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An Opportune Life: 50 Years in Human Cytogenetics

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Abstract

This article is one person's view of human cytogenetics over the past 50 years. The flowering of human cytogenetics led the way to the establishment of clinical genetics as one of the most important developments in medicine in the twentieth century. The article is written from the viewpoint of a scientist who never tired of analyzing the images of dividing cells on the light microscope and interpreting the wealth of information contained in them.

MY INTRODUCTION TO CYTOGENETICS

On the first day of World War II, my family was evacuated from London to the west coast of Scotland. My father worked in the Nobel Division of Imperial Chemical Industries (ICI), and the division was evacuated to Ardeer, where an ICI factory was situated under the sand dunes on the Ayrshire coast. The Ardeer factory was the largest manufacturer of explosives on the Allied side in World War II, and the London branch of the Nobel Division was moved en masse, together with their families, and housed in a hotel in Saltcoats just north of the factory. It was thought that the war would be over quickly and that after a few months we would return to London. When it became clear that the war was going to last longer than initially anticipated, the ICI staff had to find alternative accommodations. My parents and I moved to a rented accommodation in Saltcoats, our home for the remainder of the war.

I was given *The Observer's Book of Wild Flowers* for my sixth birthday, one of a series of children's pocket books dealing with nature. I set out with youthful enthusiasm to collect every flower illustrated in the book. I pressed my finds between sheets of blotting paper and mounted the carefully labeled specimens in jotters. At no time do I remember being given any instruction or encouragement, or sharing my hobby with any friends. It was a lone endeavor that gave me a great deal of pleasure, although I spent many fruitless hours looking for plants that were illustrated in my reference book but would never be found growing on the raised beaches and hills of Ayrshire. This hobby of collecting wildflowers ultimately led me to my career in biology.

At the end of the war I had completed primary school, and my parents, who had never really reconciled themselves to the abrupt change from north London to coastal Ayrshire, were eagerly awaiting the transfer of the ICI Nobel Division back to London. Their disappointment on learning that the division was going to remain in Scotland was intense. However, I was happy to remain in Ayrshire, where I could continue my education in the secondary school of Ardrossan Academy, which I enjoyed, and where I could continue to cycle about the pleasant and varied countryside looking for flowers. The secondary school included many additional pupils from north Ayrshire as well as those who had gone through primary school together. I became friendly with one of the new pupils and, on meeting her parents, found that her father was very knowledgeable about wildflowers and had many books that he would lend me. At last I understood why I had failed to find so many of the flowers I had been looking for—wrong soil, wrong part of the country, and wrong climate! I decided that studying wildflowers (whatever that meant) was what I wanted to do for the rest of my life.

The qualification for which everyone who stayed in school past the age of 14 studied was the Scottish "highers." I studied English, mathematics, geography, physics, chemistry, and French. I'm not sure why biology was not taught, but as a result, I received no tuition in the subject that I hoped was going to be my life's work.

The University of St. Andrews

My father's job had now moved from Ayrshire to Glasgow, and at the end of my schooling, my parents moved to Glasgow while enrolling me in the University of St. Andrews. I have no recollection of this decision having been discussed with me; I was just told I was going to St. Andrews. I didn't mind, as I had no connections in Glasgow and was not familiar with life in a large city. I "went up" to study botany; higher mathematics, physics, and chemistry were the prerequisites for studying any branch of science at St. Andrews, and the fact that I had never studied biology was not held against me. In the first year, I studied chemistry, botany, and zoology, and found to my disappointment that botany consisted not of studying wildflowers in the pleasant environs of St. Andrews but rather of studying fossil ferns and other arcane subjects. However, the

zoology course was more to my liking. It was taught by Professor H.G. "Mick" Callan, who, when appointed as head of the Zoology Department, following in the footsteps of the famous D'Arcy Thompson, was the youngest professor in the UK. Callan was an inspiring teacher, and by the end of the first year I had decided to shift my emphasis from botany to zoology. Callan's research interest was in the lampbrush chromosomes of newts, and embedded in the first-year general zoology class was a special section on chromosomes. This was my first exposure to chromosomes. Mick Callan made them sound like the most fascinating and important objects in the whole of biology, and over the subsequent 50 years I have never had any reason to doubt him.

At St. Andrews, as at all Scottish universities of that time, one could do an ordinary degree in three years or an honors degree in four years. In the Zoology Department, the honors year was entirely a research year, with the production of four essays and a thesis being the only academic requirements. However, Callan insisted that anyone accepted for an honors degree had to first pass the ordinary degree course. Thus, I took a joint degree in botany and zoology at the end of my third year at university prior to setting out on my final honors year. The honors year was a revelation to me, as it was then that I really discovered my love of chromosomes and my love of research.

After passing my ordinary degree in June 1955, I was given a topic for my honors degree: the study of meiotic chromosomes in the male centipede. I can't remember why this topic was given to me, but it was clear that I would have to obtain a large number of male centipedes, and in the summer of 1955 I started my search. Unfortunately, that summer was one of the hottest on record, and I found that centipedes had all but vanished. I devoted over two months to seeking out these normally common animals, but my total haul was only eight, which on dissection yielded only two males.

I made an appointment to see Professor Callan and explained that I thought the heat had sent the centipedes far underground and that I had failed to find a suitable number in which to study male meiosis. Callan said that he was sorry and that perhaps I would like to study male meiosis in the praying mantis instead. I was aghast at the thought of attempting to find testicles of *Mantis religiosa*, which does not even live in Britain! However, in a flash, Callan (who always spent the summer at the marine research institution run by his father-in-law in Ischia, off the coast of Naples) produced from his desk drawer a bottle containing 70% alcohol and some small brownish-gray objects and said, "There you are, testicles from 20 praying mantises, that should be enough for a project."

