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# Chromosomes in the Clinic: The Visual Localization and Analysis of Genetic Disease in the Human Genome

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**Abstract**
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# CHROMOSOMES IN THE CLINIC: THE VISUAL LOCALIZATION AND ANALYSIS OF GENETIC DISEASE IN THE HUMAN GENOME

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## ABSTRACT

CHROMOSOMES IN THE CLINIC: THE VISUAL LOCALIZATION AND ANALYSIS OF GENETIC DISEASE IN THE HUMAN GENOME

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This dissertation examines the visual cultures of postwar biomedicine, with a particular focus on how various techniques, conventions, and professional norms have shaped the 'look', classification, diagnosis, and understanding of genetic diseases. Many scholars have previously highlighted the 'informational' approaches of postwar genetics, which treat the human genome as an expansive data set comprised of three billion DNA nucleotides. Since the 1950s however, clinicians and genetics researchers have largely interacted with the human genome at the microscopically visible level of chromosomes. Mindful of this, my dissertation examines the 'observational' approaches of postwar genetics. This is accomplished through a series of case studies, which examine the visual delineation, diagnosis, and genomic localization of a number of disorders. My case studies explore various exemplary attempts to associate particular clinical disorders with specific genetic mutations. This dissertation uses archival resources, oral histories, and the published biomedical literature to examine the many successes of postwar biomedicine, and to highlight the contributions made by a wide rage of biomedical professionals. I find that the visible, tangible human genome, as conceived and depicted at the level of chromosomes, has become an important work object among a diverse array

of practitioners. Chromosomal ideograms, I argue, provide an important basis for communication and common practices among this community. While genetic data is becoming increasingly significant to our understanding of human disease, distinguishing the normal from the pathological remains a task that relies on input from the laboratory and the clinic. Thus, the success of postwar genetic medicine must be seen in light of the contributions of biomedical actors from many disciplines, who have agreed to see and communicate about the human genome – their object of study – in standardized ways.

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# INTRODUCTION

## Informational and Observational Approaches to Human Genetics and Biomedicine

This dissertation examines the visual cultures of postwar genetics and biomedicine. I trace the changing 'look' of disease during an era in which an ever-increasing number of disorders have come to be understood as having a 'genomic' basis. Since the 1960s, practitioners of biomedicine have sought to move past the variable and often confusing presentation of disorders in the clinic. Instead, they have increasingly come to rely on the genomic markers of disease to aid in clinical delineation. As part of this, a new nosological system was developed in biomedicine, which has sought to differentiate diseases by locating their causes within the human genome. In this dissertation, I explore the many successes of this approach to disease classification, while also highlighting various complications that have arisen.

Throughout the postwar period, clinicians and geneticists have looked to the genome, at the microscopically visible level of chromosomes, in the hope of improving the delineation, diagnosis, understanding, and treatment of disease. I examine how human and medical geneticists have come to see and analyze the human genome – their object of study – in standardized ways. Scholars have previously highlighted the 'informational' approaches of postwar genetics, which treat the genome as an expansive digital data set. Since the 1950s however, geneticists have largely interacted with the genome at the microscopically visible level of chromosomes. Mindful of this, this dissertation explores the 'observational' approaches of postwar genetics. Similar to the heart in cardiology, the human genome has been referred to as the 'organ' of medical

genetics. Through historical case studies of disorders like Fragile X and Prader-Willi syndrome, I examine how clinicians and geneticists locate, assess, and develop confidence in correlations between visible chromosomal markers and likely clinical outcomes. The human genome, I argue, has a tangible presence in postwar biomedicine as an anatomical entity and standardized scientific object that can be seen, analyzed, and dissected. At the same time, the genome is also an important conceptual space in biomedicine, where the conventions, interests, and questions of basic genetics and applied clinical research intersect and intermingle.

**Examining 'Mistakes of the Binder' in Postwar Biomedicine**

In 1969, Yale University School of Medicine physician Herbert Lubs reported in the *American Journal of Human Genetics* on the identification of an unusual chromosomal abnormality in a boy affected by intellectual disability. Chromosomal analysis had been performed, and a 'secondary constriction' was identified on one of the boy's chromosomes (Figure 1). The secondary constriction, which caused the appearance of large 'satellites' at the end of this chromosome, was also seen in the patient's mother and similarly affected brother. This suggested that the marker might be simply a benign genetic variant. However, analysis of the boy's extended family revealed that the secondary constriction was always associated with intellectual disability when seen in males, but seemed to have no clinical impact on females. This inheritance

pattern suggested to Lubs that the chromosomal marker, and its physical effects, were X-linked traits (Lubs, 1969).[1]

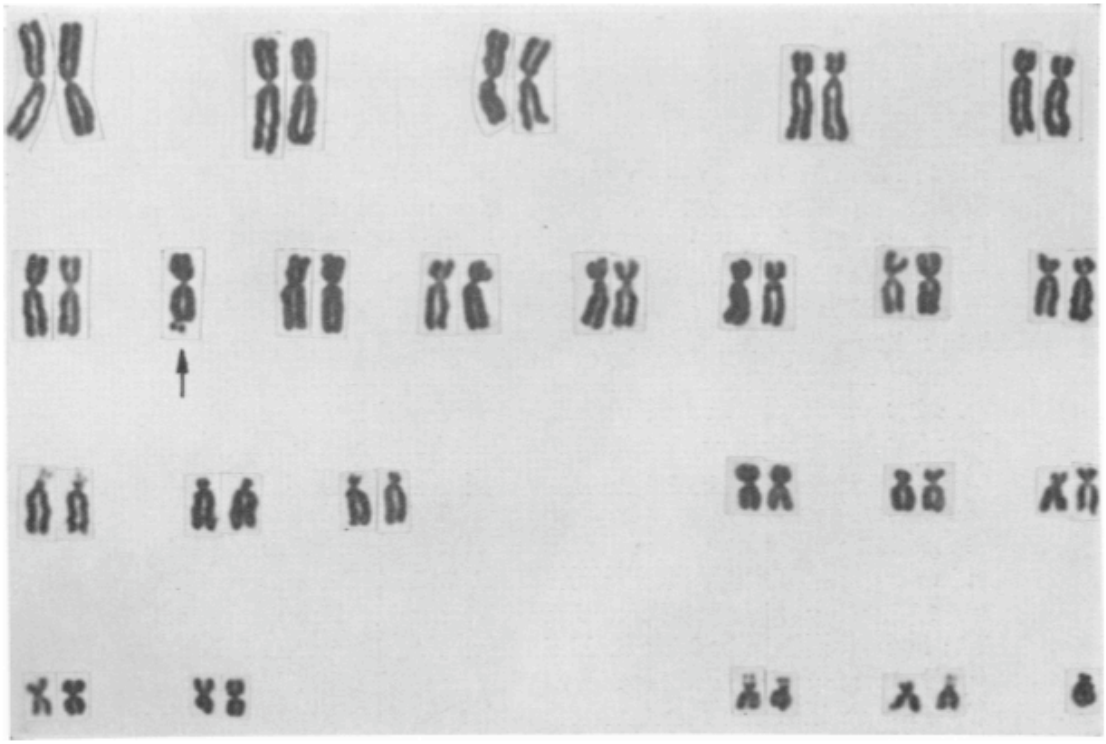

FIG. 3.—Karyotype from the proband showing the marked secondary constriction present in one-third of his cells. The resulting satellites are smaller in diameter than the chromatid but larger than any of the satellites on the acrocentric chromosomes.

**Figure 1** Human chromosome karyotype showing the secondary constriction identified by Herbert Lubs. This image was published in Lubs (1969), Copyright Elsevier (1969). Reprinted with permission.

[1] X-linked traits are so named because they are inherited on the X chromosome. Normally, males possess one X chromosome, and females have two. If such a trait is recessive, it is usually overridden by the other X chromosome in females, but is expressed in males, who have just one.

Perhaps the most clinically significant attribute of this new chromosomal marker was its heritability. Chromosomal abnormalities – such as trisomy 21, the cause of Down syndrome – that had been identified over the past decade were most often the result of random reproductive events, meaning that they were not heritable and could not be traced through families.[2] That this "marker X chromosome", as Lubs called it (231), was passed down through a family, and predictive of intellectual disability, was potentially valuable clinically. With this in mind, Lubs suggested at the beginning on his report that, "descriptive human cytogenetics [chromosomal analysis] is entering a new and important phase." Indeed, identifying smaller, heritable chromosomal anomalies, such as this one, was important because, "they may permit prevention of clinical disease by identifying high-risk marriages and allowing subsequent amniocentesis and abortion of abnormal fetuses if requested by the family" (Lubs, 1969, 231).

Lubs' chromosomal studies in this 1969 report are representative of a set of practices and conceptions that have not been adequately addressed among scholars of postwar genetics and biomedicine. In 1963, human geneticist Lionel Penrose drew a distinction between gene-level mutations, "mistakes of an imaginary printer," which are too small to be seen, and chromosomal aberrations, "mistakes of a binder," that could be observed microscopically (Penrose, 1963, 136). Much attention has been paid, in recent decades, to how researchers study these "mistakes of the printer," using various 'informational' approaches, relying on molecular level techniques. Scholars, for instance, have highlighted the cracking of the DNA code, the importance of recombinant

[2] Most often the abnormal event is non-disjunction, which generally leads to the loss or gain of one chromosome.

DNA technology, and the humbling impacts of the Human Genome Project (Lenoir, 1999; Morange, 1998; Kay, 2000; Keller, 2000; de Chadarevian, 2002).

These informational approaches to postwar genetics treat the human genome as an expansive, and abstract data set, comprised of over three billion DNA nucleotides. Scientists and scholars alike often describe the DNA code, which consists of four nucleotides abbreviated A, T, C, and G, as a 'language'. In its entirety, the human genome has often been referred to as 'the book of life', with obvious religious overtones. As Lily Kay has described, informational metaphors have driven genetics thinking and research throughout the postwar period. Even before the demonstration of the double helical structure of DNA in 1953, information theory and cybernetics were already central to the practices of molecular biology (Kay, 2000; de Chadarevian, 2002). While the 1960s marked a move away from explicit informational theory among geneticists, efforts at 'cracking' the DNA code, and learning to read, translate, and edit it, continued to drive research in the field. Indeed, a shift from more formalistic mathematical approaches to deciphering the DNA code, to material biochemical techniques did not undercut the central role of informational metaphors in molecular biology in the 1960s (Kay, 2000).

Informational approaches to postwar genetics were greatly enhanced during the 1970s by the introduction and development of recombinant DNA technology, which allowed strands of DNA to be cut up, separated out, and recombined at the molecular level. These techniques facilitated the large scale copying of specific segments of DNA through PCR and plasmid cloning, and provided a means for developing a molecular

level ‘physical map’ of the human genome (Rabinow, 1997; Gaudilliere and Rheinberger, 2004). Ultimately, these techniques, coupled with increasingly powerful computing, were harnessed and scaled up, leading to the proposal of the Human Genome Project during the mid-1980s. By 2000, the first reference sequence of the entire human genome was finished and made publically available (Kevles and Hood, 1992; Cook-Deegan, 1995; Rabinow and Dan-Cohen, 2005; Garcia-Sancho, 2012).

While these molecular innovations have been central to the development and successes of postwar genetics and biomedicine, they were not the only tools that clinicians and geneticists used during this period to analyze the human genome. In addition to these ‘informational’ methods, in recent years historians of science have begun to explore the ‘observational’ approaches of postwar human genetics: those that seek to identify and analyze various “mistakes of the binder”. Rather than molecular techniques, observational approaches are largely based on the analysis of the microscopically visible human chromosome set. During the postwar period, despite the great importance of DNA-level techniques, clinicians and geneticists have largely interacted with the genome at the visible level of chromosomes. Mindful of this, a parallel history to that of molecular biology has recently began to be told by scholars (Martin, 2004; Lindee, 2005; de Chadarevian, 2010; Santesmases, 2010; Hogan 2013).

**Observational Analysis of the Human Chromosome Set**

Lorraine Daston has recently called for a “turn towards ontology” among historians and philosophers of science, in particular, “towards ontologies created and

sustained by scientific observation" (Daston, 2008, 97). Observation is a collective practice and the building of visual ontologies is a gradual process, which takes place among a community that learns and agrees to *see* objects, both commonplace and obscure, in particular ways (Fleck, 1979; Daston, 2008). Over the past several years, historians of science have begun to explore the observational practices of postwar genetics, in particular chromosomal analysis. Unlike the theoretical, abstract, and sub-microscopic underpinnings of DNA code cracking and analysis that has been previously highlighted by scholars, the study of chromosomes is highly visual, subjective, and fraught with ambiguous findings.

