Revisiting establishment of the etiology of Turner syndrome

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Submitted for publication in August 2007.
Approved for publication in August 2008.

JUNG, Monica de Paula et al. Revisiting establishment of the etiology of Turner syndrome. *History, Ciências, Health – Manguinhos*, Rio de Janeiro, v.16, n.2, Apr.-June 2009.
Available at: http://www.scielo.br.

Abstract

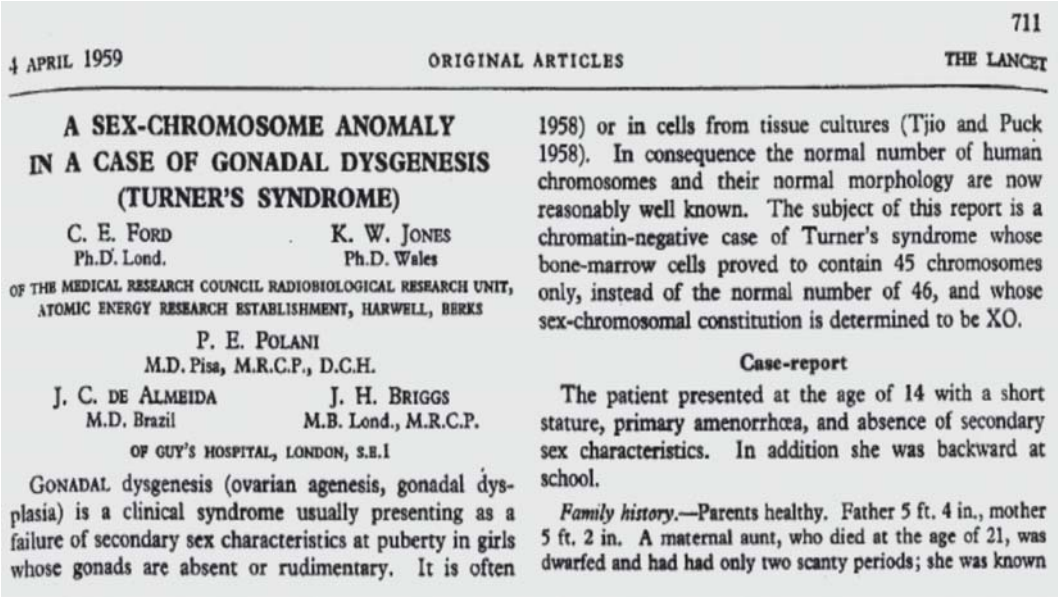
Based on an interview with José Carlos Cabral de Almeida, who took part in the investigative process, the article explores the research that culminated in the establishment of the genetic etiology of Turner syndrome. Cabral de Almeida also discusses other work that he sees as landmarks in the birth of cytogenetics and offers his current view of the development of clinical genetics and the important role played by cytogenetics, which affords more precise means of diagnosis, prognosis, and control of genetic disorders. In its conclusion, the article points to pioneer work that continues to impact medical genetics, especially the study of human chromosomes, still fundamental to the success of linking human genetics and disease processes.

Keywords: cytogenetics; medical genetics; Turner syndrome; history; José Carlos Cabral de Almeida.

In the context of both healthcare and medicine, this article looks at the period when chromosomal anomalies were first described and it became possible to uncover the genetic origin of the most common causes of birth defects, which involve seven out of every one thousand live births and also account for nearly half of all spontaneous abortions during the first semester of pregnancy (Nussbaum, McInnes, Willard, 2001). Based on an interview with Brazilian physician and scientist José Carlos Cabral de Almeida, the study evokes some of the steps in the research process leading to establishment of the genetic etiology of Turner syndrome (TS) and contextualizes his role in the process itself and thus in the history of clinical genetics and human cytogenetics.

José Carlos Cabral Almeida's testimony was obtained through a topical interview (Camargo, 1981) that served to stitch together aspects and moments of the social reality under study. We chose as our topical focus the preparation and writing of an article demonstrating the etiology of TS, written by Charles Edmund Ford, Kenneth W. Jones, Paul Emmanuel Polani, José Carlos Cabral de Almeida, and John H. Briggs and originally published in *The Lancet* in 1959, entitled "A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner syndrome)" (Fig. 1).

The interview was an open-ended free account. Although no specific questions were drawn up beforehand, some topics were outlined in order to help bring to mind the facts laid out in the article and stimulate the interviewee's recall (Jung, 2004). The interview was transcribed and then proofread twice, together with the interviewee, in order to arrive at agreement on the final document. The latter was then analyzed using: (a) open coding, which entailed a line-by-line reading of questions and answers and identification of ideas, themes, and topics; and (b) closed coding, based on topics previously identified as of special interest to the interviewer. The results of these stages were cross-referenced before moving on to the interpretation per se (Jung, 2004).



711

4 APRIL 1959 ORIGINAL ARTICLES THE LANCET

A SEX-CHROMOSOME ANOMALY IN A CASE OF GONADAL DYSGENESIS (TURNER'S SYNDROME)

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GONADAL dysgenesis (ovarian agenesis, gonadal dysplasia) is a clinical syndrome usually presenting as a failure of secondary sex characteristics at puberty in girls whose gonads are absent or rudimentary. It is often

1958) or in cells from tissue cultures (Tjio and Puck 1958). In consequence the normal number of human chromosomes and their normal morphology are now reasonably well known. The subject of this report is a chromatin-negative case of Turner's syndrome whose bone-marrow cells proved to contain 45 chromosomes only, instead of the normal number of 46, and whose sex-chromosomal constitution is determined to be XO.

Case-report

The patient presented at the age of 14 with a short stature, primary amenorrhœa, and absence of secondary sex characteristics. In addition she was backward at school.