There were three reasons that meiosis in *Mantis religiosa* males was of scientific interest. First, they had been reported to be achiasmate. Second, they had been reported to have an exceptional prometaphase stretch phase prior to the separation of the homologous chromosomes. And third, it had been suggested that they had an X_1X_2Y complement of sex chromosomes.

I was given a small cubicle in which to work. The other honors students in zoology had all chosen projects that entailed working in the Gatty Marine Laboratory, situated about a mile away on the coast. Thus, I was the only student in my year sited in the Zoology Department. I would have been very lonely indeed had it not been for Lydia Lloyd, a recently appointed lecturer who occupied a rather larger cubicle next to mine. Lydia became a source of great support, as she knew her way about the department and also instructed me in several laboratory techniques with which I was unfamiliar.

I found my project challenging, but I also quickly became fascinated, and I successfully made meiotic preparations of at least some of the testicles. I managed to answer the three questions I was addressing: Yes, the chromosomes in the first meiotic division were held together by structures that could only be termed chiasma; yes, there was a spectacular and very unusual prometaphase stretch stage prior to the bivalents separating; and yes, the three very large chromosomes were held together at meiosis by chiasma near both ends of the Y and one end of the X_1 and X_2, and thus the males had an X_1X_2Y sex-determining mechanism. This contrasted with the great majority of the Mantidae family, where sex was determined by an XO mechanism.

I also observed a relatively large number of univalents, and these failed to align on the metaphase plate. Cells containing a univalent chromosome did not proceed through meiosis, and in sectioned testis material it was quite clear that cells with univalents did not enter anaphase. This frequency of nondisjunction and its subsequent effect in blocking the cell from progression through anaphase had not previously been reported. When I look with hindsight on my career in cytogenetics, I think it astonishing that I was actually studying nondisjunction in 1955! I was awarded a first-class honors degree in zoology, and my work led to a paper entitled "The Meiotic Process in *Mantis religiosa* L. Males," published in 1957 under the authorship of Callan & Jacobs (3).

The USA

In spite of these successes, it never occurred to me to study for a PhD, so what next? A friend of mine from St. Andrews and I decided we would like to see the USA. On perusal of a tome entitled *Study Abroad* in the summer of 1956, we discovered that we should have started making inquiries at least nine months previously and that very few opportunities remained open. However, we found positions at Mount Holyoke College in Massachusetts. Our applications were accepted, and we both succeeded in obtaining a Fulbright Travel Scholarship. In September 1956, we set sail for New York.

After a five-day voyage on calm seas, we anchored in New York complete with two very large cabin trunks and spent a few days of wonder in the city before setting off for the tiny community of South Hadley in Massachusetts, where Mount Holyoke College, a small but prestigious undergraduate college for girls, was situated. The few teaching and research assistants—for such we were—were housed in two graduate houses on the campus. Aside from our jobs, there was little to do in this small college. I was a research assistant to the head of the Zoology Department, and on my first day I was shown to my room—long, narrow, and dark, next door to the mouse house, which I was put in charge of. I had no idea what this entailed, as I had never entered a mouse house before, let alone been in charge of one.

About halfway through the year, I heard from my St. Andrews mentor, Professor Callan. He had just received a letter from a Dr. Michael Court Brown, who was setting up a new Medical Research Council (MRC)–sponsored unit in Edinburgh for the study of the clinical effects of radiation. He wanted to study the chromosomes of individuals with radiation-induced leukemias and was seeking to recruit a cytogeneticist to undertake this work. I expressed interest and, after an exchange of letters with Court Brown, I found myself appointed as a scientist by the MRC, with the position to start when I returned from the USA. Luckily for me, in those days the MRC (and presumably other employers) made no distinction between individuals with a first-class honors degree and those with a PhD. Thus, my lack of a PhD appeared to not be a disadvantage.

Eventually spring came, and we discovered that we were living in one of the main asparagus-growing regions of the USA and, furthermore, that "casual labor" was needed to harvest the asparagus. We were hired by a local farmer, who collected us from our graduate house at 5:30 AM, and, armed with asparagus knives, we harvested the field before the sun rose. We were then driven back to the college in time to present ourselves for work. Every morning we cut the same field, as the asparagus grew about six to eight inches every day. I had no idea that anything could grow that fast.

We saved every cent we earned to put toward the purchase of a secondhand car, because four of us decided that we should see as much of the North American continent as possible before we returned to Europe. After about two months of roughing it, we had seen most of the USA and large parts of western Canada but had run out of money. We therefore returned to South Hadley, where we sold the car, got our luggage and ourselves back to New York, and caught the *Mauritania*

back to Southampton. We had certainly made the most of our year abroad, and returned much wiser and better informed than the rather naive girls who had set out the previous year.

HUMAN CYTOGENETICS: THE BEGINNING

On my return from the USA in 1957, I joined the newly constituted MRC Clinical Effects of Radiation Research Unit, situated in the Radiology Department of the Western General Hospital in Edinburgh. This was to be my place of employment for the next 14 years. My remit was to study the chromosomes of dividing bone marrow cells from patients who had developed leukemia after having received a significant dose of radiation and compare the results to marrow from individuals who had leukemia but no history of radiation. I first had to learn some relevant techniques, and two people helped me with this: Dr. Lazlo Lajtha, from the Radiotherapy Department of Churchill Hospital in Oxford, who had developed a technique for the tissue culture of human bone marrow, and Dr. Charles Ford, from the MRC Radiobiological Research Unit in the Atomic Energy Research Establishment in Harwell, some 30 miles south of Oxford, who was a cytogeneticist with expertise in the examination of chromosomes from bone marrow of mice exposed to radiation. Both these individuals generously agreed to have me in their laboratories, and so I set out for Abingdon, a town midway between Oxford and Harwell, where I stayed for about three months. I learned a great deal about short-term tissue culturing of human bone marrow cells and about the handling of bone marrow cells to produce cytogenetic preparations.