As Soraya de Chadarevian has noted however, chromosomal analysis was nonetheless of great value to human geneticists in the 1950s and 1960s because it "offered a glimpse of the complete genetic make up on an individual" (de Chadarevian, 2010, 180). This observational view of the human genome produced some extremely persuasive evidence for the genetic cause of disorders like Down syndrome, while at the same time demonstrating that visible genetic abnormalities are often complex, variable, and difficult to distinguish with absolute certainty.[3] Despite various complications, the ability to physically 'see' the genome revolutionized the practices of postwar human and medical genetics. The capabilities of chromosomal analysis were greatly improved during the early-1950s by the introduction of a number of new laboratory techniques. This included the use of colchicine, for arresting cells when chromosomes were visible,

[3] For instance, in some cases of Down syndrome an extra copy of chromosome 21 was not visually obvious, because the cause was a translocation, involving chromosome 21 (Cowan, 2008; Gaudilliere, 2001; Santesmases, 2010).

and the recognition that a hypotonic (low salt) medium helped to spread out the 46 chromosomes present in each human cell, thus making them easier to see, distinguish, and count. Also significant were improvements in cell culture techniques, which allowed for human tissue samples to be derived from the skin or blood or patients, rather than, much more invasively and painfully, from their bone marrow (Kottler, 1974; Martin, 2004; Lindee, 2005; Cowan, 2008).

In large part due to these developments, the human chromosome set increasingly became an object of analysis in human genetics and biomedicine beginning in the mid-1950s. Also central to this progress were multiple international standardization meetings held during the 1960s, which helped to make chromosomes more scientifically and medically useful objects of study. As scholars have previously noted, the biomedical value of chromosomes was even further revolutionized in the early-1970s, with the introduction of chromosomal banding (Lindee, 2005; de Chadarevian, 2010). The impact of this new technique on how chromosomes were seen, standardized, and used in genomic research has yet to be fully explored by scholars, despite its central role in shaping how the human genome has been depicted and communicated about ever since. As I describe, since the 1970s, internationally standardized representations of the 'banded' human chromosome set have been widely used to visually represent and analyze the organization of various genetic elements within the genome (Hogan, 2013).

My focus in this dissertation is on how these observational approaches to postwar human genetics and biomedicine have shaped conceptions and depictions of the structure and function of the human genome since just before chromosome banding was introduced

around 1970. In studying this, I am attempting to better understand the ongoing development of research, thinking, and practices in genetics and biomedicine, during the decades immediately before the Human Genome Project was completed in 2000. Indeed, as clinicians and geneticists increasingly looked to the genome, at the microscopically visible level of chromosomes, they began to see various markers that were reproducibly associated with particular clinical disorders. Identifying such markers required the development of standardized ways of seeing, reporting, and reproducing these chromosomal features. As I show, in the years before large-scale DNA sequencing was possible, researchers achieved significant, and often overlooked successes, when using observational approaches to explore the human genome and its role in genetic disease.

During postwar biomedicine, the visual genetic markers of disease have proven to be of both clinical and biological interest. The association of chromosomal locations and abnormalities with particular clinical outcomes can be useful diagnostically, but it also may be seen as an important introductory step in determining biological mechanisms of causation. Indeed, long before disease genes could be isolated and sequenced, observational analysis of the human chromosome set was used to locate disease etiologies in the genome and to explore the causative link between genetic aberrations and clinical expressions. While "mistakes of the printer" largely could not yet be identified, the 'binding' of the genome, as Penrose described it, proved to be more transparent than anticipated (Penrose, 1963, 136). During the 1970s and 1980s, observational approaches to genetics and biomedicine increasingly began to reshape understandings of the genome, leading to the broader recognition of it as a physical entity, and a distinct part of the

human anatomy. As I show, accounts of the human genome in the published biomedical literature around 1980 increasingly began discussing it, less as an informational abstraction, and instead more frequently as a discrete object, which was suitable for observational analysis.

**Classifying Disease in the Laboratory and the Clinic**

Scholars have consistently shown that the value and apparent sensibility of classification systems are always context dependent (Foucault 1970; Fleck 1979; Latour 1987; Bowker and Star 1999). In *The Birth of the Clinic*, Michel Foucault suggests that disease classification has long been influenced by a natural history tradition: since the 18th century, physicians have sought to identify and classify clinical pathologies as Linnaeus did plants, "to see, to isolate features, to recognize those that are identical and those that are different, to regroup them, to classify them by species or families" (Foucault 1973, p. 89). As those experienced in clinical practice recognize however, approaches to disease classification based on the identification of clinically visible signs, symptoms, and lesions often lead to uncertain or variant diagnoses. Indeed, while some patients may present with the 'classical' bodily indicators of a particular disorder, the clinical spectrum of expression, and various "individual idiosyncrasies", often complicate and inject uncertainty into the diagnostic process (Aronowitz, 1998, 7).

Clinical practice is heavily influenced by an 'ontological' perspective on disease: one that treats diseases are real entities, which develop within, and impact, all individuals in a similar way. As Charles Rosenberg has noted, this view of disease is part of a larger reductive trend in our society, which in this case gives individual diseases legitimate

identities (Rosenberg, 1992). The ontological characterization of a disease becomes particularly persuasive when that disorder is deemed to be genetic. In some cases, a disease may be considered to be genetic because it appears to be passed down through the generations of a family, while in others the disorder may became associated with a particular genomic mutation or visible aberration, as in the case of Down syndrome. While genetic factors may play a role in nearly all disease, to associate a disorder with a specific genetic etiology is to provide it with a unique ontological identity.

One of the most influential and demonstrative examples of this ontological perspective on genetic disease in the postwar period can be found in Victor McKusick's catalogs of disorders, *Mendelian Inheritance in Man*. Beginning in the 1960s, McKusick, a leading figure in the burgeoning field of medical genetics, who had first-hand experience with the complexities of clinical diagnosis, promoted a new way of delineating clinically described genetic disorders. This system, influenced in part by Linus Pauling's demonstration that sickle cell anemia was caused by a specific, inherited protein anomaly (Pauling *et al* 1949; Stasser 1999; Interview with Kurt Hirschhorn, New York City, January 26, 2012), sought to link clinical disorders with specific genetic inheritance patterns (dominant, recessive, X-linked) and genomic mutations. The success of this ontological system for genetic disease designation is made apparent by the growing size and scope of subsequent editions of *Mendelian Inheritance in Man*.

Closely associated with McKusick's approach to clinical classification is the 'gene-for' concept of disease, which is related to the 'one-gene-one-enzyme' hypothesis developed by George Beadle and Edward Tatum in the early-1940s. As scholars have

shown, genes are both real physical entities and powerful social concepts (Nelkin and Lindee; Kay, 2000; Moss, 2003). Throughout the postwar period, clinicians and geneticists have suggested the presence of a gene-for X often long before one (or two, or none) was ever identified and sequenced. As I trace in this dissertation, the gap between suggesting a gene-for-X syndrome and determining its DNA sequence or functionality was often quite wide in time and technique in the 1970s and 1980s.

Nonetheless, the idea that there was a gene or mutation somewhere in the human genome that could be used to delineate a particular clinical disorder was, and continues to be, a significant driver of biomedical research. Along the way, clinicians and geneticists have discovered however, both to their frustration and professional benefit, that in most cases the gene-for-X concept of disease is overly simplistic. Indeed, as biomedical researchers looked to the genome, at the visible level of chromosomes, in order to find the gene-for-X during the 1970s and 1980s, they increasingly found that the functionality of the human genome was much more complex and multi-dimensional than many had previously anticipated.

**The 'Syndrome' Concept**

The case studies that I focus on in this dissertation are all inborn genetic syndromes. Syndromes are characterized by an array of clinical symptoms, all occurring together, assumedly due to the same cause. Each of the syndromes described here were eventually associated with a specific genomic mutation and etiological mechanism, though syndromes may also be caused by environmental or developmental exposure to

toxins. The syndrome concept is generally attributed to 17th century English physician Thomas Sydenham, who sought to identify distinct disease entities as naturalists did species (Faber, 1923; Opitz, 1979; Opitz, 1994). Unlike with Linnaean binomial nomenclature however, there is no standardized, top-down naming and classification system for syndromes. During the postwar period, some medical geneticists, most notably McKusick and longtime *American Journal of Medical Genetics* editor John Opitz, played an intermediary role in disambiguating disease nomenclature.

Syndromes may be named after those who first described them (Down syndrome, DiGeorge syndrome), certain clinical features (Kabuki syndrome, Velo-cardio-facial), or particular chromosomal markers (1p36 deletion syndrome, Fragile X syndrome), among other possibilities. Many syndromes are known by multiple names, which may differ over time, by clinical subspecialty, or by city or country of diagnosis. As this dissertation describes, the stabilization of a common or universal name for a syndrome generally happens over the course of years, and may be impacted by new genetic findings, the development of research and support institutes, or the acknowledgement that an existing name, though descriptive or useful, is offensive to those affected. Indeed, while clinicians and geneticists may understand a particular syndrome to be a discrete ontological entity, this does not imply that they can easily agree on what to call the disorder, or what symptoms are components of its clinical spectrum.

McKusick sought to adjudicate the variable naming and understanding of genetic disorders in *Mendelian Inheritance in Man* (MIM) by associated specific syndromes, often known by multiple designations, with one particular MIM number and, when

possible, genetic inheritance pattern, mutation, or genomic location. To this day MIM, now published exclusively online as OMIM, plays a key role in designating the ontological existence of disorders by providing them with an OMIM number and entry. Additional medical texts, such as David Smith's *Recognizable Patterns of Human Malformation* similarly play a role in identifying and easing the diagnosis of genetic disorders. As I describe in this dissertation, clinicians do not take lightly the application of the term 'syndrome' to a particular disorder. Rather, the proper designation of syndromes in the clinic has been a hotly contested matter in some instances, for nosological, professional, and institutional reasons.

**Medical Technologies and New Types of People**

As many scholars have previously described, the introduction of new technologies into the clinic alters how physician think about diseases, what they look like, and what groups of people may be impacted by them. In the early part of the 20th century, new techniques of examining blood were central to the identification, diagnosis, and treatment of various disorders (Howell, 1995; Wailoo, 1997). Similarly, the introduction of visualization technologies into the clinic, such as X-ray and ultrasound, as well as CT, MRI, and PET scans have greatly impacted the practices of healthcare and diagnosis in the 20th century (Kevles, 1997). Indeed, new medical technologies, such as PET scans, have been used in the clinic, as well as the courtroom, to emphasize differences among people (Dumit, 2003).

New technologies in the clinic, in addition to enhancing the 'clinical gaze', also have facilitated the development of the 'molecular gaze' (Foucault, 1973; Rose, 2007). In recent years, genetic markers have also been used widely to identify new categories of people, in some cases who could not be differentiated otherwise (Rabinow, 1992; Hacking, 20077; Parthasrarthy, 2007; Hogan, 2012). A genetic categorization may designate a population with an increased risk of particular health consequences, such as breast cancer, Alzheimer's disease, or cystic fibrosis. Genomic abnormalities may also 'designate' an individual as being part of a particular social group of affected persons (Navon 2011; Navon 2012). The association of genetic markers with particular clinical risks and outcomes has led to what some scholars refer to as the 'geneticization' and 'biomedicalization' of contemporary medical thinking and practice.

In 1991, Abby Lippman pointed to the geneticization of medicine, noting that human disease and difference was increasingly being reduced to molecular explanations (Lippman, 1991, 1992). Just as Nelkin and Tancredi (1989) warned a few years earlier, the development of simple diagnostic tests for genetic conditions represented a potentially dangerous new form of social power. Such tests carry scientific legitimacy, and their simplicity makes them broadly applicable, meaning that they be in wide use before their potential social harms are recognized. In their 1995 book *The DNA Mystique: The Gene as a Cultural Icon* (1995) Nelkin and Lindee future demonstrated the social and scientific power of 'gene talk', tracing the prevalence of genetic essentialism in contemporary society, and its role in defining kinship, disease, and

responsibility. The gene, they demonstrate, has been transformed into a source for social difference, with potentially destructive ends.

