The origins of my 40-year preoccupation with gene mapping are less easily traced. Certainly, in a college genetics course I was fascinated by the intellectual elegance of gene mapping in *Drosophila*. The fascination was enhanced in the 1950s, when others found linkage of two genetic disorders, elliptocytosis and nail-patella syndrome, with blood group markers Rh and ABO, respectively. Haldane in the 1920s and John Edwards in 1956 had suggested the usefulness of linkage data in genetic counseling and prenatal diagnosis, respectively. But the potential utility of mapping was not what mainly interested me, I think; there are people — and I am one — for whom mapping has an intrinsic attraction. In the cartographic metaphor, the genome was in the 1950s *terra incognito*. Even the chromosome count was not accurately given until 1956. An anatomic metaphor is as apt as the cartographic one. Mapping chromosomes conferred a concreteness to the genes and the lesions in them causing disease. Complete mapping of the human genome — a complete anatomy, to use a double metaphor — appealed to my encyclopedist bent from an early stage.

Methods of medical genetic research

1956 — "Chromosomology"

1966 — Somatic cell genetics
- for the study of inborn errors of metabolism *in vitro*
- for mapping genes on chromosomes

1976 — Molecular genetics

1986 — Transgenic methods ("knockouts," "knockins" etc.)

1996 — Database searching ("research *in silico*")

I plead *mea culpa* also to the related crimes: chauvinism, provincialism and parochialism. I have been at Johns Hopkins uninterruptedly since arriving there to start medical school in 1943 and as of this past July, have been a member of its faculty for 50 years. I have not even taken a sabbatical during that period. It would not have been possible to do this and stay productive were it not for the great depth and breadth at Johns Hopkins. Also, Johns Hopkins is located at a crossroads — on the trade routes, one might say. Thus, I could pitch my tent beside the road and keep in touch with what was going on in far-off Cathay without ever traveling there myself.

In the third place, I must also confess dilettantism. It can be asked, How can it be more than dilettantism when one studies the physics of cardiovascular sound, maps genes on chromosomes, analyzes gene dynamics in genetically isolated founder populations and engages in delineation of genetic disorders from the entire range of clinical medicine?

I count as one of my greatest privileges to have had a large variety of exciting topics to study. For me, one of the most attractive features of medical genetics is its broad scope. The medical geneticist is the last of the generalists. He has license to swashbuckle through all of medicine, and I have enjoyed doing that — penetrating the domains of the ophthalmologist, orthopedist, neurologist, dermatologist, endocrinologist, gastroenterologist and others, including the cardiologist. A large general hospital such as Johns Hopkins has been an ideal setting for wide-ranging nosology.

It is ridiculously inappropriate, of course, to draw a parallel between my accomplishments and those of Isaac Newton; however, I have always liked Newton's statement: "I do not know what I may appear to the world; but to myself I seemed to have been only like a boy playing on the seashore, and diverting myself and now and then finding a smoother pebble or a prettier shell than ordinarily, whilst the great ocean of truth lay all undiscovered before me."

As an unabashed dilettante in the Newtonian mold, as well as an insufferable Johns Hopkins chauvinist and a shameless opportunist, I offer the following observations on intestinal polyposis, Marfan syndrome and achondroplasia. All three of these disorders illustrate the advances in medical genetics over the past 50 years, from the delineation of distinct enti-

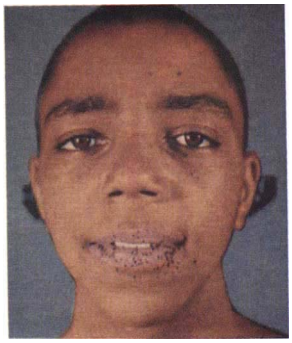

Fig. 1 Melanin spots of lips in Peutz-Jeghers syndrome.

ties from the previous nosologic hodgepodges to the definition of the precise causative mutations. All three illustrate the key role that gene mapping has played in their elucidation.

This account will reflect the evolution of medical genetics research (and clinical practice) over the past 40+ years. During this time, five nearly unique methodologic approaches have successively become available to medical genetic research (see box).