The status of human cytogenetics when I started my career in 1957 must be remembered. In 1956, Tjio & Levan (53) showed the human chromosome number to be 46 and not 48, as had been previously supposed. Their observations of the correct number of chromosomes were made on cultured embryonic fibroblasts and were confirmed by Ford & Hamerton (8) using direct preparations of human testicular material. By 1956 it was also known that a sexual dimorphism existed in the interphase nuclei of humans, a dense sex chromatin body that is present in many cells of females but not those of males, and also that in some pathological conditions, notably Klinefelter syndrome and Turner syndrome, the phenotypic sex was often at variance with the nuclear sex (34). However, it was taken as an article of faith that sex determination in humans was identical to that in *Drosophila* and thus dependent on the number of X chromosomes, with the Y chromosome playing no part. The sex chromatin body was assumed to consist of the material of both X chromosomes, chromatin-positive males and chromatin-negative females being examples of true sex reversal, with the males having a 46,XX and the females a 46,XY constitution.

Two other discoveries made an important impact on the early development of human cytogenetics. The first was the realization from the study of atomic bomb survivors in Japan and patients treated with radiation therapy for ankylosing spondylitis in Europe that radiation was associated with an increased mortality from leukemia. The second was the development by Ford & Hamerton (9) of techniques for looking at the cytogenetics of reticuloendothelial neoplasms in rodents and their demonstrations that these were often associated with chromosome aberrations.

My time in Abingdon proved rather successful, and the results of our joint endeavor of combining the culture technique of Lajtha and the cytogenetic technique of Ford to produce chromosome preparations from short-term cultures of human bone marrow were published in 1958 (10). Toward the end of my stay in the south, we were contacted by Dr. Davidson and Dr. Robertson Smith of King's College Hospital in London and asked whether we would examine the chromosomes of a patient with Klinefelter syndrome using our newly developed technique. We were excited by this prospect, and I went off to King's College Hospital to collect the bone marrow specimen and bring it back to Harwell for culture and subsequent processing for cytogenetic observation.

Unfortunately, the expedition was not a success. I found my way to the appropriate department in south London, approached a lady behind a desk, and explained that I had an appointment with Dr. Robertson Smith. She rather brusquely showed me to a seat where I might wait. After the best part of half an hour, when nobody took any notice of me, I approached the receptionist and once again explained that I had an appointment to see Dr. Robertson Smith half an hour ago. She sent me back to my seat, and after another 15 minutes had passed, a rather cross man emerged from a consulting room and spoke to the secretary, who pointed me out. I had been assumed to be a patient and treated as such! I was hustled into a room where the patient with Klinefelter syndrome was lying on a trolley waiting, together with appropriate staff, for me to turn up to receive his bone marrow. Everyone seemed rather annoyed at my being so late, but no one gave me a chance to explain why. Feeling very nervous, I prepared my culture bottles and other supplies to receive the bone marrow, and after what seemed like an age, the marrow was obtained and passed to me. As this was the first time I had ever had charge of such precious cells and as I was totally outside my environment, I was not at my best. Half an hour later, I exited King's College Hospital with my precious bottles to catch a train to Harwell, where I would culture the marrow and make cytogenetic preparations.

As it turned out, the quality of the material from the Klinefelter patient was poor, with very few dividing cells. On analysis of the metaphase plates, I thought the most reasonable interpretation was that the cells had 47 chromosomes with a Y, but Ford thought that they had 46 chromosomes and a female constitution. In truth, we should have regarded the whole exercise as a failure. However, the result favored by Ford was included in our paper, and thus the Klinefelter patient was considered to be a 46,XX male (10).

I learned a very important lesson from this experience, namely that the scoring of cytogenetic preparations should be done only on cells of reasonably good quality, and should be done blindly and preferably with an element of the unexpected. This important lesson has stayed with me for my entire career. People much more knowledgeable than I were convinced that sex determination in humans was the same as in *Drosophila*, and thus that chromatin-positive Klinefelter males were sex-reversed females with a 46,XX constitution and that the Y played no role, and this was how the poor-quality cells were interpreted. In my ignorance, I had no preconceived expectations and just recorded what I thought I saw.

Shortly after the debacle with our first Klinefelter patient, I returned to Edinburgh to set up my own laboratory to study radiation-induced leukemias, but, alas, appropriate patients were few and far between. Luckily, Dr. John Strong, a local endocrinologist, offered me marrow from a chromatin-positive male who was thought to have Klinefelter syndrome. However, I remembered the lesson I had learned in Harwell. The preparation we obtained from bone marrow was not of the high quality we have now come to expect, and there was often considerable doubt about even the number of chromosomes in any given cell. I used to record in my microscope book all possible counts for each cell and then underline the one I thought most probable. In the chromatin-positive male with Klinefelter syndrome, there seemed to be many more cells than usual in which the number 47 was underlined as the most probable chromosome count. Let me remind you that at this time, no mammal had ever been correctly reported to have an additional chromosome, and therefore a true chromosome count of 47 seemed implausible, if not downright impossible.

As I didn't know what to make of my observation, I shelved the problem and prepared to leave on my summer vacation. However, before leaving, I asked my technician, Muriel Brunton, to prepare a tray of slides for me to examine blindly on my return and to include a slide of the chromatin-positive male together with a number of slides of normal males and females. On my return, I scored the coded slides and found not one but two preparations that appeared to have 47 chromosomes. This suggested to me that false counts of 47 must be a fairly common hazard of cytogenetics, but when I reported this to my technician, her face broke into a delighted grin

because she, smart girl, had put two different preparations from the chromatin-positive male into the tray. We spent the morning analyzing the cells and concluded that the individual did indeed have 47 chromosomes and that the most reasonable interpretation for our observations was that he had a 47,XXY constitution. Luckily, no one had told us that sex determination in humans was supposed to be the same as in *Drosophila*, with the Y chromosome playing no essential role! I told Michael Court Brown of our discovery of a human being with an additional chromosome. He immediately recognized the importance of our observation and encouraged me to write a short note to *Nature*, an article that was published in the record time of five weeks (28).