Family history.—Parents healthy. Father 5 ft. 4 in., mother 5 ft. 2 in. A maternal aunt, who died at the age of 21, was dwarfed and had had only two scanty periods; she was known

Fig 1: First page of article by Ford et al, The Lancet, 1959

The first section of the present article sketches a brief professional biography of José Carlos Cabral de Almeida. The second centers on how he came into contact with cytogenetics, with particular emphasis on the role played by the third co-author of *The Lancet* article, Paul Emmanuel Polani, in designing the research; drawing up the hypothesis of the 45,X0 karyotype[1] as a correlate of the phenotypical manifestations of TS; interpreting results; and writing the article itself. The third part discusses the text per se and its meaning within the history of cytogenetics; it also compares its structure with other seminal articles produced around the same time. Lastly, it presents Cabral de Almeida's opinions on medicine, cytogenetics, molecular biology, and the union of clinical practice and research, as expressed during the interview.

Brief biography of Cabral de Almeida

José Carlos Cabral de Almeida graduated in 1955 from the Universidade do Brasil, now the Universidade Federal do Rio de Janeiro (UFRJ). He began his studies of endocrinology in his final years at the school, a reflection of the influence of renowned Brazilian endocrinologist José Sherman and of his interactions with the endocrinology team at Santa Casa da Misericórdia hospital in Rio de Janeiro. In mid 1958, Cabral de Almeida finished his specialization and received a fellowship from the British Consulate for an internship at Guy's Hospital in London, where he became part of the group that was to uncover the etiology of TS.

Upon returning to Brazil, he joined the medical staff at the Fernandes Figueira Institute, part of the Oswaldo Cruz Foundation. There, in 1960, he created the first medical genetics service in what is now the state of Rio de Janeiro, heading it until his retirement in 1998. During the same period, Carlos Chagas Filho invited him to work at the Universidade Federal do Rio de Janeiro's Institute of Biophysics, where he founded the Human Cytogenetics Unit. In mid 1960, he created the Outpatient Clinic for Genetics at the newly inaugurated Luiz Capriglione State Institute for Diabetes and Endocrinology (known by the acronym Iede).

Cabral de Almeida published over 150 scientific articles and was the only Latin American researcher to be appointed by the world geneticist community, for ten years, to serve on the international committee responsible for drawing up the International System for Human Cytogenetics Nomenclature (ISCN). He is still professionally active, maintaining a keen interest in genetics and endocrinology and promoting cooperation between Brazilian researchers and researchers from other American and European countries. He maintains a vast personal library, and for decades held weekly meetings at his home with physicians and scientists from different specialties, all connected by the field of genetics. He still keeps in touch with the services he founded, through his close ties with the scientists currently working at them. As he states in his interview, all these services continue to hold to the tradition of working with lines of research and teaching that are directly related to clinical genetics.

Discovering cytogenetics in London

In 1959, Cabral de Almeida arrived in London to work with professor Peter Maxwell Farrow Bishop, a prominent British endocrinologist affiliated with the internationally

renowned Guy's Hospital. He was sponsored by the British Consulate and recommended by Lawson Wilkins, a noteworthy figure in the history of endocrinology and arguably of medicine, whose book *The diagnosis and treatment of endocrine disorders in childhood and adolescence* is considered a landmark in endocrinological practice and research. The first edition was published in 1950; in the third edition, released after Wilkins' death, TS is discussed in chapters 12 and 13, with one of the references being Ford et al.'s article (Wilkins, 1965, p. 340). Wilkins had introduced the Brazilian physician to Bishop about one decade earlier, and now a paper to which Cabral de Almeida had contributed was cited in the bibliographical references to the section "Gonadal aplasia and its variants" in Wilkins' book (p. 339).

Cabral de Almeida's interview confirms John Money's words in the brief introduction to the third edition of the book: Lawson Wilkins continually strove to expand his and everyone's knowledge of child and adolescent endocrinology. In his interview, however, the Brazilian physician states that his greatest reward was meeting Paul Emmanuel Polani shortly after arriving in London. Polani was impressed by some of Cabral de Almeida's observations regarding a female patient, and he invited the young doctor to join the team researching the possible genetic etiology of TS.

A physician trained at the University of Pisa, Italy, Paul Emmanuel Polani was best characterized by his eclectic intellectual and scientific interests, which tightly linked such varied specialties as pediatrics, neurology, cardiology, and genetics. According to his peers, it was Paul Polani's view that a multidisciplinary approach to disease was even more effective when driven by a genetic perspective (Adinolfi et al., 1982). From the very start of Polani's clinical practice, his career was influenced by an interest in pediatric neuropathies, and in October 1960, he was appointed to the Prince Philip chair in pediatric research at Guy's Hospital Medical School.

Over the years, especially after 1983, Polani conducted a number of activities at the *Pediatric Research Unit,* part of the *Division* of *Medical and Molecular Genetics* at Guy's Hospital, and also at King's College hospital and St. Thomas Hospital Medical School, all affiliated with the University of London, where he was professor emeritus until his death on February 6, 2006, at the age of 92. Considered one of the forerunners of cytogenetics in London, Paul Emmanuel Polani passed away at a moment when the world genetics community was preparing to commemorate the fiftieth anniversary of the birth of cytogenetics, marked by the 1956 discovery of the correct chromosome number in human somatic cells (Adinolfi; Alberman, 2006).

In the 1950s, Polani turned to the application of genetic analysis to congenital heart disease. In 1954, his observation that an unusual number of women with TS displayed coarctation of the aorta[2], an anomaly more often found among men, prompted him to study their *sex chromatin,*[3] which proved negative. Given this result and knowing that color-blindness was an X chromosome marker, he began studying it in female patients, working together with Bishop (Polani, Lessof, Bishop, 1956). This clinical research led him to the hypothesis that patients with TS presented an abnormal karyotype and possibly presented X-chromosome monosomy (Harper, 2006).