While Lippman (1991, 1992) originally intended for the term 'geneticization' to have a negative connotation, scholars, following the lead of Hedgecoe (1998, 2001), have more recently approached the process of geneticization from a more symmetrical perspective. These studies of geneticization, conducted over the last 15 years, follow one of two general methodological approaches. The first is ethnographic analysis, focusing on the day-to-day process of clinical diagnosis. Ethnographers have questioned the extent to which genetic evidence has impacted clinical practice, arguing that the evaluation of patients' bodies continues to provide key evidence for the diagnosis of disorders, while genetic data is often inconclusive (Shaw, 2003; Featherstone et al, 2005; Latimer et al, 2006). These ethnographic studies have pushed back against existing accounts of the pervasiveness of genetic reductionism in medicine (Lippman, 1991; Keller, 2000; Hedgecoe, 2001).

A more middle-of-the-road ethnographic study responds to the anti-reductionist perspective by suggesting that clinical diagnosis involves analytic "triangulation" among mutations, phenotypes, and disease categories (Rabeharisoa and Bourret, 2009, 701). Rabeharisoa and Bourret also explore the epistemological status of genetic data in the clinic, and conclude that a certain mutation is not automatically considered "objective proof" that a syndrome is present. Rather, a mutation's diagnostic value depends upon the pre-existing "interpretive model" for a particular disorder (704).

Ethnographic studies provide valuable analysis of the heterogeneous evidence, social processes, and inevitable uncertainties associated with the process of clinical diagnosis. However, while these studies have exposed scholars to the breadth of possible clinical interpretations and outcomes, they do not provide an in-depth look at the diagnosis of any one disorder in particular. For instance, though the analysis of Rabharisoa and Bourret (2009) is broadly insightful, there is still a need to further unpack the historical development and impact of pre-existing interpretive models for disorders. For instance, how is a disorder initially associated with a genetic marker, and how is this correlation stabilized and made diagnostically useful?

The second methodology used recently for studying the impact of geneticization involves a close analysis of the scientific literature on a particular disorder. Certain studies have focused on the strategic attempts of researchers to 'geneticize' an existing disorder, such as diabetes and schizophrenia (Hedgecoe, 2001; Hedgecoe, 2002). While, in other instances, scholars have focused on the impact of geneticization on the clinical diagnosis of a disease. A few of these studies have looked at cystic fibrosis, and shown the ways in which the geneticization of a disorder, rather than providing simple and clear-cut diagnostic markers, has instead further complicated clinical understandings of individual risks and likely symptomatic effects (Kerr 2000; Hedgecoe, 2003; Kerr, 2005). Throughout this dissertation, I examine various instances in which such complications arose that called into question the reliability of a seemingly straightforward genetic marker. As I demonstrate, in the course of attempting to resolve these genetic

complications, clinicians and geneticists came to appreciate new levels of complexity in genomic function and disease causation.

**Perspectives on Biomedicine and 'Biomedicalization'**

This rise of 'biomedicine' has similarly been associated with the increasing centrality of molecular genetic conceptions of disease. Nicholas Rose (2007) has argued that medical practice has shifted away from the "clinical gaze" that Foucault describes, towards a "molecular gaze". Understandings of disease at the molecular level, argues Rose (2007, p. 8), as well as Clarke et al. (2010), have led to new conceptions of "life itself", and a novel "somatic ethics" of individualized biomedical knowledge, responsibility, and intervention. Jean-Paul Gaudilliere (2002) has similarly pointed to the central role of molecular analysis in the rise of biomedicine, and has argued that the biological laboratory has replaced the pathology clinic as the primary location and focus of medical practice. For Gaudilliere, and other scholars, this suggests that there is a largely unidirectional flow of knowledge in biomedicine from the basic biological laboratory to the clinic (Gaudilliere, 2002; Fujimura, 1992).

Other scholars however, have pushed back against this perspective on biomedicine in which knowledge in the laboratory supersedes and directs clinical diagnosis and understanding of disease. Keating and Cambroiso (2000, 2003, 2004) have repeatedly argued that, in contemporary biomedicine, pathology has not been reduced to biology. Rather, they suggest that biomedicine represents a realignment of the 'normal' and the 'pathological' (Canguilhem 1991), not a fusion of the two, or a reduction of one

into the other. Biomedical practice, they argue, takes place in intellectually and institutionally collaborative spaces or 'platforms', which are, "benches upon which conventions concerning the biological or normal are connected with conventions concerning the medical or pathological" (Keating and Cambrosio 2000, p. 386).

Still other scholars have expressed doubt about the transformative nature of biomedicine. Based largely on ethnographic analysis of the practice of dysmorphology, the identification of genetic disorders through bodily characteristics, these researchers maintain genetic results are often quite uncertain, necessitating a return to the clinic. Indeed, as these scholars suggest, clinicians do not simply rely on genetic testing to slot patients into pre-existing and discrete categories. Nor does genetic testing always make diagnosis a simple and immediate process. Rather, the clinic has always been, and continues to be, central to medical knowledge production: genetic testing may supplement clinical judgment, but certainly has not supplanted it, as Rose (2007) suggested (Shaw, 2003; Latimer *et al*. 2006; Featherstone and Atkinson 2012). In this dissertation, I explore how clinicians and geneticists locate, assess, and develop confidence in correlations between genomic markers and clinical outcomes. The genome, I argue, has become an important conceptual space in biomedicine, where the questions, interests, and conventions of basic genetics and applied clinical research intersect and intermingle.

**Outline for this Dissertation**

This dissertation draws on archival resources, informational interviews, and a thorough analysis of the published medical literature to examine the evolving look and 'genomic' understanding of a number of inborn genetic disorders. Because of the contemporary focus on this research, available archival collections for this project are relatively limited. As a result, I draw heavily on journal articles and medical textbooks relevant to my case study disorders, and medical genetics more broadly. In line with Hannah Landecker's *Culturing Life* (2007), this dissertation draws on the published biomedical literature to examine the broad impact of a specific concept: the genomic basis of disease. I also rely on interviews with over 30 biomedical professionals. These interviews helped to clarify the published literature, and often revealed what was thought and debated by clinicians and researchers, but not published in journals. In some cases, these individuals also provided relevant materials that are not yet archived, including meeting minutes and correspondence concerning professional committees or joint publications.

In chapter one of this dissertation, I explore the development of human cytogenetics in the postwar period. Multiple scholars have thoroughly examined the early decades of human cytogenetics (Martin, 2004; Lindee, 2005; de Chadarevian, 2010; Santesmases, 2010), so my research primarily focuses on the 1970s and 1980s. Over this period, I trace the parallel evolution of standardized chromosomal depictions and changing conceptions of the human genome. I argue that, during this period, conceptions of the human genome shifted from abstract references to 'all the human genes or genetic

material' to more physical and bounded descriptions, physically embedded in the visual representations of the human chromosome set.

I explore how the nomenclature system, initially developed to help standardize the laboratory and clinical analysis of banded chromosomes, was also adopted for gene and disease etiology mapping. As part of this, I demonstrate how descriptions of the human genome became increasingly anatomical during this period. Like heart in cardiology, the genome was described as the 'organ' of medical genetics, and the mapping of it as a 'neo-Vesalian' revolution. All of this together provided the basis for new ways of delineating, diagnosing, and understanding human disease. Medical geneticists were now no longer dependent on clinical signs of disease alone: they could also rely on genomic locations to identify unique disorders.

Chapter 2 offers a case study of the clinical and laboratory history of Fragile X syndrome. I begin with a discussion of the debate over X-linked intellectual disability in the 20$^{th}$ century, and then address the first clinical description, in 1943, of what was later termed Martin-Bell syndrome, and eventually Fragile X syndrome. From there, I discuss the identification of a cytogenetic marker, initial confusion over its laboratory expression, and its ultimate use as a diagnostic basis for delineating and naming Fragile X syndrome in the late-1970s and early-1980s. Next, I explore the challenges of using this cytogenetic marker to identify carriers of the disorder, and attempts to identify treatments for Fragile X syndrome based on a limited cytogenetic understanding on the fragile X site.

During the mid-1980s, clinicians and researchers were perplexed by the unusual inheritance pattern of Fragile X syndrome in families. I explore the various theories developed to explain the abnormal pedigrees, and the experimental demonstration of one of these theories based on observational chromosomal analysis in 1986. This cytogenetic research was done five years before the fragile site could be molecularly characterized, and offered important evidence that a novel genomic mechanism was at play in causing Fragile X syndrome and other disorders with similar inheritance patterns. This chapter explores how clinicians and researchers took advantage of chromosomal analysis in the years before large-scale DNA sequencing was possible to delineate, diagnose, prevent, treat, and explain Fragile X syndrome, while at the same time exploring unanticipated structural and functional characteristics of the human genome.

In chapter 3, I explore the genetic characterization of another clinical disorder, based upon chromosomal analysis. Prader-Willi syndrome was first described in 1956, and mounting cytogenetic evidence suggested a genomic basis during the 1970s. In 1981, Prader-Willi syndrome became one of the first genetic disorders to be associated with a microscopically visible, de novo deletion on a human chromosome. As a result, during the early-1980s, Prader-Willi syndrome was seen as an exemplar of a new type of disorder, which could be delineated and diagnosed based on a discrete cytogenetic marker. It became associated with a discrete genomic abnormality and location, on the long arm of chromosome 15.

To the surprise of researchers and clinicians however, in 1987 another clinically and historically distinct genetic disorder was associated with the same exact visible

chromosomal deletion. Angelman syndrome looked nothing like Prader-Willi syndrome in the clinic, and yet it seemed to be caused by the same exact genomic etiology. This posed a problem for medical geneticists, who had argued that genetic diseases could be thought of as either the same or different based on the mutation that caused them. Clinicians and researchers never suggested that Prader-Willi and Angelman syndromes might in fact be the same, but instead began to explore alternative explanations. Cytogenetic analysis provided the first evidence for the mechanistic different between them. It was demonstrated chromosomally, and later molecularly, that which parent a patient inherited the deletion from made the difference in terms of which syndrome affected them. This finding revolutionized the ways in which clinicians and researchers thought about the structure and functionality of the human genome. In 1989, this was the first demonstration in humans of an 'epigenetic' phenomenon known as genetic imprinting. This history offers a window into the continuing development of biomedicine during this period, as the interests and aims of basic and clinical researchers became closely aligned.

Chapter 4 looks at a second case of what one might call 'genetic intersection': an unusual instance in which two clinically and historically distinct disorders are found to be associated with the exact same genomic abnormality. DiGeorge and Velo-Cardio-Facial syndromes were both independently described and named, in distinct times and places, based on clinical analysis alone. During the 1980s, chromosomal analysis suggested a genomic cause for DiGeorge syndrome, which was eventually determined to be a small deletion on chromosome 22. Similar to the case of Prader-Willi and Angelman

syndromes, DiGeorge and Velo-Cardio-Facial syndromes became associated with the exact same genomic deletion in the early-1990s. Unlike the previous case however, this finding was widely pointed to as evidence that these two disorders were in fact two historically distinct forms of the same clinical disorder.

In making this argument, some clinicians and geneticists pointed to the parable of the blind men and the elephant. As they described it, researchers had been distracted all along by their own specialties and interests, and overlooked what this instance of genetic intersection had finally made apparent to them. DiGeorge and Velo-Cardio-Facial syndrome were the same exact disorder: there had always been just one elephant in the room. Following the association of these two disorders with the same genomic deletion, debates have been ongoing over the appropriate name for this syndrome. Some have promoted 22q11 deletion syndrome, a designation that directly links that clinical disorder to a genomic location and chromosomal band. The distinctions between this story, and the one presented a chapter early offer a useful opportunity to reflect on the constantly evolving relationship between clinical and laboratory findings in medical genetics as well as the professional and institutions implications of disease nosology.

My concluding chapter examines how and why chromosomal depictions continue to shape the ways in which biomedical professionals interact with the genome in the post-Human Genome Project era. Indeed, even with a complete DNA reference sequence at their fingertips, clinicians and geneticists have persisted in thinking and communicating about the human genome using a visual nomenclature originally developed for chromosomal analysis in the 1970s. An excellent example of this is the prominent place

that standardized depictions of the human chromosome set continue to have as signposts and navigation tools in online genomic databases, such as the University of California, Santa Cruz Genome Browser. Mindful of this, I examine how and why older, less exacting – and often incommensurable – languages of description are maintained, and relied upon, for the analysis and presentation of results in science and medicine. In doing so, I explore how clinicians and researchers use techniques of visualization to help make the genome, more legible for themselves and their colleagues.