I recount this story in some detail for two reasons: first, because it illustrates the state of the art in 1958 and the huge strides that have been made since then, and second, because it embodies three principles that I hold dear. The first of these principles is that in a largely observational discipline such as cytogenetics, in which there is no way the findings can be subjected to rigorous proof, it is mandatory that observations be made blind and desirable that the blind scoring have an additional element of the unexpected. While this simple scientific principle appears too obvious to need saying, it is surprising how often it is ignored. The second principle is that it is important to give people the opportunity to make original and independent research contributions while they are still young enough to question, or even to be totally ignorant of, accepted dogma. I was 23 and my technician 18 when we discovered the male-determining properties of the mammalian Y chromosome. The third point is that, in spite of my never having written a paper before, I was encouraged to write up my findings on my own, even though my painful struggles in writing even a two-page paper delayed the production of a final manuscript by two or three months. However, I was left with the satisfaction of seeing a piece of work right through to the end on my own, something that I think is important, especially to young scientists, and something that is all too easily forgotten in these days of team research and concerns over priority.

The year 1959 was indeed the *annus mirabilis* of human cytogenetics. In addition to our observation of the XXY male, we published our finding of an additional chromosome 21 in Down syndrome (referred to at the time as mongolism) (23), and this was done quite independently of Lejeune, Gautier & Turpin (31). Ford and his colleagues also published their observation of a 45,X constitution in a chromatin-negative female with Turner syndrome (11). In the same year, we described the first female with an XXX sex chromosome constitution, who, incidentally, was found to have two sex chromatin bodies in a proportion of her cells (22). This observation was not understood because the phenomenon of X inactivation was not yet known, but it did cast doubt on the previously accepted notion that the sex chromatin body was formed from two X chromosomes. In addition, the first mosaic was described by Ford and his colleagues (12); the first structural rearrangement, a D-D translocation, was described by the French group (54); and the first example of sex reversal, the 46,XY chromosome constitution of females with testicular feminization, was described by our group in Edinburgh (21). Thus, in the space of one year, a new discipline had been established in the field of human genetics, and the first steps had been taken into an entirely new realm of human pathology.

EDINBURGH IN THE 1960s: POPULATION CYTOGENETICS WITH SPECIAL REFERENCE TO THE SEX CHROMOSOMES

The findings published in 1959 were only a start: 1960 saw the description of the blood culture technique by Moorhead and his colleagues in Philadelphia (35). This method revolutionized human cytogenetics by allowing us to routinely obtain high-quality cytogenetic preparations from an easily sampled tissue. For the first time, population cytogenetic studies became a practical possibility. In 1960, Michael Court Brown decided to link the techniques of cytogenetics, which

were established in our unit in the persons of myself and David Harnden, with the techniques of epidemiology, his own major discipline. This was done because of our desire to obtain information on the frequency of cytogenetic abnormalities carried by the population, a task that could not be accomplished by the study of only the physically and mentally handicapped. This information, in addition to being of interest in its own right, was vital to the correct interpretation of chromosome variation ascertained among the clinically abnormal. Thus, the discipline of human population cytogenetics was born, a field that our unit made very much its own for the next decade. While others took the lead in clinical cytogenetics, we in Edinburgh were concerned almost wholly with the study of population groups, both those defined by a medical condition and those considered to be representative of the population at large. Thus, we began to have an understanding of just how frequent cytogenetic aberrations were in the population.

Now let me turn to sex chromosome abnormalities. In the early 1960s, all surveys done to determine the incidence of sex chromosome abnormalities in both normal and clinically defined populations were based on sex chromatin surveys of cells from buccal mucosa. Such nuclear sexing surveys were usually followed by chromosome analysis of the individuals found to have an abnormal sex chromatin pattern (32). These sex chromatin surveys played a pivotal role in early studies of sex chromosome abnormalities, but, of course, they provided information only about the number of X chromosomes. Such surveys showed that in institutions for the mentally handicapped, some 1% of males were chromatin +ve (i.e., had more than one X chromosome). When the chromosomes of these chromatin +ve males were examined, the great majority were found to have an XXY sex chromosome constitution (i.e., Klinefelter syndrome). However, while sex chromatin surveys provided us with information about abnormalities of the X chromosome, they told us nothing at all about the Y.

In 1964, I learned from a colleague in Sheffield that his group had just completed a sex chromatin survey of the mentally handicapped individuals in the three English maximum-security hospitals, which housed those who had committed a criminal offense but were judged to be not responsible by virtue of being mentally handicapped or having a neurologic disease. The Sheffield group found 2% to be chromatin positive, in contrast to the 1% found in ordinary institutions for the handicapped. But more interesting, at least to me, was the observation that about one-third of the chromatin-positive males did not have a simple 47,XXY constitution, as expected, but a 48,XXYY constitution—that is, they had an additional Y chromosome as well as an additional X. From our own population studies, I knew that such men were extremely rare among the inmates of ordinary hospitals for the mentally handicapped, and I wondered whether their extra Y chromosome might have contributed to their antisocial behavior and resulting admission to maximum-security hospitals. I reasoned that if this were the case, then an additional Y chromosome might be associated with antisocial behavior in males in whom it was the only chromosome abnormality, that is, males with a 47,XYY constitution. Such males might therefore also be found in increased numbers in maximum-security settings.