According to medical historiography (Tesch, Rosenfeld, 1995), the Italian anatomist Giovanni Morgagni was the first researcher to describe what would come to be called Turner syndrome, when, in 1768, he reported on his autopsy of the body of a woman of short stature with underdeveloped breasts, an absence of pubic hair, kidney malformations, and gonadal dysgenesis.[4]

In 1902, a 15-year-old girl of short stature with *congenital lymphedema*,[5] a webbed neck,[6] and gonadal dysgenesis caught the attention of Otto Funke, who recorded the case in medical literature; it is now considered one of the first cases illustrating the condition (Tesch, Rosenfeld, 1995). At the Pediatric Society in Munich in December 1929, Otto Ullrich reported on the case of an 8-year-old girl who had been born with *congenital lymphedema* and had a webbed neck, palpebral ptosis,[7] micrognathia,[8] narrow palate, short stature, a low hairline at the back of the neck, and inverted and hypoplastic nipples.[9] This still stands as the definitive description of gonadal dysgenesis.

Nine years later, Henry Turner (1938) published his study on seven women patients at the *Oklahoma State University* Medical Center's Endocrine Clinic who presented characteristics similar to those reported by Ullrich, in addition to elbow deformities (*cubitus valgus*), an aspect that he emphasized as much as gonadal dysgenesis. He then began hormone therapy of his patients, and the condition became known as Ullrich-Turner syndrome. The name Ullrich was gradually abandoned.

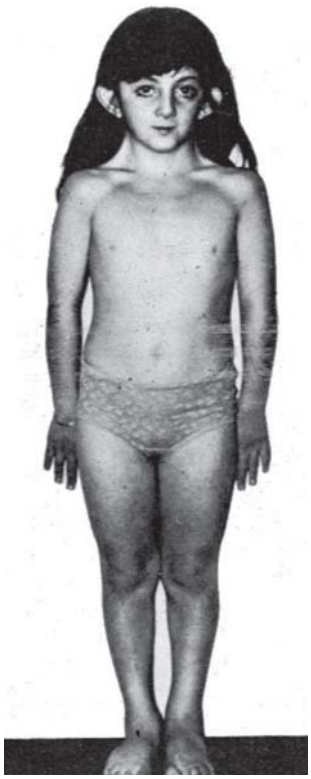

Fig 2: Girl with phenotypical characteristics of Turner's syndrome (source: http://medidacte.timone.univ-mrs.fr/Learnet/webcours/genetique/mutations_chromo/AMCJPG/AMC11.jpg)

In brief, TS is defined today as a chromosomal anomaly in which only one X chromosome functions normally and the other sex chromosome may be missing or abnormal (Hall, Lopez-Rangel, 1995). The phenotype is characterized by short stature, gonadal dysgenesis, and other congenital malformations, with infertility one of its marks. The rate is 1:2,500 live female births. It is postulated that women with TS have a high risk of low-self esteem and, consequently, a social life of compromised quality (Schmidt et al., 2006).

In his interview, Cabral de Almeida talks about Paul Emmanuel Polani's participation in the next milestone in the historiography of TS. He says that Polani prepared the entire study, from formulating the hypothesis to concluding how Turner patients should be classified sexually. The fact that Charles Edmund Ford and Kenneth W. Jones were listed as the first two authors was in keeping with the day's convention; in addition to the fact that both held PhDs, Ford headed the laboratory at the Atomic Energy Research Establishment's Medical Research Council Radiobiological Research Unit in the city of Harwell and had conducted the cytogenetic cultures and analyses together with Jones.

Charles Edmund Ford (1912-99) was born in England and received his doctorate at King's College, London. The history of cytogenetics recognizes him both for the incredible technical skill he displayed in adapting methods and for his application of Tao-Chiuh Hsu's hypotonic solutions[10] (1952) to the visualization of chromosomes in metaphase. Ford is also internationally known for devising new techniques that made possible chromosome analysis in difficult tissues like that of the testicles (Kent-First, 1999). Together with John Hamerton, he unequivocally demonstrated the presence of a haploid number[11] of 23 chromosomes in the germinative cells of normal men (Kent-First, 1999).

Ford and Hamerton had always engaged in research using animal models, the latter having investigated intersexuality in goats (Hamerton et al., 1969). For years this was also a topic of studies for Cabral de Almeida (Just et al., 1994). The interviewee emphasized the importance of this kind of research in sex differentiation and determination; he in fact remarked that it was no coincidence that Hamerton and he both devoted themselves to the same kind of research, though kilometers apart and during different periods; in his words, within each of its fields, science is produced by specialists sharing the same research interests and curiosity.

Further according to the interviewee, cytogenetics began to be applied to medicine in 1959, broadening interest in human cytogenetics (Harper, 2006).

As to the article on the etiology of TS, Ford carried out his work on the laboratory bench, preparing tissue cultures together with Kenneth W. Jones. In his interview, Cabral de Almeida explained that the clinical aspects of the research were undertaken by him and John H. Briggs, under the supervision of Polani.

The article attests to Paul Emmanuel Polani's intelligence, medical culture, and rigor in research and interpretation. Cabral de Almeida underscored these same attributes and decisively stated that this scientist's brilliant mind underpinned the entire paper.

The article on Turner syndrome and the origins of cytogenetics

The introduction to the paper "A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome)" defines gonadal dysgenesis, describes the role of chromatin negativity in gonadal dysgenesis, indicates two approaches to studying the problem, and briefly discusses the development of techniques that made possible the study of human chromosomes. It then reports on the clinical case of a female patient with TS, presenting her social identification, family and personal history (including development and growth), and the results of complementary tests, along with a description of the 45-chromosome karyotype. The article states that "these observations of themselves strongly suggest that the chromosome constitution is X0" (Ford et al., 1959, p. 711)[12].

The discussion addresses the unsuitability of the expression chromatin-negative in defining the sex of these patients, because they are "female anatomically and psychologically" (Ford et al., 1959, p.712). This implied an important shift in focus, which in turn had a fundamental impact on these girls – as Cabral de Almeida stresses in his interview – since they had until then belonged to a category dubbed 'intersex'. The article also proposes an explanation for the origin of the *sex chromosome anomaly* in gonadal

dysgenesis and confirms the X0 hypothesis. The conclusion warrants a complete quotation, since it determined a new way of viewing children born with TS, in scientific, social, and cultural terms: "The X0 patient should not be referred to as an instance of 'sex-reversal', as a '*chromosomal male', or a 'genetic male': she is a female, with an abnormal* genotype" (p. 713).