## CHAPTER 1
## Seeing and Analyzing the Human Genome at the Level of Chromosomes

In 1982, Victor McKusick, Physician-in-Chief of the Johns Hopkins University School of Medicine, published a commentary entitled, "The Human Genome Through the Eyes of a Clinical Geneticist." At the time, McKusick was a central figure in the burgeoning field of medical genetics, a discipline populated by both Ph.D. trained geneticists and physicians interested in the role of genetics in human disease. In his article, which reflected on the previous 25 years of advancement in medical genetics, McKusick noted,

> The advances which started in 1956 have provided the clinical geneticist with his organ. Now the clinical geneticist is in the same position as the nephrologist with his kidney, the cardiologist with the heart, and so on. He has an organ that he can biopsy, of which he can analyze disordered structure and function, and which he can attempt to repair (McKusick, 1982, 7).

As the title of his paper suggests, the *organ* that McKusick was referring to is the human genome. During the early-1980s, McKusick, and other prominent figures in the genetics community, increasingly began discussing the human genome using anatomical points of reference. As McKusick put it in his 1982 paper, genomic analysis provided the field of medical genetics with a "neo-Vesalian model" for identifying and understanding genetic diseases (McKusick, 1982, 22). In this chapter, I explore how the human genome was

visualized, conceptualized, dissected, and mapped at the level of chromosomes during the 1970s and 1980s.

The human genome is often referred to as an abstract informational database, comprised of billions of DNA nucleotides. Indeed, it has widely been presented in the postwar period as a code to be 'cracked', as well as scanned and analyzed, at the molecular level. Analysis of the human genome however, frequently has also taken place at the level of its most basic, and visible, components: the human chromosomes. Since the 1960s, the observational approach of chromosomal analysis has provided human and medical geneticists with the opportunity to look for and locate various genes and disease etiologies within the human genome. Throughout the 1970s and 1980s, McKusick maintained and updated a visual depiction of this process, which was based upon standardized depictions of the human chromosome set (McKusick and Ruddle, 1977; McKusick, 1982, 1984, 1986a, 1988).

As I will demonstrate throughout this chapter, during the 1970s and 1980s, the human genome became an important, an increasingly well-defined, object of both scientific and anatomical investigation. While descriptions of the genome in the 1970s often referred to it quite abstractly as being composed of 'all the human genes or genetic material', by the early-1980s, medical textbook definitions of the human genome were becoming increasingly bounded by, and literally 'embodied' within the human chromosome set. Over this time, conceptions of the human genome, among clinicians and biomedical researchers, became increasingly chromosomal and anatomical, at the

same time that standardized depictions of the human chromosome grew more-and-more linearized and 'genomic'.

Conceptions and representations of genetic disease paralleled these developments. In this chapter, I also explore the making of a new system of disease nosology within the field of medical genetics. Rather than being dependent on clinical presentations to delineate, diagnose, and classify genetic disorders, during the postwar period, medical geneticists increasingly looked to the genome as a new and valuable arbiter. It was assumed that mutational analysis could provide a more accurate and reliable means of distinguishing different diseases, compared to the diverse and often confusing array of signs and symptoms seen in the clinic. Indeed, embedded within depictions of what McKusick called the 'morbid anatomy' of the human genome was the idea that every genetic disease could be associated with a unique location in the genome, thereby giving it a distinct identity.

Throughout this dissertation, I explore the application of this nosological system in postwar biomedical thinking and practice. My case studies highlight various problems and complications that arose over this time, issues that transformed chromosomal analysis into an unexpectedly productive experimental system for geneticists more broadly. A historical analysis of these cases offers a window into the evolving ways that clinicians and biomedical researchers have thought about the human genome, and its role in human disease, since the late-1960s.

**Counting Chromosomes in the 1950s**

1953 is often pointed to as a landmark year in the history of genetics, because it marked the proposal of a double helical structure for DNA. This important finding shaped the trajectory of molecular biology for years to come, by helping to explain DNA replication and facilitating the cracking of the DNA code over the next decade, thus adding to the informational basis of genetics research. While the identification of the DNA double helix is also regarded as an important moment in the history of medical genetics, Victor McKusick often pointed to another event, three years later, as being central to the origin of the field, "medical genetics has become established as a clinical specialty, as the culmination of developments that began in 1956 with the description of the correct chromosome number in man" (McKusick, 1997a, 1).

Until the early-1950s, the chromosome number in man was believed to be 48, not 46 (Kottler, 1974; Martin, 2004). Identifying the correct chromosome number is widely regarded as a significant finding in medical genetics, because in the years after its stabilization, a variety of syndromes associated with abnormal numbers of chromosomes were identified. The most notable among these, Down, Turner, and Klinefelter syndromes, were each linked to specific chromosomal abnormalities within five years of 1956. As McKusick put it in his 1997 history of medical genetics, "With the discovery of specific microscopically visible chromosomal changes associated with clinical disorders, beginning with Down syndrome in January 1959, medical genetics acquired an anatomic base. Medical geneticists now had their specific organ – the genome – just as cardiologists had the heart and neurologists had the nervous system" (McKusick, 1997a,

1). Indeed, the year 1956 matters for medical geneticists because it marks the moment at which the size and scope of the human genome, the field's *organ*, was for the first time concretely defined.

Histories of medical genetics often highlight the 1956 demonstration, by Joe-Hin Tijo and Albert Levan, that humans normally possess 46 chromosomes (Tijo and Levan, 1956). As Aryn Martin has pointed out however, while "communal closure around the new number," did not occur until 1956, the revolution in medical genetics associated with 1956 actually began with the introduction of a number of new techniques beginning in the early-1950s (Martin, 2004, 936). Foremost among these was the uptake of the chemical colchicine, which arrests cells at a point in their reproductive cycle when chromosomes are visible (they usually are not), and the use of a hypotonic (low salt) solution to spread out the chromosomes in a cell, thus making them easier to differentiate and count (Martin, 2004).

As Martin has suggested, the introduction of these new techniques began to change the way that human geneticists thought about chromosomes, even before 1956. Referencing a paper by American cytogeneticist T.C. Hsu (1952), in whose lab the hypotonic technique was first (accidently) identified and used,[4] Martin notes that Hsu,

> Also lined up the chromosomes in pairs by length and named them (by number). This, I suggest, changed the counting game from the question 'How many?' to the question 'Are all members accounted for?' An

[4] As Martin (2004) notes, an 'invisible technician' was initially responsible for 'the *hypotonic* miracle', having made a 'mistake' in mixing solutions.

analogy would be counting how many people are in a room versus taking attendance (Martin, 2004, 935-6).

Establishing the correct human chromosome number therefore, was an important step in bounding the human chromosome set, allowing for 'normal' versions of it to be distinguished from various 'pathological' ones (Canguilhem, 1991).

During the early-1950s, human geneticists developed new expectations for the counting of chromosomes. And, by the end of the decade, the number of chromosomes that an individual was seen to possess became much more meaningful medically, "A count of 47 is no longer indicative of a poor counting methodology or a challenge to the established count, but of a body marked by genetic difference" (Martin, 2004, 938). Indeed, by the close of the 1950s, abnormal chromosomal counts were seen as very significant observational findings in the clinic. And, as I explore here, over the coming decades, the human chromosome set would be further transformed into a standardized object for biomedical thinking and research, thereby creating various new methods for 'doing' genetics that were based on the observational approaches of cytogenetics.

In 1959, French clinician Jerome Lejeune famously reported that patients with Down syndrome appeared to possess one additional chromosome: for a total of 47 instead of 46 (Lejeune et al, 1959). Once it was recognized that chromosomal abnormalities could be associated with particular clinical syndromes, a call went out for the establishment of a system for naming individual chromosomes. The development of an internationally standardized chromosomal nomenclature system was undertaken at a 1960

meeting of cytogeneticists in Denver. After much social and technical negotiation, the participants decided upon a system in which each of the non-sex chromosomes (the autosomes) would be numbered by their relative size, with the largest becoming chromosome one. The sex chromosomes remained named X and Y, even though the X chromosome is closer in size and shape to chromosome 7 and the Y is most similar to chromosome 22 (Denver Study Group, 1960; Lindee, 2005).

This standardized nomenclature system has, since 1960, frequently been the basis for constructing human karyotypes, where all of the chromosomes in one cell are represented together in one picture. During the 1950s, karyotypes were often drawn by hand with the assistance of a camera lucida. However, as cytogeneticist Malcolm Ferguson-Smith has described, by the end of the decade, photographic cameras were being affixed directly to microscopes (Interview with Malcolm Ferguson-Smith, 5 December 2003, conducted by Peter Harper).[5] When a karyotype is constructed in this way, a photograph is taken of a cell is in which all of the chromosomes appear to be visible and out enough to be differentiated. After the photograph is developed, each individual chromosome is cut out and rearranged by its relative size. Karyotyping is used to help identify and systematically name abnormalities in the chromosome set, for instance an extra copy of chromosome 21, the cause of Down syndrome.

[5] Interview with [Malcolm Ferguson-Smith, 5 December 2003]. Interviews with Human and Medical Geneticists series, Special Collections and Archives, Cardiff University, Cardiff, UK.

**Creating a Standardized Representation of the Human Chromosome Set**

The human chromosome set may be visually represented in a variety of ways. While karyotypes involve the direct cut-and-paste organization of microscopic photographs, chromosomes have also been represented throughout the postwar period using idealized drawings called 'ideograms'. Chromosomal ideograms depict the distinctive features of each human chromosome, including two arms (each chromosome has a long and a short arm), a centromere, which separates the two arms, and 'satellites' (additional material at the end of certain chromosomes). Chromosomal locations are always identified relative to the centromere: those close to the centromere are 'proximal' and those further away are referred to as 'distal'.

In 1960, the Denver Study Group individually numbered each chromosome, and developed ideograms to represent the human chromosome set (Denver Study Group, 1960). Throughout the 1960s however, it remained quite difficult, if not impossible, to distinguish various chromosomes that were of similar size and shape. Reflecting this reality, cytogeneticists often referred to the human chromosomes as being members of seven visually distinguishable groups: A (1-3), B (4, 5), C (6-12, X), D (13-15), E (16-18), F (19, 20), and G (21, 22, Y). These groupings were officially recognized during a follow-up meeting to the Denver Study Group, held in London in 1963 (London Conference, 1963). Within each group, individual chromosomes were very difficult to tell apart, a situation that was particularly problematic for the C group, which is made up of eight members, including the X chromosome.

The inability to differentiate individual chromosomes greatly limited the specificity with which chromosomal abnormalities could be identified and communicated. For example, one could report that a 'B group' chromosome was lacking its short arm in patients with a certain clinical disorder. However, one could not say for certain if the impacted chromosome was 4 or 5. Later, another cytogeneticist might find a similar aberration in a different group of patients, but not know if the same chromosome was affected. Indeed, as the 1960s went on, this inability to differentiate chromosomes greatly limited the capability of medical geneticists to associate clinical disorders with particular, visible chromosomal aberrations (Hirschhorn et al, 1973).

Following the Third International Congress of Human Genetics meeting, held in Chicago in 1966, a committee similar to the Denver Study Group met to further improve the existing cytogenetic nomenclature (Chicago Conference, 1966; Lindee, 2005). One of the most significant outcomes of this 1966 gathering was the designation of a standard abbreviation for the long and short arms of each human chromosome. It was quickly agreed upon that the short arm of each chromosome should be abbreviated 'p' for petit. According to American cytogeneticist Kurt Hirschhorn however, the debate over what to call the long arm of each chromosome was extended and contentious. Multiple participants at this, and other, nomenclature meetings have suggested to me that much of the disagreement took place along national lines, particularly among French and German members (Interviews with Uta Francke, February 27, 2012; Kurt Hirschhorn, January 26, 2012; Dorothy Warburton, May 11, 2011).

At the 1966 meeting, a debate lasted late into the night over what to call the long arm of chromosomes, particularly given that a francophone designation had already been attached to the short arm. As Hirschhorn tells the story, sometime after midnight, Lionel Penrose, that year's Congress president, walked into the room where the meeting was being held, and was surprised to find that the discussion was ongoing. Informed that the designation 'p' had been decided upon for the short arm of chromosomes, Penrose immediately suggested that the long arm should be called 'q'. This was not because 'q' had any sort of linguistic significance, but instead because 'p + q = 1' was a well-known equation (named after Hardy and Weinberg) in population genetics. Hirschhorn paraphrased Penrose's successful argument in this way, "If you have 'p' for the short arm, use 'q' for the long arm: 'p + q = 1', you got the whole chromosome" (Interview with Kurt Hirschhorn, January 26, 2012).[6] Apparently, this settled the debate. Ultimately, this decision was just one of many compromises in an ongoing international discussion about how to divide the human chromosome set into standardized subsections.