To test this hypothesis, we undertook a complete cytogenetic survey of the only Scottish maximum-security hospital. We received a coded blood sample from each patient who had agreed to participate, together with a small piece of paper that gave the patient's date of birth, height, and clinical diagnosis. In the second week of the survey—which, it must be remembered, was done long before the era of banding and instant recognition of the Y chromosome—my delighted technician rushed into my office and said that she had found either our first XYY male or a 6′ 4″ Down syndrome patient! In total, we found that 3% of the men in the institution had an XYY constitution, and, furthermore, that the men with an additional Y chromosome were several inches taller than their XY counterparts (24). The observation that some 3% of males in medical-penal settings had an additional Y chromosome was subsequently confirmed in virtually

every similar population examined (20). One would have thought that the finding of a more than 30-fold excess of men with an additional Y chromosome in medical-penal settings and the suggestion that the additional Y chromosome might contribute to the antisocial behavior of such men would have provided an objective tool in the rational study of human behavior. Instead, it aroused fierce and acrimonious controversy that resulted in the cessation of virtually all research aimed at identifying sex-chromosomally abnormal individuals and understanding the effect of the additional chromosomes on their development and behavior.

I think there were two reasons for this. The first was the enormous amount of publicity given to these observations in the lay press. Unfortunately, this resulted in a great many people, scientific and lay alike, receiving their first information about the possible association between an additional Y chromosome and antisocial behavior from the sensationalist and incorrect accounts in the media. In the long term, even more harmful than the irresponsible attitude of the press was the reaction of the large number of dedicated environmentalists who felt threatened by the suggestion that there might be a genetic component to behavior. Remember that this all happened in the touchy-feely 1960s. It was inconceivable to me that there was a large body of people, including biologists and psychiatrists, who did not accept that both genes and environment played an integral role in human behavior. Thus, a groundswell of opinion made itself heard criticizing work on population screening in general and on identification of XYY individuals in particular. At its most extreme, in the USA, these objections took the form of intense personal harassment of individuals involved in this research and their families. This ultimately resulted in the curtailment of virtually all inquiry in this area.

The XYY story was the end of an era for me because, after consolidating our investigations of individuals in penal and subnormal populations (27) and carrying out a large cytogenetic survey of newborns that was designed, at least in part, to determine the frequency of the XYY genotype in the general population (26), I left Edinburgh to join my husband in Hawaii.

CYTOGENETICS IN HAWAII

I left a thriving organization rich in equipment and trained personnel for an empty laboratory in a corner of the Anatomy Department of a newly constituted medical school. At the same time, chromosome banding was starting to revolutionize human cytogenetics. Thus, I reviewed my future in human population cytogenetics in the light of both the advances created by the banding techniques and my lack of resources. As my main interest was investigating the origin and effect of constitutional chromosome abnormalities, I decided the way forward for me was to study a population with a high proportion of such abnormalities. Naturally, I turned to spontaneous abortions.

Spontaneous Abortions

Many others had already published data showing an impressive proportion of abortions to be chromosomally abnormal (1, 4), but nobody had yet used banding techniques to precisely identify the abnormalities and to provide a range of heteromorphisms with which to study their parental origin. As a population cytogeneticist, I found the study of spontaneous abortions, with their 50% abnormality rate, to be amazing. Spontaneous abortions are a population cytogeneticist's dream, and their study certainly brings home the extraordinary frequency of chromosome aberrations in our species.

Using the limited tool of cytogenetically visible heteromorphisms, we started to study the parental origin of some of the abnormalities seen among spontaneous abortions. Triploids—that is, conceptuses that have three haploid complements and therefore 69 chromosomes—are one of the

most common classes of aberrations among spontaneous abortions. Together with Aaron Szulman, an expert placental morphologist, we showed that the placenta in triploid spontaneous abortions with two paternal and one maternal haploid set had the phenotype of partial hydatidiform moles. In contrast, the placenta in triploid spontaneous abortions with two maternal and one paternal haploid set had a normal placental morphology (29). This observation fit well with the observation by Kajii & Ohama (30) that complete hydatidiform moles—that is, pregnancies consisting of a huge abnormal placenta but no fetus—had two paternal but no maternal genetic contributions. These observations were among the first in the new field of imprinting and epigenetics.

In Hawaii, we were particularly fortunate in the ethnic diversity of our study population, which comprised people of Chinese, Japanese, Filipino, Hawaiian, and Caucasian ancestry. This enabled us to study conditions that varied in prevalence among the ethnic groups, most notably partial and complete hydatidiform moles, the latter being much more prevalent among Asian women than among Caucasian women. A case–control epidemiological investigation of the complete moles in our population (33) showed maternal age and race to be important, with moles being significantly more prevalent in women under 20 and over 40 years of age and also in women of Japanese, Filipino, or other Asian ancestry. However, no difference was seen in the prevalence of moles between Asian women born in Asia and those born and raised in Hawaii. No significant difference was found in paternal age, paternal race, socioeconomic status, or reproductive history, suggesting that these factors do not play an important role in the etiology of complete hydatidiform moles. Incidence rates for complete moles were calculated with age and race taken into consideration and ranged from a high of 1 in 150 to a low of 1 in 2,000 naturally terminating pregnancies.

The use of cytogenetically visible heteromorphisms to give information on parental origins soon gave way to highly polymorphic minisatellite probes, which were much more reliable molecular markers for investigating the parent and mechanism of origin of cytogenetic abnormalities. Thus, it was demonstrated that trisomy, by far the most common class of microscopically detectable abnormality in our species, was usually the result of abnormal patterns of recombination during maternal meiosis (17). Surprisingly, every chromosome seemed to have its own pattern of abnormality. For example, trisomy 18 is due largely to errors arising at the second maternal meiotic division, which occurs after the egg is fertilized (2), whereas trisomy 16, the most common of all human trisomies, occurs exclusively as the result of errors arising at the first maternal meiotic division, which occurs while the mother is a five-month-old fetus (18)!