Intestinal polyposis

Harold Parker (Fig. 1), my patient when I was an intern, had suffered a rectal prolapse due to rectal polyp at the age of 6 months. Through his first 10 years, he had chronic anemia and repeated episodes of colic, and early in his second decade he suffered major intussusceptions, leading to bowel resections for jejunal polyps. I was impressed by melanin spots of his lips, buccal mucosa and digits, and during the next year, collected four other cases with the combination of melanin spots and intestinal polyps, three of them being members of the same family. Harold Jeghers at Boston City Hospital, had collected five cases over a period of several years. When Jeghers came to Washington in 1948 as the first full-time professor of medicine at Georgetown, he invited me to write-up the ten cases with him for publication in two successive issues of the *New England Journal of Medicine* in December 1949. As we noted, the syndrome had been described in a Dutch kindred in the 1920s by Peutz. In the 1950s, the disorder became known as the Peutz-Jeghers syndrome (PJS).

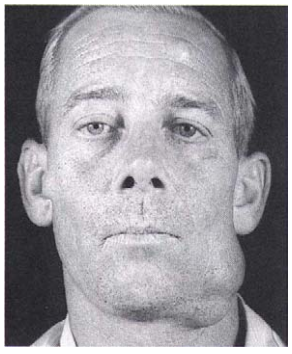

Fig. 2 Osteomas of mandible and frontal bone in Gardner syndrome.

Fig. 3 Total colectomy specimen in familial polyposis of colon.

My interest in other forms of polyposis was obviously stimulated by the experience with PJS, partly because of the necessity to distinguish the newly recognized disorder from classic familial polyposis coli (Figs 3,4), especially in its prognosis. We recognized that the polyps of PJS are hamartomatous in type and concluded that they are not premalignant — an impression that subsequently required revision.

A leading nosologic question concerning familial polyposis coli was whether the disorder that combined multiple polyposis of the colon with soft tissue and bony tumors (Fig. 2) was fundamentally the same condition. The latter condition, Gardner syndrome, is one of the few clinical disorders named for a Ph.D. geneticist, in this case Eldon Gardner (1906 to 1989) of Salt Lake City (Fig. 5). I was of the view that these were separate entities; others claimed to have seen them in different members of the same kindred and maintained that they were different expressions of the same basic disorder.

In 1973, we established at Hopkins a polyposis registry covering six states and the District of Columbia. Organized by Anne Krush, a former associate of Henry Lynch of Omaha, the registry was a resource for subsequent studies by many, including Bert Vogelstein and Stanley Hamilton, into the basic biology. Of interest was the finding that Gardner syndrome (defined by the occurrence of associated extra-bowel manifestations) was more frequent than "garden variety" familial polyposis coli. That the gene for colonic polyposis is located on the long arm of chromosome 5 was suggested by description (by Avery Sandberg's group) of a patient with that disorder (actually Gardner syndrome) and deletion of one band of the long arm of chromosome 5. Pursuing this clue, Bodmer and colleagues demonstrated linkage of familial polyposis to DNA markers in that region, and Bert Vogelstein and colleagues in Baltimore and Ray White and colleagues in Salt Lake City isolated the gene that was symbolized APC (for "adenomatous polyposis coli"). It turned out that there are a large number of mutations in the APC gene, any one of which can cause the disorder. It turned out, furthermore, that Gardner syndrome and polyposis coli without extra-bowel manifestation are both due to mutations in the APC gene. Indeed, the large Mormon kindred in which Gardner originally observed the association of polyps with jaw osteomas and skin tumors was found to carry an APC mutation. In some members of the family, the extra-bowel manifestations were inconspicuous or seemingly absent.

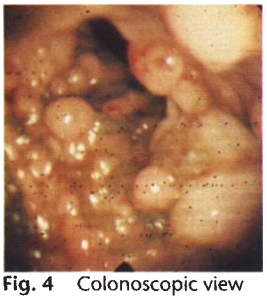

Fig. 4 Colonoscopic view of multiple polyps in familial polyposis coli.