**Moving Out of "The Doldrums" in the Early-1970s**

As the 1960s went on, the "golden age" of cytogenetics, during which a number of clinical disorders were associated with visible chromosomal abnormalities, came to close. The continued inability to distinguish individual human chromosomes greatly limited the potential specificity of cytogenetic diagnosis. In reference to this very

[6] Peter Harper offers a similar account involving 'p + q = 1' during his interview with David Harden. Interview with [David Harnden, 18 March 2004]. Interviews with Human and Medical Geneticists series, Special Collections and Archives, Cardiff University, Cardiff, UK.

problem, McKusick, quoting his colleague Margery Shaw, has referred to the late-1960s as a time when clinical cytogenetics was "in the doldrums" (McKusick, 1997a, 8). The 1970s, on the other hand, proved to be a revolutionary time for the field, with the development new chromosome staining techniques.

Quinacrine (Q) banding was introduced in 1968 (Caspersson et al, 1968), followed by Giemsa (G) banding and reverse (R) banding around 1970 (Seabright, 1971). These new techniques produced distinguishable banding patterns on each human chromosome, allowing for them to be visually differentiated under the microscope. The banding techniques take advantage of the differential condensation of DNA within each chromosome. On G-banded chromosomes, more densely compacted regions stain more darkly, while on R-banded chromosomes the 'reverse' happens. The density of a chromosomal region often reflects the number of genes present within it: less dense regions usually have more genes (Sumner, 1990).

The ability to concretely differentiate each chromosome was a key contribution of new banding techniques, but this was only the beginning. These chromosomal bands also created visible and reproducible landmarks on each chromosome, meaning that they could be broken down into additional regions and sub-regions based on their bands. This advance was very important in that it allowed cytogeneticists to speak reliably in terms of much more than just the long or short arm of a certain chromosome. Now, a chromosomal aberration, such as the deletion, duplication, or translocation of genetic material, could be defined by its specific physical band location along a chromosomal arm. This improved the visual resolution of chromosomal analysis, made laboratory

results more reliable and reproducible, and represented a first important step towards creating a genomic map at the visual level of human chromosomes.

In order to make use of banding, cytogeneticists once again had to agree on a nomenclature system. An international committee was convened to do so in 1971 following the Fourth International Congress of Human Genetics meeting in Paris. The 1971 meeting featured many of the same members as the one five years earlier in Chicago, and similar international tensions. At the time, G-banding and R-banding were the two most common methods for creating chromosomal bands. Mary Seabright (1971), a British cytogeneticist, had developed the most commonly used technique for G-banding, which was widely adopted in the US and much of Europe. R-banding was a French innovation, and its use was largely exclusive to France (Interview with Dorothy Warburton, July 21, 2011).

The Paris Committee's primary challenge was to create a nomenclature system that incorporated both G and R banding. As American participant Dorothy Warburton has told me about coming to a consensus, "it wasn't easy". The patterns created by G and R bands were essentially exact opposites: dark G-bands went unstained by R-banding, and dark R-bands were not stained by G-banding. As a result, the debate was over which regions of each chromosome were banded and which were not. Those who did R-banding wanted to number the visible bands down each chromosome as 1, 2, 3. G-banding proponents wanted the same numbering system, but with the dark bands that they saw (Interview with Dorothy Warburton, July 21, 2011).

A breakthrough came, according to Warburton, when committee chairperson John Evans suggested a compromise: chromosomes should not be thought of as having regions that were either banded or not, but instead, the bands on each chromosome should be understood as continuous: light then dark then light again. This way, a standard numbering system could be adopted, and the darkness or lightness of a band would simply depend on which banding method was used. So, following the 1960 chromosome numbering system and the 1966 arm-lettering compromise, members of the 1971 committee created a consensus nomenclature that named each visible chromosomal band, whether dark or light. Chromosomal arms were broken down into anywhere from one to four regions, which were then further divided by the bands visible in each. The committee worked in groups to decide how many bands could be seen on each chromosome and to draft representative ideograms (Interview with Dorothy Warburton, July 12, 2011).

Each band was identified by the chromosome (1-22, X, Y) and arm (p or q) on which it was located, followed by a number identifying the region (beginning with 1 at the centromere), and finally with a second number to designate the specific band. For example, the band 15q12 (pronounced 15 – q – 1 – 2) is located on the long (q) arm of chromosome 15, and is the second visible band (2) in the first region (1) from the centromere. Ideograms showing all of the (about 400) visible chromosomal bands in the human chromosome set were included in the conference report (Paris Conference (1971), 1972) (Figure 2). This standardized nomenclature provided the basis for the system that

geneticists use to the present day, for identifying the genomic location of various chromosomal abnormalities, genes, and disease etiologies.

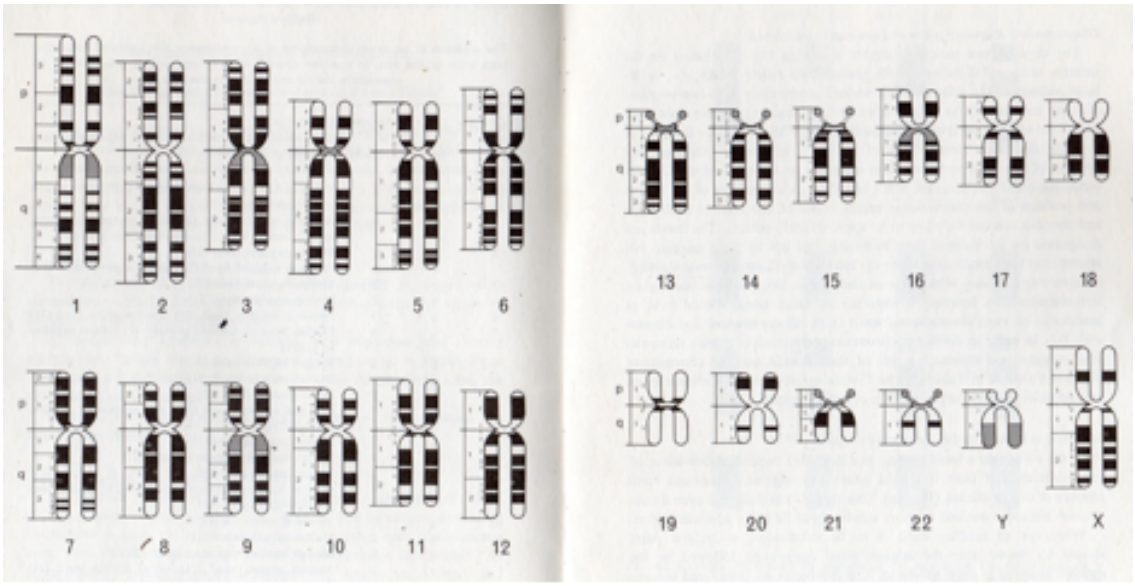


**Figure 2** X-shaped, banded chromosomal ideograms, first developed in 1971. This image was originally published in Paris Conference, 1971 (1972). Reprinted with permission from the March of Dimes.

**Visualizing 'High-Resolution' Chromosomes**

During the 1970s, cytogeneticists began to experiment with capturing chromosomes in a somewhat less condensed state under the microscope, in the hope that they would reveal additional, visible bands. This new technique, known as 'high-resolution' chromosomal banding, began to spread by the end of the decade (Yunis, 1978). High-resolution chromosomes had more than double the number of visible bands, and with this technique in place, another international standardization meeting was held

in Paris in 1980 to update the existing nomenclature. Committee members maintained the 1971 system, and divided existing bands into sub-bands by adding a decimal to the end of the current designation. For instance, the second sub-band of 15q13 became known as 15q13.2 under the revised nomenclature (ISCN, 1981).

Debates arose once again however, over which original band was being divided into new sub-bands. In G-banding, light bands are understood to be less condensed than dark bands. Therefore, it did not make logical sense that a new high-density dark band could be derived from an existing, low-density light band. Because of this and other ongoing debates (which continued in correspondence afterwards), the meeting turned into what participant Uta Francke described to me as a "shouting match" (Interview with Uta Francke, February 27, 2012). As a result, the report from this meeting, held in May of 1980, was not published until well into the next calendar year. Ultimately, the goal of what was now formally called the 'International System for Cytogenetic Nomenclature (ISCN)' Standing Committee was to maintain an internationally acceptable and standardized system for identifying chromosomal bands. To accomplish this, small or theoretical discrepancies and disagreements had to be sidelined in favor of having one system that everyone, whether they primarily practiced G or R-banding, could use.

In a 1982 letter sent to ISCN committee member David Harnden, one committee member, a human geneticist, commented on the continuing disagreements among committee members concerning band standardization by noting, "The guiding principle of ISCN is clearly the written word, not the ideogram. To worry about minor artistic inaccuracies in such a highly stylized diagram is pedantic" (From the ISCN Papers, May

1980 meeting records).[7] Indeed, the ideograms were not necessarily meant to capture the most accurate or logical description of what chromosomes looked like. Instead, they depicted a nomenclature system that was created through compromise. The most important goal of the standardization committee was to promote a single naming system, and not necessarily the most theoretically accurate one.

This said, with the advent of high-resolution chromosomal banding, it had become increasingly clear that the black-and-white limitations of the 1971 compromise failed to capture what cytogeneticists were actually seeing under the microscope. While the official ISCN report in 1981 maintained the all black-and-white banding pattern, committee member Uta Francke was allowed to publish, as part of the report, a separate set of G-banded ideograms. These high-resolution ideograms had the same nomenclature as the official ISCN bands, except that instead of being exclusively black and while, they showed multiple shades of gray. Francke's goal was to more accurately represent what cytogeneticists actually saw under the microscope, so as to improve the visibility of small chromosomal aberrations (Francke, 1981; ISCN, 1981) (Figure 3).

[7] The ISCN papers are currently privately held, and were made available to me, by geneticist Uta Francke. The collection is located at the Stanford University School of Medicine, Palo Alto, CA.

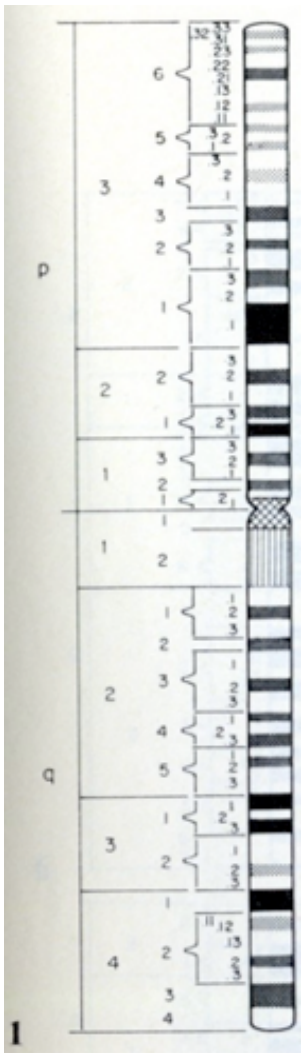

**Figure 3** Rod-shaped ideogram of chromosome 1 showing shades of grey. This series of ideograms was published in the same report as the ISCN (1981) ideograms and largely looks the same, except for the differential coloring (Francke, 1981). Reprinted with permission from Uta Francke.

The cytogeneticists involved in creating and maintaining this chromosomal nomenclature system clearly recognized that they were working with fluid entities, which were in a constant state of flux during the life of a cell. Any drawing of a chromosome only captures it at one brief moment and cannot necessarily be directly and logically related to how that same chromosome looks at a different point of condensation.

However, the intention of ideograms was not demonstrate or explain the physiological process of chromosome condensation, but instead to identify and designate the most reliable, and therefore useful visual landmarks that could be used for analyzing, and communicating about, the human chromosome set in a standardized manner.

While standardization committee meetings were often contentious and drawn out affairs, in the end cooler heads had to, and did, prevail in favor of compromise and consistency. The ideograms produced by these meetings were important for the exchange of clinical and scientific findings. In addition, these ideograms became an internationally shared representation of what the human genome looked like, and an important basis for its physical mapping, as well as for increasingly commonplace efforts to 'locate' genetic disease etiologies within the genome.

**The Changing Look of the Human Chromosome Set**

Since the first set of human chromosomal ideograms were proposed by the 1960 Denver Study Group, ten standardization committee updates have been published, with the most recent coming in 2009. Many of these revised editions suggested only small adjustments to the existing system, but a few provided a significant overhaul of the previous edition, most noticeably in the form of chromosomal ideograms with new banding patterns. Such major revisions occurred in 1971, 1981, 1995, and 2005. While each of these updates revealed the impact of novel cytogenetic techniques on chromosomal analysis, I argue that the new sets of chromosomal ideograms also offered novel ways of seeing the human genome. In addition, they reflected new conceptions of

how genes and disease etiologies could be located and analyzed within it. Here I focus on the changes that were made to chromosomal ideogram depictions in 1971 and 1981.