In his interview, Cabral de Almeida mentions Polani's line of reasoning, highlighting how he approached the hypothesis of the X0 karyotype based on his studies of the rate of coarctation of the aorta and color-blindness in the women examined. For Cabral de Almeida, it was thanks to Polani's astute mind and to his vast clinical experience – also mentioned by Harper (2006) – that he noted that coarctation of the aorta in sexually normal individuals was more common in men and in patients with TS. This is why he suspected that women with TS were neither XY nor XX but probably X0.

Next, according to the interview, Polani began studying color-blindness (Harper, 2006); Cabral de Almeida himself was assigned to conducting eye exams of the female patients with TS. It was observed that their rate of color-blindness was similar to that found in the male population in general and that the parents of color-blind girls with TS were not themselves color blind. Polani then concluded that the mothers were the carriers of the color-blindness gene.

In order to better represent Polani's hypothetical deductive method and the importance of the heuristic hypothesis of the X0 karyotype, Cabral de Almeida decided to read the article in order to translate what had just been explained as faithfully as possible. After repeating his affirmation that he considers this article one of the best written in the history of cytogenetics, he read the excerpt about the two proposed approaches for sex identification in TS: direct cytological observation and the study of color-blindness, which is a sex-linked recessive factor and X-chromosome marker. Cabral de Almeida then pointed out that cytological examination showed the female patients to be chromatin-negative, that is, their cells presented no Barr body, contrary to what occurs in the cells of individuals of the female sex.

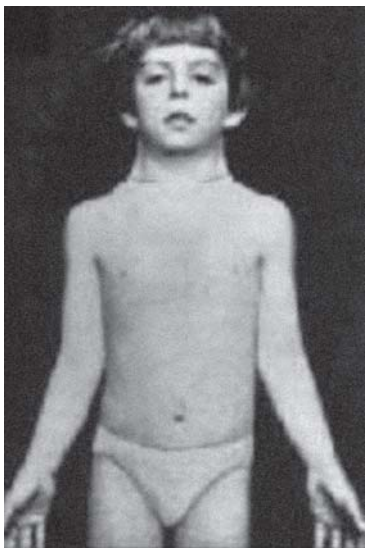

Fig 3: Child with phenotypical characteristics of Turner's syndrome, particularly elbow deformities and webbed neck (source: http://www.caihand.com/images/turner.jpg)

In the same article, Polani suggested doing away with the terms 'nuclear sex' and 'genetic sex', attributed to this chromatin. According to Cabral de Almeida, the scientist's caution regarding the designation of this sex chromosome pattern reflects not only his concern with how it might impact patients psychologically but also his concern with scientific exactitude, since it had already been shown that chromatin positivity or negativity did not indicate whether a child was X0 or XY, XX or XXY.

During this part of the interview, Cabral de Almeida recalled the role Brazilian scientists played in the development of genetics; he emphasized the group with ties to Luciano Décourt, an endocrinologist

from the Escola Paulista de Medicina, whose paper (Décourt et al., 1954) was referenced in the article. Décourt was the first scientist to apply Barr and Bertram's 1949 discovery about the sex chromatin of cats to humans (Ford et al., 1959). It is worth mentioning that Décourt and his co-authors were still using the term 'genetic sex' to refer to chromatin positivity or negativity, even though Paul Polani's proposal to drop the expression from the scientific vocabulary and replace it with chromatin-positive or chromatin-negative had already appeared in a publication written with Maurice H. Lessof and Peter Maxwell Farrow Bishop (Polani, Lessof, Bishop, 1956). Cabral de Almeida underscores the inarguable importance of Polani's accomplishment: the suggestion was embraced by the worldwide scientific community, significantly alleviating the stigma attached to 'intersexuality', the term most often used to (non)define children born with TS.

For Cabral de Almeida, revisiting this research and the article that reported on it took him back to the origins of cytogenetics and the trajectory of the researchers who wrote the history of a fundamental episode in the development of human genetics research. In his opinion, what was most phenomenal about it were the emergence of cytogenetics and the names associated with it, like Jo Hin Tjio, Jerome Lejeune, Patricia Jacobs, Paul Emmanuel Polani, Mary Lyon, and Susumu Ohno. In point of fact, the articles published by these researchers between 1956 and 1959 are cited as the founding landmarks of human cytogenetics, according to works that retrace the history of the field of human genetics, such as the collection of papers edited by Boyer IV (1963) and the book by Harper (2004).

In his interview, Cabral de Almeida revisited each of the personalities with whom he interacted professionally, through Polani, during the three years in which cytogenetics was taking root as the study of chromosomes. Since then, chromosome analysis has evolved in precision and established itself as an ever more important procedure in various areas of medicine, in addition to clinical genetics (Nussbaum, McInnes, Willard, 2001).

Jo Hin Tjio, the first name cited by Cabral de Almeida, was the earliest to ascertain the correct chromosome number in human somatic cells, in 1956, that is, 46. It had been previously believed that the total was 48 (Wright, 2001), but new methods and greater technical accuracy made it possible to establish the correct number beyond a shadow of a doubt, in turn laying the ground for the study of chromosomal anomalies. The clarity of the metaphase cells[13] was probably explained by the use of fibroblasts from *embryonic lung tissue* (Tjio, Levan, 1956) removed from aborted fetuses; back then, Sweden was one of the few countries where abortion was legal, and that was where Tjio and Levan's laboratory was located (Harper, 2004).