Fig. 5 Eldon Gardner

Through the work of the Vogelstein group at Johns Hopkins, it was demonstrated that the APC gene plays a gatekeeper function in the prevention of most colorectal cancer, that mutation in the APC gene is the first in a multi-step process of colon carcinogenesis.

Whereas mapping and positional cloning played a crucial role in the definition of the APC gene in colonic polyposis and as a somatic mutation in isolated colorectal cancer, both mapping research and research *in silico* (see box) were critical to the discovery of the basis of hereditary non-polyposis colon cancer (HNPCC). There are several genetic forms of HNPCC due to mutation in different DNA mismatch repair

Fig. 6 Unusual height is a feature of the Marfan syndrome, a condition that Abraham Lincoln may have had.

genes. The Vogelstein group found abnormalities in repeat sequences in many sites in the genome in patients with colon cancer. Repeat sequences are subject to error through slippage mispairing during DNA replication. Ordinarily, mismatches are repaired under the control of a class of genes first identified in bacteria and yeast. One form of HNPCC was mapped to chromosome 2, and a gene in that chromosome region was shown to have sequence homology to a specific mismatch repair gene of *E. coli* and yeast.

Since 1949, we have learned that PJS in fact has malignant potential. Studying 31 PJS cases followed for 12 years through the Johns Hopkins polyposis registry, Frank Giardiello and colleagues in 1987 reported that gastrointestinal carcinoma had developed in four, non-GI cancer in ten and multiple myeloma in one. There were four cases of pancreatic cancer. The second of my five original cases (not included in the Giardiello series) had died of pancreatic cancer. Females with PJS are prone to granulosa cell tumors of the ovary and males to sex-cord tumors of the testis.

Fig. 7 The Hopkins Marfan team in 1991, with the author (in rear center) and (left to right) Hal Dietz, Clair Francomano, Reed Pyeritz and Garry Cutting.

Earlier this year — 50 years after I first saw Harold Parker — the gene for PJS was mapped on chromosome 19; that is, the disorder was found to show linkage to DNA markers on the short arm. This first step in positional cloning of the gene itself was achieved by an interesting approach. It was assumed that the gene which, in mutant form, causes PJS polyps is a tumor suppressor gene and that individual polyps require the occurrence of a mutation on the normal chromosome, according to the Knudson two-hit model. Often in such cases, the second mutation is a deletion. By a special method of comparing DNA in a polyp with that in other cells of the patient, it was possible to show deletion in the short arm of chromosome 19 (19p) in the polyps from many of the cases. Then standard linkage studies in PJS families using markers from 19p showed the PJS gene to be located there.

Recent work also suggests a relationship of PJS to familial juvenile polyposis, Cowden disease and Bannayan-Zonana syndrome, all of which have hamartomous intestinal polyps but are determined by genes on chromosome 10, not 19. Bannayan-Zonana syndrome, like PJS, has a spotted pigmentary peculiarity — namely, melanin speckling of the penis. The precise nature of the gene defect and the mechanism of the curious pleiotropism (polyps and spots) in PJS should become clearer with positional cloning of the gene on 19p.

Marfan Syndrome

The Marfan syndrome (Fig. 6), with its life-threatening aortic complications, came to my attention as a budding cardiologist in 1950. I collected all available cases (and families) identifiable in the Johns Hopkins Hospital. Schooled in the principle of pleiotropism of gene action by experience with the polyps-and-spots syndrome and the tutelage of Bentley Glass at Hopkins, I concluded that the subluxation of the ocular lenses, loose-jointedness, scoliosis, anterior chest deformity due to long ribs, long bones of the extremities and weakness of the aorta must reflect a defect in one element of connective tissue wherever it is found in the body. This was the principle put forward in 1956 in the first edition of *Heritable Disorders of Connective Tissue*, which also analyzed Ehlers-Danlos syndrome, osteogenesis imperfecta, pseudoxanthoma elasticum and Hurler syndrome (the prototypic mucopolysaccharidosis) in similar terms. The monograph suggested that when it is known what the suspensory ligament of the lens and the media of the aorta have in common, we will know the site of the basic defect in Marfan syndrome. In the 35 years between 1956 and 1991, we learned much about Marfan syndrome: the differentiation from the simulating inborn error of metabolism, homocystinuria; the intra- and interfamilial variabil-