The chromosomal ideograms developed by the 1960 Denver Study Group have two distinguishing features when compared to those produced in later decades. First, the chromosomes are depicted as solidly colored bodies: no banding techniques were yet available to provide each ideogram with a distinctive pattern. Secondly, each chromosome looks like some variation of the letter X (Figure 2). In a 1963 follow-up meeting held in London, the chromosomes were officially broken down into seven, lettered groups, as described above. These groupings were based on variations in the chromosomes' X-like shapes, as well as their relative size. Group A chromosomes are large and 'metacentric', meaning that their centromere is centrally located, making their long and short arms close to the same size. Group B and C chromosomes, on the other hand, are among those called 'submetacentic', since their long arms are significantly larger than their short arms. Group D and G chromosomes are 'acrocentric', meaning that the short arm is too small to easily be seen (London Conference, 1963).

In 1971, the chromosomal ideograms went from being solidly colored to banded, reflecting the development of Q, G, and R banding techniques. Otherwise, these ideograms remain X-shaped, like those published by the Denver Study Group (Paris Conference (1971), 1972) (Figure 2). An entirely new set of ideograms, with new banding patterns, was not published again until the 1981 ISCN report (ISCN, 1981). However, two intervening updates in 1975 and 1978 included human chromosomal

ideograms that were not X-shaped, but rather looked like long, narrow rods (Paris Conference (1971), Supplement (1975), 1975; ISCN, 1978).

These ideograms were created to facilitate comparisons among the banding patterns of human and other primate chromosomes (Interview with Uta Francke, February 27, 2012; Paris Conference (1971), Supplement (1975), 1975) (Figure 3). The revisions were regarded at the time as supplements to the 1971 Paris conference, as opposed to a full overhaul (ISCN, 1985). However, this new way of depicting chromosomal ideograms makes it clear that, in the mid-1970s, geneticists were beginning to think differently about what chromosomal analysis could reveal about the physical organization of the genome, and about how standardized ideograms of the human chromosome set could be used to depict these findings.

Undoubtedly, there were multiple reasons for this move from depicting chromosomal ideograms as X-shaped to rod shaped. As Uta Francke, who was a consultant to the 1981 Paris committee, has explained to me, part of the reason to switch from X-shaped to rod-shaped ideograms was for efficiency: more ideograms could be fit on one page (Personal Communication with Uta Francke, via email, March 28, 2012). Indeed, during the mid-to-late-1970s, when comparing the chromosomal ideograms from different primates was a major feature of the nomenclature committee's publications, this made a lot of sense because rod-shaped chromosomes were easier to line up next to each other (Paris Committee (1971), Supplement (1975), 1975; ISCN, 1978). Another factor by the late-1970s was the introduction of high-resolution chromosome banding, described in the previous section. Because high-resolution chromosomes were captured in a less

dense state, they tended to look more rod-like than X-shaped. Indeed, by 1980 rod-shaped chromosomes were closer to what geneticists actually saw under the microscope.

I want to suggest however, that this incremental shift from X-shaped to rod-like ideograms between 1971 and 1981 also reflected new conceptions of the sort of knowledge that chromosomal analysis could provide to human and medical geneticists. X-shaped chromosomal ideograms, as they were depicted up through 1971, represented important functional units of cellular reproduction. These ideograms appeared X-shaped because they were actually depicting two exact copies of the same chromosome (joined at the centromere), which were about to split apart in the formation of two genetically identical cells. Chromosomal analysis up to this point was mostly focused on identifying large chromosomal abnormalities. With the wider uptake of chromosomal banding in the early-to-mid-1970s however, the aims of cytogenetic analysis began to shift. It was now possible to identify, and communicate about, more specific locations than entire chromosomes, or large portions of them. As a result, the human chromosomes were coming to be understood as more than just units of cellular reproduction: they were also increasingly seen as the basic, observable, and map-able subsections of the genome.

This facilitated early attempts to produce a 'physical map' of the human genome, which involves the direct association of genetic characteristics with distinct landmarks in the genome. This is different from, but often closely associated with, the older technique of 'linkage' or 'genetic' mapping, famously practiced in T.H. Morgan's fly lab to identify the relative location of genes or other traits on individual chromosomes.[8] Rather than

[8] For more on mapping in the Morgan Lab see: Kohler (1994).

determining the relative distance between genes, physical mapping seeks to associate genetic traits not with fixed genomic locations, which in the 1970s and 1980s were often defined by visibly distinct chromosomal bands (McKusick, 1988).

The human genome is generally conceived of at the molecular level as being a linear chain of DNA. As the medical genetics textbook *The Metabolic Basis of Inherited Disease* put it in 1978, "All genetic mapping data are consistent with the hypothesis that the genome is a linear unbranched structure" (Stanbury et al 1978, 41). With this conception of the human genome in mind, it would not have made much visual or logical sense to map genes and other genetic traits onto X-shaped chromosomes. This said, there was no indication in the reports of the standardization committee published in 1975, 1978, or 1981 that this shift from X-shaped to rod-like chromosomes was meant to make the human genome more map-able. Nor is it my intention to argue for a causal link in either direction between ideogram linearity and genome map-ability.

Rather, I suggest that this alteration in depiction reflects a shift in how geneticists conceptualized the relationship between the assumed linearity of the human genome and the associated observational characteristics of the human chromosome set. Chromosomes went from being countable entities, with which certain clinical disorders could be associated, to visibly comparable and 'dissectible' linear units of the human genome, within which the etiologies of disease could be definitively located. In the next section, I demonstrate this same shift in another way: by tracing evolving definitions of the term 'genome' within the published biomedical literature specific to human genetic disease. I argue that, during the 1970s and 1980s, the human genome was being

conceptually and visually remade, at the level of chromosomal analysis, into a clinically and biologically important, physical part of the human anatomy.

**Evolving Conceptions of the Human Genome**

It is commonly held that German botanist Hans Winkler coined the term 'genome' in 1920 as a hybrid of the words 'gene' and 'chromosome' (McKusick and Ruddle, 1987). However, an alternative interpretation, offered by Joshua Lederberg and Alexa McCray in 2001, holds that the suffix 'ome', used by Winkler in 1920, instead referred to, "a holistic abstraction, an eventual goal, of which only a few parts may be initially at hand," as in the use of "biome" to refer in a general sense to all life in a particular earth environment (Lederberg and McCray, 2001, 9).[9] In this section, I trace the term 'genome' in biomedical textbooks from the late-1960s through the 1980s. My findings suggest a shift in the use of 'genome': from being an abstract way to identify all of an individual's genetic material or genes, to a term referring more specifically to a physically embodied and discretely bounded anatomical entity.

A number of texts aimed at geneticists interested in human disease were in print during the 1970s. 'Genome' only appears in a couple of these texts, and when it does, it is described in quite abstract terms. The glossary of McKusick's text *Human Genetics* (1969), defines the genome quite simply as, "The total genetic endowment" (203), while the 1973 edition of *Genetics in Medicine*, a textbook by physician James S. Thompson and Ph.D. geneticist Margaret W. Thompson, defines genome in its glossary as, "The full

[9] All of the world's deserts or oceans constitute a biome.

set of genes" (361). Beyond this, the term genome does not appear in the index or the main text of other medical genetics texts available during the 1970s such as, *An Introduction to Medical Genetics* (Roberts, 1973), and *Medical Genetics: Principles and Practice* (Nora and Fraser, 1974).[10]

Discussions and definitions of the human genome were equally absent in more general medical texts during the 1970s. The term genome is not used in either the 1971 or 1975 editions of the Cecil *Textbook of Medicine* (Beeson and McDermott, 1971; 1975). In the 1979 update, genome is not listed in the textbook's index. However, in a chapter on genetics, physician Alexander G. Bearn does muse, quite abstractly, "It is apparent that despite the acceleration in discovery of new genetic entities 90 per cent of the human genome remains to be discovered" (Beeson et al, 1979, 31). Genome does, in fact, appear the 1970 and 1974 editions of another prominent general medical text, Harrison's *Principles of Internal Medicine*. The term can be found in a chapter contributed by McKusick, which refers to the genome in 1970 as "the rest of the genetic make-up", and in 1974 as "the genetic background" (Wintrobe et al, 1970, 14; Wintrobe et al, 1974, 323). Following McKusick's departure as author of this chapter however, genome completely disappears from the 1977 and 1983 editions of Harrison's, in which physicians Joseph L. Goldstein and Michael S. Brown contributed a similar chapter on genetics and disease (Thorn et al, 1977; Petersdorf et al, 1983).

[10] The medical texts chosen for this survey were influenced in large part by a list, meant for small medical libraries, of recommended selections by topic (Brandon and Hill, 1979).

During the 1980s, the term genome became both more prominent and more precisely defined in medical genetics and general medicine textbooks. The 1986 edition of Thompson and Thompson's *Genetics in Medicine* states, "The term genome refers to the full DNA content of the chromosome set" (Thompson and Thompson, 1986, 6). *An Introduction to Medical Genetics* (1985) by physicians J.A. Fraser Roberts and Marcus Pembrey refers to genomic DNA as, "the nuclear DNA of the chromosomes" (Roberts and Pembrey 1985, 104). Physician James J. Nora and Ph.D. geneticist F. Clarke Fraser define genome as, "The complement of genes found in a set of chromosomes", in the 1981 edition of *Medical Genetics: Principles and Practice* (Nora and Fraser 1981, 497). In the 1985 update of the Cecil *Textbook of Medicine*, contributor John L. Hamerton, a human geneticist, notes, "The term *genome* refers to the full DNA content of the chromosome set" (Wyngaarden and Smith 1985, 138). Genome also appears once again in the 1987 edition of Harrison's *Principles of Internal Medicine*, in which physician and cell biologist Arthur Beaudet compares the genome to a series of books which, "can be envisioned as being bound into 46 volumes, each the equivalent of one chromosome" (Braunwald et al 1987, 296).

While the human genome was certainly associated with the human chromosome set by geneticists before 1980, the findings from these medical textbooks clearly shows that there was a shift in the importance and meaning of the term genome for medical geneticists, and physicians more broadly, between the early-1970s and mid-1980s. When used in the 1970s, genome generally referred to the abstract concept of 'all the genetic material or genes' possessed by an individual. By the mid-1980s however, as the term

became increasingly commonplace, it was more often defined in the context of the human chromosome set. Indeed, during 1980s, the human genome came to be understood as a discrete object of scientific interest among human and medical geneticists, and one that was physically and conceptually embodied within a visible part of the human anatomy: the chromosomes.

**Victor McKusick and Postwar Medical Genetics**

This chapter has, so far, described how the genome became increasingly understood as embodied by the human chromosome set during the 1970s and 1980s, and in turn how standardized representations of chromosomes themselves became increasingly linearized and 'genomic' over this time. My focus now shifts to how chromosome level depictions of the human genome were used to shape conceptions of genetic disease at this time. Central to this story is the work of Victor McKusick, and his influence on the field of medical genetics during the postwar period. After a brief overview of McKusick's biography, I turn to a discussion of the new basis for genetic disease noslogy, which he helped to create and promote through his well-known catalog of human genetic disorders, *Mendelian Inheritance in Man*.

McKusick has frequently been referred to as the 'father' of postwar medical genetics. Obituaries of McKusick appearing in *Science*, *Nature Genetics*, and the *Lancet* after his death in 2008 highlight his status in the field, as does the Award Description provided for the Lasker Award for Special Achievement in Medical Science, which McKusick received in 1997 (Lasker Foundation, 1997; Collins, 2008; Oransky, 2008;

Rimoin, 2008). Reed Pyeritz, a student of McKusick's, has told me that he was a "pretty towering figure" in the field of medical genetics, even in the mid-1970s, and that he is among a group of just four or five individuals who could be considered as having founded this medical specialty in America (Interview with Reed Pyeritz, April 18, 2012). Indeed, McKusick was heavily influential in the fields of human and medical genetics during the last five decades of this life, and certainly one of the most important figures who shaped conceptions of the human genome, including its size, scope, functionality, and impact on biology and medicine in the postwar period.[11]

Born in Parkman, Maine in 1921, McKusick received an M.D. from Johns Hopkins University School of Medicine in 1946, where he became a faculty member a year later, specializing in cardiology. As part of his cardiology research, McKusick became interested in Marfan syndrome, a genetic disorder associated with heart defects. McKusick tracked the inheritance pattern of this disorder in his patients, ultimately leading to a wider interest in other inherited disorders (McKusick, 1980, 2006; Stafford, 2008). As he recounted in a 2006 autobiography, McKusick was surrounded by multiple other, more senior, faculty members at Hopkins, during the 1950s, who were also interested and knowledgeable in genetics, including Bentley Glass and Barton Childs. Additionally, he suggested that he was heavily influenced by Curt Stern's 1949 textbook *Principles of Human Genetics*. In 1957, McKusick was installed as director of the J. Earl Moore Clinic at Johns Hopkins, where he developed a Division of Medical Genetics. As

[11] For more on the status and role of McKusick in postwar medical genetics see: Lindee, 2005; Comfort, 2012.