Of all the geneticists named by Cabral de Almeida, perhaps the most well known is Jerome Lejeune, a French pediatrician who devoted himself to scientific research, especially on Down syndrome (DS). In 1953, Lejeune began publishing papers on this topic, in which he described the palmar features of DS children. The results of his investigations were published in the journal of the French Academy of Sciences in 1959, and it was based on this work that the scientist received his doctorate, in 1960.

Lionel S. Penrose – a British physician trained in psychiatry and in caring for children with mental deficits, whose work on phenylketonuria is considered a milestone in the transition from biochemical to molecular genetics studies[14] – and other renowned geneticists

had already suggested that DS was likely the result of a chromosomal anomaly (Harper, 2004). But the paper that can be considered the starting point for human cytogenetics applied to clinical practice was written by Lejeune, Gautier, and Turpin in 1959. Lejeune's research continued until his death in 1994; it included the investigation that led to discovery that the missing segment of the short arm of chromosome 5 resulted in what he called cri du chat, or cat cry, syndrome (Miranda Evaristo, 2003).

Human genetics, cytogenetics, and molecular genetics form the tripod underpinning Patricia Jacobs's work. This British geneticist inaugurated her work with the Medical Research Council's Radiobiology Unit and with Charles Edmund Ford's group in Harwell with her seminal article on the etiology of Klinefelter syndrome; her collaboration with them continues today (Grinstein, Biermann, Rose, 1997). The article that Cabral de Almeida mentions in his interview was written together with John A. Strong and was published in *Nature* in 1959 under the title "A case of human intersexuality having a possible XXY sex-determining mechanism." It describes a patient, "an apparent male aged twenty-four," with gonadal dysgenesis, *gynecomastia*, and small testicles, associated with sparse facial hair and a high-pitched voice (Jacobs, Strong, 1959, in Boyer IV, 1963, p. 241). From collected bone marrow tissue, it was possible to count 44 cells in metaphase, the majority of which contained 47 chromosomes. Study of these cells showed the presence of a normal Y, but also a medium-sized extra chromosome with a sub-median centromere. The authors concluded that there were strong observational and genetic grounds for believing that people with chromatin-positive nuclei are genetic females who possess two X chromosomes. The fact that the patient in this study was chromatin-positive and had an extra chromosome the same size as the X, as well as a normal Y, suggested a genetic constitution XXY.

In a way, the basically descriptive nature of the article, which provides no details of its line of reasoning, resembles that of Lejeune et al.'s article on the etiology of DS. There is a brief introduction about the number of cells examined, as in Jacobs and Strong's paper, ending with the statement: "The 'perfect' cells of non-mongoloid individuals never present these characteristics; it would thus seem legitimate to conclude that mongoloids present a small supernumerary telocentric chromosome, which is responsible for the abnormal 47 number" (Lejeune, Guatier, Turpin, 1959, p. 602).

Cabral de Almeida points out another major revolution in the history of genetics – subsequent to the revolution caused by the work of James Dewey Watson and Francis Henry Compton Crick (1953) – that is, the birth of cytogenetics, which included the following events: Joe Hin Tjio and Albert Levan's establishment of the correct number of chromosomes in human somatic cells; Lejeune et al.'s discovery of the etiology of DS; Jacobs and Strong's etiological description of Klinefelter syndrome; and the establishment of the TS karyotype. However, he emphasizes that the first two studies focused on verifying the correlation between trisomy 21[16] and DS and between the XXY karyotype and Klinefelter syndrome[17]. Despite his respect and admiration for Lejeune and Jacobs, he highlights the import of Paul Polani's work, which did not only verify the X0 karyotype in TS but also brought development of nearly prophetical, and still current, methods of research and presentation, anticipating aspects of the disorder that would only later be confirmed by recombinant DNA techniques.

In 1956, the Japanese researcher Susumu Ohno, also mentioned by Cabral de Almeida, showed that the chromatin found in women's cells was not associated, as believed, with two X chromosomes but only with a heterochromatic X chromosome (Beutler, 2002). Ohno likewise suggested that the sum of gene material is the same across species and, around the same time, submitted that in most higher-order organisms, DNA consists of sequences that do not code protein; he called this extra DNA 'junk DNA'.

Further according to Cabral de Almeida's interview, British scientist Mary Lyon, together with Ohno, made fundamental contributions to the development of human cytogenetics research. Her current research focuses on mammalian genetics, radiation genetics, mutagenesis, gene mapping, mutant genes and the analysis of bonds, X chromosome inactivation, and haplotypes in mice (Beutler, 2002). Lyon's innovative work became known for detailing the principle of X-inactivation, now called the Lyon hypothesis, which postulates that while men only possess a single copy of each gene on the X chromosome and women have two, the total amount of gene product produced by a single allele in a man or by a pair of alleles in a woman is generally the same (Lyon, in Boyer IV, 1963).

On the one hand, Cabral de Almeida's mention of these particular studies reflects his training under the influence of certain scientists, especially Polani, with whom he grew closer over time. On the other, it reaffirms his predilection for joining basic and clinical research, grounded on the notion that there should be a very tight relationship between the clinician and the geneticist, the molecular biologist and the clinician. For Cabral de Almeida, such a connection is indispensable, because when you study the gene that may be responsible for a disease or predispose people to it, you must be sure the patient does indeed carry the disease. The interviewee posed the following question to illustrate his argument: if 70% of those with a certain disease present a certain mutation, what do the other 30% have? He goes on to say: one must adopt a stance of constant questioning, asking, for example, if the gene under study is really the promoting gene or if the clinical diagnosis is really correct. In our view, this emphasis evinces a dynamic scientific posture and the adoption of an interdisciplinary philosophy according to which the point of the arrow, shot from the bow of research, aims at clinical practice and not at research per se.

Current positions shaped by the past

It is because of studies like those mentioned by Cabral de Almeida that geneticists such as Therman and Susman (1993), transforming themselves into historians of cytogenetics, have roughly divided the field into different ages and eras: the dark ages, the hypotonic era, the trisomy period, the chromosome banding era, and, lastly, the molecular era. These designations indicate how the chronology has been based on the history of the evolution of techniques, with the first era corresponding to the time of hypotonic solutions and the second, to counting the precise number of chromosomes in human somatic cells, which made it possible to uncover the genetic etiology of various syndromes.