Fig. 8 The Amish madonna

Fig. 9 Annual national convention of LPA, 1965, at Gloucester City, New Jersey, USA.

ity; the natural history of the mitral valve and aortic complications; use of beta-blockers to slow or stay the progression of the aortic dissection; and when this failed, replacement of the ascending aorta with a composite (valve-tube) graft, the Bentall operation. But identification of the precise molecular defect — in fibrillin — had to await map-based gene discovery. The Marfan phenotype was mapped to chromosome 15 by the group of Peltonen in Finland, with confirmation by my colleagues and others, and the fibrillin gene — a strong candidate because of histopathologic findings in Marfan patients and because it mapped to the same region of chromosome 15 — was shown by Hal Dietz and others of our group at Johns Hopkins to carry causative mutations (Fig. 7).

Achondroplasia

Achondroplasia, as the most frequent form of skeletal dysplasia causing dwarfism, naturally came early to my interest as a medical geneticist; in general, the skeletal dysplasias share pathologic features with the heritable disorders of connective tissue. I undertook studies of the Old Order Amish in 1963 partly because "achondroplastic dwarfism" was said to be unusually frequent in the group. Achondroplasia is an autosomal dominant disorder with almost 80% of cases occurring as new mutations. It seemed likely that the skeletal dysplasia in this inbred group was a recessive disorder. Indeed, two recessive types of dwarfism were found in the Amish: the rare and little-known Ellis–van Creveld syndrome ("six-fingered dwarfism") (Fig. 8) and a "new" entity we called cartilage-hair hypoplasia (CHH).

From these studies in the Amish, I became involved with Little People of America, the fraternal organization ("genetic support group," we now call it) of dwarfs, midgets and their families. At the first meeting I attended (Fig. 9), it was evident that there are many different forms of skeletal dysplasia leading to dwarfism. In addition to the numerous cases of achondroplasia, Morquio syndrome (in the sisters on the ground), osteogenesis imperfecta (in the woman on crutches), spondyloepiphyseal dysplasia congenita and cartilage-hair hypoplasia (in LPA founder Billy Barty) were identifiable.

The active nosology of skeletal dysplasia that started in the mid-1960s has defined several dozens of different types, and the molecular basis of many of them has been defined on the basis particularly of gene mapping and positional cloning. (The two Amish dwarfism genes, *EvC* and *CHH*, have been mapped to chromosomes 4 and 9, respectively, but no one has thus far succeeded in positional cloning of the genes.)

In 1994, the achondroplasia gene (*ACH*) was mapped to the tip of the short arm of chromosome 4, independently by Clair Francomano and her colleagues at Johns Hopkins and by two other groups, on the basis of linkage studies in families with affected members in two or more generations. The *ACH* gene mapped to the same region at the tip of chromosome 4 where the Huntington disease gene (HD) had been mapped. HD had been mapped there in 1983; the gene was not isolated until 10 years later after an intensive search in the region. It took no great insight to suspect that the Huntington disease researchers had in their freezers the very gene that is mutant in achondroplasia. This proved to be indeed the case: the late John Wasmuth and his colleagues had previously cloned the fibroblast growth factor receptor 3 gene (*FGFR3*) and shown that it is conspicuously expressed in cartilage. They went back to the *FGFR3* gene and identified mutations in achondroplasia (as well as in a related lethal disorder called thanatophoric dysplasia). Remarkably, essentially all cases of achondroplasia had a mutation in only one nucleotide, guanine (G), at position 1138; in most cases it was changed to an adenine (A), in a few to cytosine (C). In either case, the amino acid glycine, number 380 in the FGFR3 protein, was changed to arginine. (GGG was changed to either AGG or CGG.) 1138 G must be the most mutable nucleotide yet identified in the human genome.

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