McKusick has told it, colleagues warned him about shifting focus from cardiology to rare genetic disorders, calling the move "professional suicide" (McKusick, 2006, 5).

During the late-1950s and 1960s, McKusick went on to train a number of major figures in the field of medical genetics, including David Rimoin and Alan Emery, editors since 1983 of the text *Principles and Practice of Medical Genetics*, as well as David Weatherall, author of *The New Genetics and Clinical Practice*, Malcolm Ferguson-Smith, long-time editor of the journal *Prenatal Diagnosis*, and Peter Harper, author of *A Short History of Medical Genetics* (2008). McKusick also helped to develop the Short Course in Medical and Experimental Mammalian Genetics, which is held annually at the Jackson Lab in Bar Harbor, Maine. This two-week course has been responsible for educating thousands of clinicians about the research and practices of medical genetics since it began in 1960 (Stafford, 2008; Comfort, 2012).

Victor McKusick however, is perhaps best known for his catalog of genetic disorders, *Mendelian Inheritance in Man* (MIM). First published in 1966, MIM grew out of a series of annotated reviews of medical genetics that McKusick and colleagues had been compiling since 1958. Ultimately, these were organized into catalogs on X-linked, recessive, and dominantly inherited disorders, which were put into a computer database beginning in 1964. Successive editions of MIM were published in 1966, 1968, 1971, 1975, and so forth until the final print edition in 1998. The catalog expanded with each edition, providing a visible demonstration of the growth and success of the field of medical genetics. Students occasionally referred to the brightly colored books as "green genes" and "blue genes" when distinguishing among 1970s era editions (McKusick,

1981, 67). In 1987, MIM became available electronically as *Online Mendelian Inheritance in Man* (OMIM) (McKusick, 2006).

In MIM, McKusick offered a particular conception of and perspective on human genetic disorders. He argued that the study of genetic disorders provided valuable insights concerning the normal human genetic make-up,

> Genetic disorders give us insight into the normal. These catalogs of hereditary traits are like photographic negatives from which a positive picture of man's genetic constitution can be made . . . Physicians have a unique opportunity to contribute to knowledge of what Richard Lewontin referred to as 'man's mutational repertoire' (McKusick, 1968, ix).

Additionally, McKusick emphasized the direct correlations that medical genetics expected to find between genetic mutations and specific clinical disorders,

> In medical genetics there is little place for expressions such as 'spectrum of disease,' 'disease A is a mild form, or a variant, of disease B,' and so on. They are either the *same* disease, if they are based in the same [genetic] mutation, or they are *different* diseases. Phenotypic [clinical] overlap is not necessarily any basis for considering them fundamentally the same or closely related (McKusick, 1968, xi).

A major goal of medical genetics was to help clarify the delineation of particular disorders, whose identity was confused by variable or overlapping clinical expression. Indeed, if every genetic disorder could be mapped to one discrete location (or perhaps multiple locations) in the genome, this could greatly improve diagnosis, clinical understanding, and potentially treatment. McKusick's contribution to this process was in the collection and organization of diseases and other genetic traits in MIM. As I describe in the next section, this also included participation in workshops, which facilitated the mapping of genes and disease etiologies in the human genome.

**Mapping the Human Genome at the Level of Chromosomes**

In her book, *Moments of Truth in Genetic Medicine* (2005), which looks at the first decade of medical genetics, Susan Lindee argues,

> [McKusick] was an early and eloquent proponent of what I call the cataloging imperative: the increasingly powerful idea among medical geneticists that the compilation of a list (or, later, map) of genetic traits, birth defects, and diseases in human populations could transform medical practice and patient care (Lindee, 2005, 81).

Lindee suggests further that McKusick was, "collecting with a remarkable passion and with an explicitly medical agenda that has been fully realized in the international effort to map and sequence human genes, the Human Genome Project" (Lindee, 2005, 81).

McKusick's interest in 'collecting' genetic diseases, and mapping their etiologies to particular genomic locations, in many ways defined, throughout the 1970s and 1980s, the way that human and medical geneticists thought about the geography and scope of the human genome. McKusick himself was not among the first researchers to propose what became the Human Genome Project (HGP) during the mid-1980s (McKusick, 1997a). However, he did feel that the Gene Mapping workshops, which he helped to found, lead, and promote during the 1970s and 1980s, represented an important foundational basis for the HGP.

In a draft history of the HGP, written in 1998 McKusick suggested, "Since mapping all the genes in the human is a goal of the Human Genome Project, the Human Genome Project (HGP) can be said to have begun in the summer of 1973 when the first human genome mapping workshop was convened in New Haven by Frank Ruddle" (McKusick Papers, Box 509623, 'Hx of HGM 98' folder, 1). Indeed, McKusick's leadership, in the 1970s and 1980s, of the Human Gene Mapping workshops, offered geneticists with an early glimpse of what mapping the genome would look like and mean for the study of human genetics and disease.

In 1986, at a Cold Spring Harbor symposium on mapping and sequencing the human genome, Victor McKusick gave a presentation about the status of the human gene map. At the time, approximately 900 genes had been mapped to specific human chromosomes and chromosomal locations (McKusick, 1986b). McKusick later described this presentation as being "an eye-opener to the molecular geneticists present" (McKusick, 1997a, 18). Indeed, as McKusick notes, and in line with the history of

Robert Cook-Deegan (1994), the initial impetus for the Human Genome Project came not from the human or medical genetics community, but rather from molecular biologists (McKusick, 1997a). As it turned out however, these molecular biologists were largely unaware of the extensive human genome mapping that had already been taking place since 1968, and in an organized manner since 1973 (McKusick, 2006).

The first genes mapped in man were located on the X chromosome, for the simple reason that X-linked disorders could be identified based on pedigree analysis because they generally only affected males, as in the case of hemophilia and color blindness. Roger Donahue, a student of McKusick's, linked the first gene to an autosomal chromosome in 1968. Donahue traced a visible abnormality that he had identified in his own karyotype through various other members of his family. He was eventually able to link this visible marker, located on chromosome 1, to a set of genes known as the Duffy Blood group, which code for red blood cell molecules. Reflecting on Donahue's research process, McKusick later mused, "As every good graduate student in genetics should, Donahue studied his own chromosomes" (McKusick, 1981, 67).

Donahue's finding happened around the same time as other significant innovations that contributed significantly to human gene mapping in the 1970s. The first, as I have already discussed, was the development of chromosomal banding techniques, which offered hundreds of unique and reproducible chromosomal locations (Caspersson et al, 1968; Seabright, 1971). Another was the development of somatic cell hybridization techniques involving the fusion of rodent and human cells. The formation of these hybrid cells, which initially contain full human and rodent genomes (usually mouse or hamster),

occurs very rarely, but can be facilitated by chemical manipulation and particular viruses. As the hybrid cells go through continuous rounds of reproduction, most of the human chromosomes are lost, while all rodent chromosomes remain. Some human chromosomes however, may be retained, especially if they contain a gene that is necessary for cell survival in a particular selective medium (Harris and Watkins, 1965).

Somatic cell hybridization proved to be of great value to human gene mapping because researchers could test for hybrid cells that continued to express a particular human protein (Weiss and Greene, 1967). If a human protein was still produced by a hybrid cell, this meant that its gene must be on one of the human chromosomal fragments still present. In combination with the uptake of G-banding in the early-1970s, somatic cell hybridization helped to facilitate the mapping of hundreds of human genes to certain chromosomal locations over the next decade. Because it brought about the mixing and recombination of chromosomes from different cells, a cellular process that generally only occurs as part of sexual reproduction, J.B.S. Haladane famously referred to cell hybridization as "an alternative to sex" (McKusick, 1981, 76).

In 1973, the first Human Gene Mapping workshop was held in New Haven, Connecticut under the leadership of Frank Ruddle, a Yale geneticist and early adopter of somatic cell hybridization for gene mapping. The gathering was funded by the March of Dimes organization, which already had a longstanding relationship with McKusick, and role in the funding education of medical geneticists through supporting the annual Short Course in Medical and Experimental Mammalian Genetics. At the time of the first workshop, very few genes had been mapped to specific chromosomes. However, by the

fourth workshop held in Winnipeg in 1977, at least one gene had been mapped to each human chromosome (McKusick, 2006).

This was a significant intellectual accomplishment for those involved in gene mapping, even if it was only the tip of the iceberg as far as the entire human genome was concerned (McKusick, 1980). Why was it so meaningful to have associated at least one gene with every chromosome? Part of the answer to this question can be found in a lecture that McKusick had given in 1969 at the 3rd International Conference on Congenital Malformations. It was in this forum that he first publically proposed that the entire human genome should be mapped on a detailed level. In this talk, he spoke about human chromosomes metaphorically as continents,

> The chromosomes of man are still largely *terra incognita*. The developments in human cytogenetics in the last 10-15 years have shown us the gross outlines of the continents . . . In a pitifully small number of instances we know pairs of neighbors residing somewhere on one of the continents, which are the chromosomes in this geomorphic anatomy. But in few instances do we know which chromosomes continent carries which gene . . . Combined with a mapping of the fine structure of the gene should be an all-out effort at mapping the chromosome continents (McKusick, 1970, 408).

By mid-1976, human geneticists had succeeded in at least planting a flag on every continent in the human genome, and from there would further explore the landscape of each. Indeed, by identifying at least one gene on each human chromosome, human geneticists had expanded the reach of their knowledge and capabilities to the entirety of the human genome.

The next year, McKusick and Frank Ruddle published a report on the status of the human gene map in *Science*. Included in this paper was a map of the human genome, depicted at the level of banded chromosomes. The chromosomes were each represented by an ideogram, based on the rod-shape ideogram drawings provided by the 1975 supplement to the 1971 Paris conference. Each chromosome had one or more genes mapped to it, designated most often by a three-letter abbreviation, placed at the approximate location on the chromosome to which that gene had been mapped. The result is a one-page, schematic view of the human genome, divided into 24 chromosomes, with one or more genes mapped to each. As depicted in the image, the genome is 'haploid' and male: only one copy of each chromosome is shown and both an X and Y chromosome are present (McKusick and Ruddle, 1977; McKusick, 1980) (Figure 4).