Along with papers by Lejeune et al., Jacobs and Strong, Lyon and Ohno, and others, the article co-authored by Cabral de Almeida falls into the trisomy period while also moving into the next era. According to Therman and Susman, it was then that

cytogeneticists turned their attention to patients with congenital anomalies. Chromosomal anomalies, especially numerical ones, formed the focus of research and were definitively associated with specific clinical phenotypes described years before, like Down syndrome and TS.

It is impossible to disagree with the interviewee when, while offering his own historical synthesis of cytogenetics, he states that even today this constitutes an extraordinary field for research in medicine, as far as establishing diagnostic hypotheses and genetic counseling. For Cabral de Almeida, cytogenetics quite clearly went through a number of phases, the first being the 'big boom,' that is, the period when syndromes like Patau and Edwards were first described[18] – in short, those whose phenotype is associated with structural and numerical chromosome abnormalities.

Next, the interviewee goes on, cytogenetics began to be applied in fields like radiobiology, birth defects, oncogenesis, and sexual anomalies. Molecular biology was an advance that corresponded to a new revolutionary phase, which neither diminished nor devalued cytogenetics; to the contrary, the use of cytomolecular genetics is nothing but one more step forward in the history of cytogenetics. Cabral de Almeida is quite clear about how he thinks these questions should be viewed. It can be stated, he suggests, that molecular biology is here to afford cytogenetics greater resources and not to finish it off. This is so true that one cannot deny the importance of this field in cancer research or overlook the many chromosomal anomalies whose genes were located and/or possibly cloned thanks to what Niels Tommerup – "a great researcher" – called mendelian cytogenetics. In Cabral de Almeida's opinion, it was structural chromosomal anomalies, for example, that indicated the likely location of certain genes, thus mapping breaks and translocations. Once again referring to Tommerup, he calls attention to what this cytogeneticist called dynamic mosaicism,[19] in response to such questions as: why does ring chromosome 13 produce retinoblastoma, and why is ring chromosome 22 associated with a meningioma? Based on the concept of dynamic mosaicism, the answer is that the ring chromosome breaks and loses genes (a tumor suppressor gene, for example), thereby prompting a loss of heterozygosis[20] and allowing a neoplastic process to set in.

Niels Tommerup is professor at the *University* of *Copenhagen*, director of the Wilhelm Johannsen Centre for Functional Genome Research, and coordinator of the Mendelian Cytogenetics Network. He coined the expression 'mendelian cytogenetics' to designate the area of cytogenetics dedicated to mapping the genes responsible for mendelian diseases by identifying translocation breakpoints and by combining data from apparent balanced gene expressions, linked to gene mapping (Wilhelm Johannsen Centre, n.d.). In a way, we can say that this reference to Tommerup's work illustrates the tradition Cabral de Almeida has followed and that still guides research, teaching, and healthcare at the current Department of Genetics at the Oswaldo Cruz Foundation's Fernandes Figueira Institute, founded by this scientist and the first in the state of Rio de Janeiro devoted to medical genetics. The interview leaves it clear that, in keeping with this guideline, a geneticist focused on human genetics must first and foremost be familiar with the clinical aspects of the diseases with which he works. For this reason, Cabral de Almeida makes it a point to state that he has always been a clinical researcher and that in his view the clinical research

method means that the history the patient tells of his own disease is the first step in raising any diagnostic hypothesis. He also ponders the enormous difference between diagnosing and labeling something, since a diagnosis demands commitment to and involvement in the patient's process of sickness.

Cabral de Almeida devotes himself to the study of sex determination and differentiation, congenital malformations, and mental retardation. He was admittedly influenced by the time he spent in England, where he lived through the dawn of cytogenetics. For him, there is no way to separate patient care from research. During the 38 years in which he was on the staff at the Fernandes Figueira Institute and also while at the Universidade Federal do Rio de Janeiro's Institute of Biophysics and the Luiz Capriglione State Institute for Diabetes and Endocrinology, his career was framed by research, teaching, and healthcare, although he recognizes that his central focus is on healthcare; in other words, in his view, basic scientific research and teaching are tools essential to medical-clinical performance.

He believes that some of the greatest errors in research, teaching, and especially in healthcare are pre-judging, fearing new information, and accepting new models of scientific outreach and education without critical reflection, merely because they are in fashion. In a way, this attitude was already taking shape when he traveled to England. Before and immediately after graduating, his ties with endocrinology and, within this field, with genetic diseases suggested he would be a researcher who would ground the construction of his diagnostic hypotheses on the signs he observed and interpreted.

Throughout his interview and especially at the end, Cabral de Almeida strives to highlight the contribution of cytogenetics and its historical role in human genetics and clinical practice. He finishes by intentionally placing two genetic diseases on the stage – Down and Turner syndromes – both of whose etiologies were uncovered by cytogenetic analyses. In his opinion, both disorders constitute worlds under construction, with a wealth of information yet to be established, in part because of the progress made possible by molecular biology techniques. In regard to Down syndrome, he says that he knows of no other genetic disease that has produced more knowledge during the 140 years it has been studied. He reproaches clinical geneticists who tend to trivialize it as the most common genetic disease and instead place priority on very rare disorders that will certainly ensure easier publication, while they ignore the great efforts that still remain to be done if experimental and clinical research are to join together to provide a better quality of life for people with genetic diseases.