Fragile X

While I was immersed in the study of spontaneous abortions, along came another exciting cytogenetic phenomenon: the observation of a cytogenetically demonstrable marker on the X chromosome associated with a common form of X-linked mental retardation (13, 15). The cytogenetic marker came to be called a fragile site, and it was the study of fragile X-linked mental retardation that led to the recognition of an entirely novel type of mutation, trinucleotide repeat expansions. This class of mutations is now known to be the basis of at least 20 different neurological diseases.

Study of fragile X syndrome has shown that the degree of expansion is related to the phenotype, with full mutations—that is, those with more than 200 repeats—resulting in fragile X syndrome in both affected males and a proportion of female carriers. Lesser expansions—or so-called premutations, which have between 55 and 200 repeats—are associated with premature ovarian insufficiency in 20% of carrier females and a late-onset neurological condition called fragile X tremor/ataxia syndrome in a proportion of male as well as a small number of female premutation carriers. The mechanism by which the CGG expansion causes these very different conditions is not fully understood. The expansion of the CGG repeat occurs only when transmitted through a female, and this

helps to explain the bizarre pattern of inheritance, which came to be called Sherman's paradox, after our colleague Stephanie Sherman, who did much of the population genetic analysis of families in which the fragile X syndrome was segregating (42). To have had the privilege and excitement of working on this novel class of mutations was certainly one of the highlights of my career.

RETURN TO THE UK

In 1985 we left Hawaii and, after a brief spell in New York City, relocated to the UK, where I became the director of the Wessex Regional Genetics Laboratory, which had as its remit the provision of laboratory genetic services to a population of some two and a half million people. I accepted this, for me, somewhat novel situation because my research interests were still focused on the use of molecular polymorphisms to determine the mechanism of origin of common chromosome abnormalities. For this, I needed access to a large number of chromosomally abnormal patients. Directing a laboratory service covering both cytogenetic and molecular investigations has exposed me to a fascinating range of genetic abnormalities and has indeed provided a wonderful source of material on which to undertake research.

The Origin of Numerical Chromosome Abnormalities

Until the 1980s, the origin of chromosome abnormalities could be determined only when a rare cytogenetic polymorphism was segregating with the abnormality. However, the 1980s saw the development of restriction fragment length polymorphisms, and these molecular probes made it possible to determine the parental origin and the stage of gametogenesis where the error had occurred. We thus set out to determine the origin of a variety of chromosome abnormalities, both numerical and structural.

The 45,X genotype is the only monosomy that occurs in large enough numbers, among both spontaneous fetal deaths and live births, to determine the parental origin. In both the spontaneous abortion and the live-born groups, the single X is paternal in about 20% of the cases and maternal in about 80% (16). Thus, the error giving rise to this common aneuploidy usually results from the loss of a paternal sex chromosome, one of the very few aneuploidies where this is the case. Furthermore, there is no effect of parental age on this common aneuploidy. Among the remaining sex chromosome aneuploidies, the XXY genotype results from an error of paternal gametogenesis in about 50% of patients and of maternal gametogenesis in the remaining 50%. In the XXYs of paternal origin, there is again no effect of parental age, with the majority resulting from failure of recombination in the pairing region of the X and Y chromosomes followed by nondisjunction at the first meiotic division (49). In contrast, the XXYs of maternal origin are associated with advanced maternal age and a variety of unusual patterns of recombination in the first or second meiotic division (51). The great majority of XXX females, whether ascertained as spontaneous abortions or live births, are the result of nondisjunction of the maternal X chromosome, usually occurring at the first meiotic division. This is also associated with a marked increase in maternal age (51). XYY males are not found in excess among spontaneous fetal deaths, are not associated with an increase in paternal age, and are the result of fertilization of a 23,X oocyte by a sperm with an additional Y chromosome. Thus, XYY males arise as the result of segregational errors during spermatogenesis, and the great majority result from nondisjunction at the second meiotic division after a normal chiasmate first division (38).

Our knowledge of the origin of most autosomal nondisjunctions comes from the study of spontaneous fetal deaths. For all autosomes that have been studied, the majority of nondisjunctions arise from maternal nondisjunction at either the first or, less frequently, the second meiotic

division. However, there are considerable differences among different autosomes. The most common trisomy in our species is trisomy 16, which always leads to spontaneous abortion. Furthermore, trisomy 16 is virtually always the result of a nondisjunctional error at the first meiotic division of the oocyte (18). About 90% of most other trisomies are of maternal origin, with the majority resulting from an error at the first meiotic division and only a minority resulting from an error at the second (17). The only clear exception to this is trisomy 18, where the vast majority, if not all, are the result of an error of maternal meiosis, and in the great majority of these the error occurs at the second meiotic division (2). Irrespective of the precise mechanism of the nondisjunction event leading to the trisomic conceptus, all are associated with increased maternal age, although there are significant differences among different chromosomes. Interestingly enough, although the majority of nondisjunctional errors (with the exception of those in chromosome 18) arise at the maternal first meiotic division, most nondisjunctional errors of paternal origin arise at the second meiotic division (25).

The Origin of Structural Chromosome Abnormalities

One of our more recent interests has been the study of the parent and mechanism of origin of structural as opposed to numerical chromosome abnormalities. Our initial focus was on unbalanced structural abnormalities visible in the light microscope. We and others showed that the great majority of de novo unbalanced structural rearrangements were of paternal origin, and our recent restudy of these individuals has found no significant effect of either maternal or paternal age on the genesis of this class of abnormality (50).

Recently, technical advances have made it possible to determine the origin of balanced structural chromosome abnormalities. We studied a series of de novo balanced translocations and found virtually all to be paternal in origin. In contrast to our observation in unbalanced rearrangements, we found the balanced translocations to be associated with a significantly increased paternal age (52). I believe this is the first observation of an effect of paternal age on the genesis of a structural chromosome abnormality. Our observation suggested that the majority of balanced translocations might arise during one of the numerous mitotic divisions that occur in the spermatogonial germline prior to meiosis.