Final considerations

Cytogenetics has been evolving since 1956, when Tjio and Levan developed efficacious techniques for chromosome analysis and established the normal number of chromosomes in human somatic cells. By incorporating tools from molecular biology, the field has helped medicine to address issues previously explored solely through clinical resources and has thus fostered the incorporation of genetic diseases into the collective health setting. Furthermore, when advances in genetic research moved beyond the boundaries of mendelian conditions, they were assimilated by numerous sectors responsible for promoting

health and eventually came to influence the classic conceptual bases of public health (Cardoso, Castiel, 2003). Indeed, when growing interest in the study of m*ultifactorial conditions* – *especially* congenital malformations, mutagenesis, teratogenesis, carcinogenesis, hemoglobinopathies, and thalassemies – came to encompass cytogenetics, cytomolecular genetics, and molecular genetics, this testified to the link between collective health and genetics.

On the other hand, the media's coverage of the application of knowledge from the field of genetics has fed the public's imagination and instilled a new way of thinking about our bodies and shaped new ideas about what is natural and normal (Petersen, 2002). We often find major newspapers and magazines printing promises about the miracles of genetic techniques. Such controversies notwithstanding, there is an undeniable need for an inclusive scientific policy and for social engagement regarding investments and decisions about means and ends, given the social consequences of the advances brought by recombinant DNA technology (Gaskell, Bauer, 2006).

There are those who praise technique and extol the vital role to be played by researchers, like James Watson (1995, p. 197): "If we don't play God, who will?" But there are also those – as Cabral de Almeida's interview bears witness – who look towards a new narrative of life without however losing sight of the story that has been constructed over time, in which the discourses of cytogenetics, molecular biology, and medicine entwine with those of people suffering from etiologically genetic conditions, whose identities cannot be reduced to the genic dimension and whose complaints and symptoms are the point of departure and of return.

Our effort in these pages has been to bring to life the memory of an article published in 1959, with the help of the testimony of a Brazilian scientist and physician. Along with three other papers published that same year, this article was inarguably part of the process of recognizing chromosomal anomalies as the basis of serious genetic diseases, which could only be studied following the finding that human somatic cells contain 46 chromosomes. It is worth noting that although the structure of DNA was understood, it was impossible to count the correct number of human chromosomes, much less rely on cytogenetics to uncover numerical and structural chromosome variations.

All the papers cited by Cabral de Almeida were pioneering works, and their contributions to clinical genetics are still recognized today. The study of human chromosomes was and still is the source of information for geneticists in general, but it makes a special contribution to the success of human genetics as it relates to disorders.

NOTES

[1] Karyotype refers to a person's chromosomal constitution. In the case of Turner syndrome, the most common karyotype has only one X chromosome instead of the normal XX or XY.

[2] A narrowing that decreases the aortic lumen, blocking blood flow.

[3] Also called the Barr body or Barr chromatin, sex chromatin refers to the complex of DNA and proteins making up chromosomes. It is seen in female somatic cells.

[4] Dysgenesis refers to any developmental anomaly.

[5] Edema of the lymphatic vessels.

[6] Thus called because the neck is connected to the shoulders by a broad band of skin.

[7] Palpebral ptosis indicates excessive drooping of the upper lid, which covers the eye abnormally.

[8] Micrognathia is a term that describes an abnormally small lower jaw.

[9] Hypoplastic nipples are underdeveloped nipples.

[10] A hypotonic solution presents a lower osmotic pressure than other solutions.

[11] The haploid number is the number of chromosomes in a normal gamete, which contains only one chromosome from each chromosome pair.

[12] All quotations are from Samuel H. Boyer IV's collection entitled *Papers on human genetics* (Boyer IV, 1963), which reproduces the articles in full.

[13] Metaphase cells are found during a certain stage of cell division. Their chromosomes align in the middle of the cell, facilitating their observation.

[14] Prior to publication of Penrose's paper, little attention had been paid to the genetic aspects of Phenylketonuri. Penrose not only demonstrated the genetic basis of the disease, linking it with the loss of a specific enzyme, but also confirmed that it is autosomal recessive.

[15] The syndrome earned its name based on the infant's cry, which is so weak that it sounds like a meowing cat.

[16] This chromosome is characteristic of pair 21. Lejeune observed that there were three chromosomes instead of two; hence the name. In a trisomy, there are three copies of a chromosome instead of the normal two. Patau and Edwards syndromes are also trisomies: the first involves chromosome 13 and the second, chromosome 18.

[17] Klinefelter syndrome is characterized by a *47,XXY karyotype*. Its carriers are tall, thin, infertile men.

[18] This condition is characterized by trisomy of the X sex chromosome and may be associated with infertility. Those with the syndrome may have a normal phenotype.

[19] Mosaicism is a condition where two or more genetically different cell lineages are derived from one single zygote because of mutation or non-disjunction.

[20] Heterozygosis refers to an individual or genotype with two different alleles at a given locus along a pair of *homologous chromosomes*.

REFERENCES

ADINOLFI, Matteo William et al.
Pediatric research: a genetic approach. Cambridge: Cambridge University Press. 1982.

ADINOLFI, Matteo; ALBERMAN, Eva.
Paul Polani: pioneering research into chromosomal abnormalities. *The Guardian*, London. Available at: http://www.guardian.co.uk/science/2006/mar/17/obituaries. highereducation. Accessed on: Nov. 6, 2007. 2006

BEUTLER, Ernest.
Susumo Ohno – 1928-2000: a biographical memoir. *Biographical Memoir*, Washington DC, v.81. Available at: http://www.nasonline.org/site/PageServer?pagename=MEMOIRS_O. 2002.

BOYER IV, Samuel H. (ed.).
Papers on human genetics. Englewood Cliffs: Prentice-Hall. 1963.

CAMARGO, Aspasia Alcântara de.
Apresentação. In: Camargo, Aspásia Alcântara de (Coord.). *Programa de história oral*: catálogo de depoimentos. Rio de Janeiro: Centro de Pesquisa e Documentação de História Contemporânea do Brasil/Fundação Getúlio Vargas. p.11-15. 1981.

CARDOSO, Maria Helena C. de Almeida; CASTIEL, Luis David.
Saúde coletiva, nova genética e a eugenia de mercado. *Cadernos de Saúde Pública*, Rio de Janeiro, v.19, no.2, p.653-662, 2003.