After determining the origin of unbalanced and balanced structural abnormalities visible in the light microscope, we undertook studies to determine the origin of small, unbalanced structural rearrangements that are below the resolution limit of the light microscope. These are detected using fluorescence in situ hybridization or, more recently, array comparative genomic hybridization (aCGH). We and others have shown that the origin of many of the more common microdeletion and microduplication syndromes—e.g., DiGeorge syndrome and Williams syndrome—is equally likely to be maternal or paternal, and there appears to be no effect of parental age (43).

Variations detected by aCGH can be considered to fall into two general classes based on their mechanism of formation. First is those recurrent abnormalities that result from nonallelic homologous recombination (NAHR) mediated by low-copy repeats (LCRs). LCRs are region-specific DNA blocks that are usually 10–300 kb in size and more than 95% identical to one another. NAHR at meiosis results in the gain or loss of the genomic region flanked by the LCRs. NAHR is the mechanism giving rise to the common deletions that cause DiGeorge syndrome and the other well-documented genomic syndromes recognized before the advent of aCGH.

LCRs have now been defined throughout the human genome, and it is possible to distinguish rearrangements mediated by LCRs from rearrangements formed by other, mainly unknown, mechanisms. Furthermore, NAHR mediated by LCRs is now known to be the basis of a large and growing number of new clinical syndromes that have been recognized only since the advent

of aCGH. We have recently examined a series of 36 de novo deletions and duplications that were ascertained by aCGH and resulted from NAHR at meiosis. We found that they were equally likely to be maternal or paternal in origin and that they were not associated with any parental age effect. In both these respects, they are the same as the common conditions such as DiGeorge and Williams syndromes, which is hardly surprising as they all appear to result from the same mechanism (43).

However, there is a much more frequent type of variation detected by aCGH, and this can most simply be defined as variation not resulting from NAHR. We recently investigated the origin of 76 de novo copy-number variants (CNVs) of this type and showed 58 to be paternal and only 18 to be maternal in origin, a significant ($p = 0.023$) excess of CNVs of paternal origin. An excess of paternal origin for this class of CNV was also found in our previous study of de novo deletions associated with apparently balanced translocations (14) and in results reported from Orsetta Zuffardi's laboratory among clinically abnormal individuals with apparently balanced rearrangements (6). Thus, the weight of evidence suggests that there is a significant excess of aCGH-detected abnormalities of paternal origin in the major class not mediated by LCR. The most reasonable explanation for such abnormalities is that they arise because of errors in spermatogenesis. These errors may arise during the many premeiotic cell divisions that occur in the production of the male gametes, which contrasts with the relatively few that occur during the formation of the oocytes.

Mortality and Morbidity Among Chromosomally Abnormal Patients

The UK has excellent mortality and cancer incidence data and a relatively small number of diagnostic cytogenetic laboratories that serve the entire country; it is therefore relatively easy to investigate mortality and cancer morbidity in defined groups of individuals with a chromosome abnormality. In collaboration with Professor Antony Swerdlow and his colleagues, we have been involved in a series of such studies. We have shown that men with an additional X chromosome have a significantly increased mortality, with particular emphasis on diabetes, epilepsy, pulmonary embolism, and peripheral vascular disease (44). They also have elevated risks of several types of cancer, including lung cancer, breast cancer, and non-Hodgkin lymphoma, but a significantly lowered risk of prostate cancer (45). Women with an additional X chromosome have increased mortality from cardiovascular and respiratory disease and also show a significant excess of non-Hodgkin lymphoma (46).

Women with Turner syndrome show an increased mortality from almost all causes of death, an increase that manifests itself at all ages (40). They also have an increased risk for tumors of the central nervous system and for cancer of the bladder, urethra, and corpus uteri (39).

Males with an additional Y chromosome have a significant overall increased mortality, with special emphasis on diseases of the nervous system, notably epilepsy. They also have increased mortality from diseases of the circulatory system, respiratory system, and genitourinary system as well as congenital abnormalities. In contrast, their cancer incidences are not significantly different from those in the general population (19).

Among patients with autosomal deletions recognized at the level of the light microscope, the most common deletions observed are of 22q, 15q, and 7q, and the least common are 19q and 20q. The prevalence of visible deletions is significantly inversely correlated with the gene density of the chromosome arm ($p < 0.001$). Mortality is significantly increased for all deletions with ≥ 25 subjects in the study. The data suggest that the viability of fetuses with visible chromosome deletions is inversely related to gene density and that all visible deletions lead to increased mortality, with the extent and causes of the mortality varying among the specific deletions (48).

It is well established that individuals with a deletion in 11p have an increased risk of Wilms tumor and that individuals with a deletion in 13q have an increased risk of retinoblastoma. Among

the remaining patients with a deletion, we observed an increased risk of anogenital cancer in those with an 11q24 deletion, suggesting that there are tumor suppressor genes within 11q24 that greatly affect anogenital cancer risks (47).

CURRENT RESEARCH

Mosaicism and Down Syndrome

Finally, I will touch on a project in which I am currently involved. We are testing a hypothesis originally postulated by one of my scientific heroes, Lionel Penrose, based on two observations in Down syndrome patients: dermatoglyphics (i.e., hand and finger prints) and grandmaternal age. Penrose showed that Down syndrome patients have characteristic dermatoglyphic patterns that are diagnostic for the condition and also showed that some parents of Down syndrome offspring have a dermatoglyphic pattern intermediate between those of Down syndrome patients and controls. Penrose also reported an association of increased grandmaternal age in Down syndrome patients, and specifically in those born to younger mothers and where there was more than one Down syndrome conception in the sibship.

To explain his observations on dermatoglyphics and grandmaternal age, Penrose postulated that at least 10% of young mothers of Down syndrome patients were likely to be gonadal mosaics for an additional chromosome 21, and that the majority of these mosaics were conceived as a trisomy, in which trisomy correction, i.e., selective loss of the extra chromosome 21, resulted in a clinically normal individual (36). It seems reasonable to assume that, in addition to recognized Down syndrome gonadal mosaics, there may be many other mosaics where the trisomy 21 cells are absent from the gonads or have not resulted in Down syndrome offspring but are present in other tissues.

It is well established that a large proportion of Down syndrome individuals who survive to 40 years of age develop Alzheimer's disease. This is thought to result from their having three copies of the amyloid precursor protein gene on chromosome 21, which results in the overproduction of β-amyloid in the brain. The realization that there is a strong association between Down syndrome and Alzheimer's disease has led to a number of suggestions that mosaicism for an additional chromosome 21 might cause the development of Alzheimer's disease in a proportion of individuals who, while not manifesting any symptoms of Down syndrome, are in actuality trisomy 21 mosaics (37).

A compelling paper was published by Schupf and her colleagues in 1994 (41). In a study of the parents of adult Down syndrome individuals, these authors showed that the mothers, but not the fathers, of Down syndrome patients were at an increased risk of developing Alzheimer's disease. Furthermore, they demonstrated that the increased risk was restricted to those mothers who had a Down syndrome offspring when they were 35 years of age or younger. Such women were found to have a fivefold-increased risk of developing Alzheimer's disease. Schupf and her colleagues concluded that their results supported the hypothesis of a shared genetic susceptibility to Down syndrome and Alzheimer's disease. We, among others, think this shared susceptibility could well be mosaicism for chromosome 21.

We hypothesize that a substantial number of people may be unrecognized mosaics for trisomy 21. Currently, the only pointer to such individuals is that they have Down syndrome offspring at a relatively young age, because they are gonadal mosaics. However, there may be many more where the trisomy 21 cells, while not present in the gonads, are present in other somatic tissues. When these include the brain, such cells may contribute to the development of Alzheimer's disease.

Encouraged by recent developments in noninvasive prenatal diagnosis in which a very small amount of fetal DNA is obtained from a maternal blood sample (5, 7), we decided that the time might be ripe for testing DNA from Down syndrome parents for the presence of trisomy 21

mosaicism. We are extremely fortunate that our colleague Stephanie Sherman in Atlanta, Georgia, has been studying the origin of the additional chromosome 21 in Down syndrome for many years and has a large number of DNA samples from Down syndrome patients and their parents in which the parental origin of the additional chromosome 21 is known. We are currently undertaking a pilot project together with the Sherman laboratory with the aim of searching for evidence of mosaicism in the peripheral blood of four groups of individuals: young and old mothers of individuals with Down syndrome of maternal origin, fathers of individuals with Down syndrome of paternal origin, and the spouses of such fathers.

Our approach is two pronged. Initially, we tested the DNA using quantitative fluorescent polymerase chain reaction (QF-PCR) (QSTAR-21, Gen-Probe Inc.) in which seven chromosome 21–specific polymorphic repeat sequences are amplified and quantified. This technique detects levels of mosaicism of 5–10%, although confident interpretation of mosaicism usually requires the presence of three alleles of different sizes for at least one of the polymorphic repeat markers. This, of course, is indicative of trisomy rescue following meiotic nondisjunction. Our second and more powerful approach is to test the samples using massively parallel sequencing. Overrepresentation of sequences from chromosome 21 will be indicative of mosaicism. We think this technique has the potential to detect mosaicism of 3% or greater, and, furthermore, it is polymorphism independent.

We recently obtained preliminary results on our first 136 samples using the QF-PCR technique. The 136 samples successfully tested consisted of 47 young mothers and 41 old mothers of individuals with Down syndrome of maternal origin, 21 fathers of individuals with Down syndrome of paternal origin, and 27 spouses of such fathers. We found no fewer than six putative mosaics (i.e., 4.4% of the tested samples). We found three mosaics among the young mothers (i.e., 6.4%), none among the old mothers, two among the fathers (i.e., 9.5%), and, surprisingly enough, one among the spouses of the fathers. Furthermore, this mosaic lady was the spouse of one of the two mosaic fathers. What an amazing example of assortative mating!

If we continue to demonstrate that our techniques can detect mosaicism for chromosome 21 in the DNA from blood cells of the population of the Down syndrome–related individuals we are currently studying, we plan to extend the study to individuals with Alzheimer's disease. In the first instance, we will test blood samples. However, we also hope to extend our observations to neural tissue. If we can show that a significant proportion of individuals with Alzheimer's disease are indeed trisomy 21 mosaics, it will be both intellectually satisfying and, more important, may provide an early marker for the condition and thus allow treatment to be instigated at a much earlier stage of the disease than is currently possible.

Concluding Remarks

I will conclude by saying how very fortunate I have been in pursuing a career in human cytogenetics for over 50 years. I have been extraordinarily lucky in at least four respects. The first is in the timing of my career: I entered the field of human cytogenetics just as it became the laboratory discipline that kick-started the whole development of modern clinical genetics. The second is in the support I received as a young scientist from Michael Court Brown, whose guidance, encouragement, and example taught me all I know about research. The third is in the wonderful colleagues with whom I have been fortunate enough to collaborate. Almost without exception, they have been stimulating, enthusiastic, and great fun to work with. Finally, I would like to thank my husband, Newton Morton, for his support and for convincing me that human cytogenetics, while a very visual and beautiful discipline, was also one that could and should be subjected to rigorous mathematical proof. I sometimes had difficulty in following his mathematical equations, but I soon learned that he was almost always correct.

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