DÉCOURT, Luciano et al.
Sobre o sexo genético nas pacientes com síndrome de Turner. *Revista da Associação Médica Brasileira*, São Paulo, v.1, no.2, p.203-206. 1954.

FORD, Charles Edmund et al.
A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *The Lancet*, London, no.1, p.711-713. 1959.

GASKELL, George; BAUER, Martin W.
The genomic society and its public: introduction. In: Gaskell, George; Bauer, Martin W. (eds.). *Genomics and society*: legal, ethical & social dimensions. London: Sterling. p.1-6. 2006.

GRINSTEIN, Louise S.; BIERMANN, Carol. A.; ROSE, K. Rose (eds.).
Women in the biological sciences: a biobibliographic sourcebook. Westport: Greenwood Press. 1997.

HALL, Judith G.; LOPEZ-RANGEL, Elena.
Introduction. In: Albertsson-Wikland, Kerstin; Ranke, Michael (ed.). *Turner syndrome in a life-span perspective*. Amsterdam: Elsevier. p.3-9. 1995.

HAMERTON, John L. et al.
Genetic intersexuality in goats. *Journal of Reproduction and Fertility*, Colchester, no.7, suppl., p.25-51. 1969.

HARPER, Peter S.
Paul Polani. *British Medical Journal*, London, v.332, no.7542, p.670. Available at: http://www.bmj.com/cgi/content/full/332/7542/670/DC1. Accessed on: Nov. 6, 2007. 2006.

HARPER, Peter S.
Landmarks in medical genetics. Oxford: Oxford University Press. 2004.

HSU, Tao-Chiuh.
Mammalian chromosomes in vitro: the karyotype of man. *Hereditas*, Landskrona, v.43, p.167-172. 1952.

JACOBS, Patricia A.; STRONG, John A.
A case of human intersexuality having a possible XXY sex-determining mechanism. In: Boyer IV, Samuel H. (ed.). *Papers on human genetics*. Englewood Cliffs: Prentice-Hall. p.240-243. 1963.

JUNG, Monica de Paula.
Síndrome de Turner revisitada: pesquisa bibliográfica e reconstituições narrativas. Master's thesis – Instituto Fernandes Figueira/ Fundação Oswaldo Cruz, Rio de Janeiro. 2004.

JUST, Walter N. et al.
The male pseudohermaphrodite XX polled goat is ZFY and SRY negative. *Hereditas*, Landskrona, v.120, p.71-75. 1994.

KENT-FIRST, Marijo.
Charles Edmund Ford Ph.D. FRS, in loving memory of my mentor and friend. *Cytogenetics and Cell Genetics*, Basel, v.85, nos.3-4, p.193-195. 1999.

LEJEUNE, Jérôme ; GUATIER, M. ; TURPIN, R.
Études des chromosomes somatiques de neuf enfants mongoliens. *Comptes Rendues Hebdomadaires des Seances de L'Academie des Sciences*, Paris, v.248, p.602-603. 1959.

LYON, Mary F.
Sex chromatin and gene action in the mammalian X-chromosome. In: Boyer IV, Samuel H. (ed.). *Papers on human genetics*. Englewood Cliffs: Prentice-Hall. p.31. 1963.

MIRANDA, Evaristo Eduardo.
Quem foi o professor Jérôme Lejeune. Available at: http://www.portaldafamilia.org/artigos/artigo122.shtml. Accessed on: Jan. 20, 2004.

NUSSBAUM, Robert L.; MCINNES, Roderick R.; WILLARD, Huntington F.
Thompson & Thompson genetics in medicine. Philadelphia: Saunders. 2001.

PETERSEN, Alan.
Introduction. In: Petersen, Alan; Bunton, Robin. *The new genetic and the public's health*. London: Routledge. p.1-34. 2002.

POLANI, Paul E.; LESSOF, Maurice H.; BISHOP, Peter Maxwell F.
Colour blindness in ovarian agenesis (gonadal dysplasia). *The Lancet*, London, v.2, p.118-120. 1956.

SCHMIDT, Peter J. et al.
Shyness, social anxiety, and impaired self-esteem in Turner syndrome and premature ovarian failure. *Scandinavian Journal of Psychology*, Stockholm, v.47, no.3, p.219-223. 2006.

TESCH, Lynn G.; ROSENFELD, Ron G.
Morgagni, Ullrich and Turner: the discovery of gonadal dysgenesis. *Endocrinologist*, Baltimore, v.5, p.327-328. 1995.

THERMAN, Eeva; SUSMAN, Millard.
Human chromosomes: structure, behavior and effects. New York: New Springler. 1993.

TJIO, Jo Hin; LEVAN, Albert.
The chromosome number in man. *Hereditas*, Landskrona, v.42, no.46, p.1-6. 1956.

TURNER, Henry.
A syndrome of infantilism, congenital webbed neck, and cubitus valgus. *Endocrinologist*, Baltimore, v.23, p.566-574. 1938.

WATSON, James Dewey.
The double helix: perspective and prospective at forty years. New York: New York Academy of Science. 1995.

WATSON, James Dewey; CRICK, Francis Henry C.
The structure of DNA. Cold Spring Harbor Symp. *Quantitative Biology*, Cold Spring Harbor, v.18, p.123-132. 1953.

Wilhelm Johansen Centre...

Wilhelm Johansen Centre for Functional Genome Research. Staff. Available at: http://www.wjc.ku.dk/staff/. Accessed on: Oct. 12, 2003. N.d.

WILKINS, Lawson.
The diagnosis and treatment of endocrine disorders in childhood and adolescence. Springfield: Charles C. Thomas. 1965.

WRIGHT, Pearce.
Joe Hin Tjio: the man who cracked the chromosome count. *The Guardian*, London. Obituary. Available at: www.tccusa.org-www.eicaa.org. Accessed on: Oct. 12, 2003. 2001.