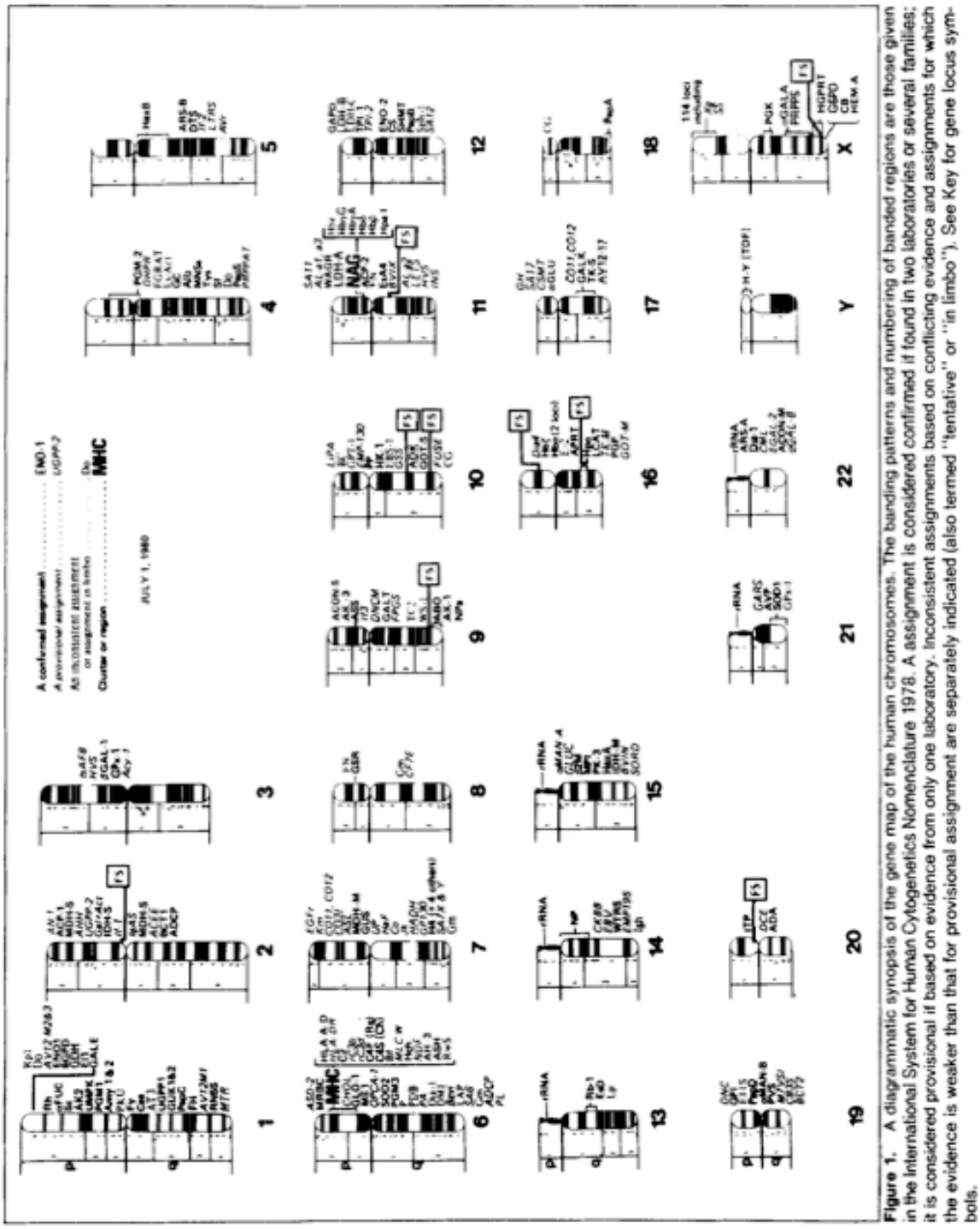

**Figure 1.** A diagrammatic synopsis of the gene map of the human chromosomes. The banding patterns and numbering of banded regions are those given in the International System for Human Cytogenetics Nomenclature 1978. A assignment is considered confirmed if found in two laboratories or several families; it is considered provisional if based on evidence from only one laboratory. Inconsistent assignments based on conflicting evidence and assignments for which the evidence is weaker than that for provisional assignment are separately indicated (also termed "tentative" or "in limbo"). See Key for gene locus symbols.

**Figure 4** Map of human genes with known genomic locations, depicted on ideograms similar to those from the 1975 supplement to the 1971 Paris conference. This image was initially published in McKusick (1980). Reprinted with permission from The Alan Mason Chesney Medical Archives of The Johns Hopkins Medical Institutions.

A similar one-page image of the human gene map was also published in the 1978 and 1983 editions of MIM as well as various papers that McKusick published on the anatomy of the human genome in the early-1980s (McKusick, 1978, 1980, 1981, 1982, 1983). The density of genes on each chromosome expanded quickly over this time, and by 1986 the gene map depiction spanned four pages instead of just one (McKusick, 1986a). Why represent the human gene map in this way? I would suggest that the depiction of genes laid out on chromosomal ideograms quite successfully represents the human genome visually as both a cartographic and anatomical object. As Sismondo (2004, 215) has argued in reference to the Human Genome Project, the practice of cartography has a "fiction of completeness . . . Maps ask to be completed" through the filling in of missing information. The growing gene map as depicted on chromosomal ideograms had a similar effect: it showed which areas of the genome were well represented, and what regions remained largely unexplored.

One set of genes that were of particular interest to McKusick, and other medical geneticists, were those directly involved in the etiology of various genetic diseases. During the 1970s, McKusick's maps were largely limited to specific gene designations. However, in the early-1980s, McKusick began creating separate maps, which he called the "Morbid Anatomy of the Human Genome," depicting the genomic location of disease etiologies as well. As I describe in the next sections of this chapter, these maps were part of McKusick's larger interests in making geneticists and clinicians see and understand the genome as part of the human anatomy.

**The Human Genome as an Anatomical Entity**

I began this chapter by noting that Victor McKusick often spoke of the human genome (and alternatively the human chromosomes) as being the "organ" of molecular genetics, equating it with the heart for cardiologists and the kidneys for nephrologists (McKusick, 1982, 1997a, 2001). This fit into McKusick's larger conception of the genome as being part of the human anatomy, "The chromosomes and the linear arrangement of the genes they carry are part of human anatomy" (McKusick, 1981, 78). McKusick cites multiple sources for this conception of the genome, including the influential human geneticist Curt Stern and biochemical geneticist Charles Scriver (McKusick, 1997b, 2001). Clearly McKusick found the reference to be of great descriptive and rhetorical value, as he used it in most every paper he published on the human genome between 1980 and his death in 2008.

McKusick was not alone in his use of anatomical analogies when speaking about the human genome in the early-1980s. In this Nobel lecture, the molecular biologist Paul Berg also made a similar anatomical reference in talking about the genome and its relevance to medicine,

> Just as out present knowledge and practice of medicine relies on a sophisticated knowledge of human anatomy, physiology, and biochemistry, so will dealing with disease in the future demand a detailed understanding of the molecular anatomy, physiology, and biochemistry of the human genome . . . We shall also need physicians who are as

> conversant with the anatomy and physiology of chromosomes and genes as the cardiac surgeon is with the structure of the heart and the circulatory tree (Berg 1981, 285).

Charles Scriver also adopted an anatomical metaphor in speaking about the genome during the early-1980s, referring to genomic mapping as akin to a "neo-Vesalian anatomy" (Scriver, 1982, 496). In the early-1980s, McKusick also picked up on this concept, publishing a paper entitled "The Human Genome Through the Eyes of Mercator and Vesalius". The article looked at the use of both cartographic and anatomical metaphors for thinking and speaking about the genome. In terms of the cartography of the genome, McKusick states, "The landmarks in the maps are the [chromosomal] bands revealed by special staining" (McKusick, 1981, 77). However, as he goes on to explain in the paper, "This is a cartographic metaphor, but an anatomic metaphor is equally apt" (McKusick, 1981, 78).

Indeed, analyzing the human genome was not just as a mapping project, but also as an anatomical exercise, in the tradition of Vesalius. Adopting the same phrasing as Scriver in a 1986 paper, McKusick noted, "Knowledge of the chromosomal and genic anatomy of *Homo sapiens* has given clinical genetics (and medicine as a whole) a neo-Vesalian basis" (McKusick 1986b, 19). In his account of the early history of the Human Genome Project, *Gene Wars* (1994), Robert Cook-Deegan recounts that such references to the neo-Vesalian nature of genome mapping were quite successful in attracting funding sources for the project, particularly from the Howard Hughes Medical Institute (Cook-

Deegan 1994, 120). Seemingly, these historical and anatomical references made mapping the human genome more legible to a wider audience.

When pointing to the 'neo-Vesalian' basis of human genetics, McKusick and Scriver were referencing the work of Andreas Vesalius, a 16th century physician, famous for his anatomical images published in *On the Fabric of the Human Body* (1543). The frontispiece of this text, which McKusick published as part of his 1981 paper, "The Human Genome Through the Eyes of Mercator and Vesalius", depicts Vesalius teaching human anatomy by directly pointing to a newly dissected human body. This was in contrast to the existing norm during the 16th century, when an instructor would read directly from the fourteen hundred year-old text of Galen, while standing apart from the dissected body (Carlino, 2001).

To the present day, Vesalius is remembered in the western medical community as having brought direct observation of the dissected human body back to the forefront of research and teaching in human anatomy. In *The Secrets of Women: Gender, Generation, and the Origins of Human Dissection* (2006) however, Katherine Park counts Vesalius among various 15th and 16th century figures who have been inaccurately remembered as heroes because they, "braved persecution and censure in the service of art and science" (21). As Park suggests, continued reference to these individuals does, "important cultural work", providing, "foundation stories that confirm deep-seated Western institutions about the scientific origins of modernity – institutions that continue to inform the writing of even specialists in the field" (Park 2006, 21).

Indeed, late-20th century physicians like McKusick and Scriver regarded Vesalius as a revolutionary figure, who had an impact on future centuries of medical thinking and practice. References to the 'neo-Vesalian' nature of late-20th century human genetics were rhetorically valuable for making the argument that the genome mattered to medical practice because it was physically and visually a part of the human anatomy. In addition, just as the work of Vesalius was seen as reshaping medicine in the 16th century and beyond, during the 1980s, anatomical exploration of the human genome was similarly presented by McKusick and Scriver as being likely to have revolutionary implications for medicine in the decades to come.

**The 'Morbid Anatomy' of the Human Genome**

As part of addressing the anatomy of the human genome in his 1981 paper, McKusick commented on the localization of disease genes,

> For an ever increasing number of diseases the chromosomal location of the mutant gene responsible is known. In many of these instances this location is known because the enzyme which is deficient has been assigned to a specific location. In most of these disorders the evidence is strong that it is indeed the structural gene for the enzyme that is mutant in the given disease (McKusick, 1981, 79).

Similar to the ways in which diseases may be located in the bodies of patients, their etiological cause may also be located in the human genome. McKusick referred to this practice as looking at the 'morbid anatomy' of the human genome, pointing to the 18th century work of Giovanni Morgagni in locating the 'clinical pathology' of a disease in certain bodily organs (McKusick, 1997a, 422; McKusick 2001, 2289).

Additionally, McKusick often spoke of "dissecting the human genome", further playing up a neo-Vesalian interpretation of the human genome and what researchers could physically do to it (McKusick, 1980, 1981, 1982 1997b).[12]

In his 1982 paper, "Window Panes of Eternity. Health, Disease, and Inherited Risk", Scriver similarly pointed to this way of thinking about the human genome noting, once again in reference to Vesalius' 1543 anatomy text, "Another revolution in anatomy is occurring; it is chromosomal and genetic cartography achieved by mapping of genes to specific chromosomes and bands on chromosomes and the delineation of nucleotide sequences in specific genes, respectively. We are beginning to possess chromosomal

[12] McKusick sought more than just a morbid anatomy of the human genome, however. He also called for a comparative evolutionary anatomy, a functional anatomy, and a developmental anatomy (McKusick, 1981, 79). These terms closely resemble various sub-disciplines of classical biological study. The human genome's evolutionary anatomy would allow it to be compared to the genomes of various other organisms, its functional anatomy would describe the way its genes, and interactions among them, led to particular clinical outcomes, and its developmental anatomy would reveal various ways in which the arrangement of particular genes affected their functionality (McKusick, 1981).

Indeed, McKusick seems to have hoped to re-appropriate the human genome as an important feature in both biological and medical research. As previous scholars have demonstrated, much of genetics research since the 1930s had been biochemical in nature, and often was not specifically oriented toward human characteristics (Abir-Am, 1982; Kay, 1993, 2000; Keller, 2000; Rheinberger, 2008). The ability to visualize and compare human chromosomes made possible by the introduction of banding in the 1970s however, made these anatomical entities newly useful for more classical medical and biological research.

addresses for Mendelian disease" (Scriver 1982, 496). Scriver now refers to gene mapping during the 1970s and early-1980s as "the genome project of the day". As a member of the HHMI medical advisory board during the 1980s, he was a major proponent of funding this ongoing research. Scriver has described his interest in gene mapping at the time to me in this way, "There was lots of initial episodic work, where a certain gene might be mapped to a certain particular region of a chromosome, and so a mosaic was being built up. I was interested in seeing the whole picture being completed" (Interview with Charles Scriver, May 30, 2012). During the 1970s and 1980s, it was McKusick who laid out the conceptual and visual framework for doing just this.

Starting with his 1982 paper, "The Human Genome Through the Eyes of a Clinical Geneticist", McKusick began publishing what he called "The Morbid Anatomy of the Human Genome". To illustrate this 'morbid anatomy', McKusick began with the 24 human chromosomal ideograms (1-22, X, Y), which were shown with banding patterns based on the 1981 Paris Conference, and arranged them into an idealized karyotype. Along each chromosome, genetic diseases, which had been mapped to certain genomic locations, were identified. Some disorders were known only to be linked to a specific chromosome, while others were associated with a particular chromosomal region or band (McKusick 1982).

Reed Pyeritz, a medical geneticist, and former student of McKusick's, has described the impetus for maps depicting the morbid anatomy of the human genome to me in this way,

> When people started laying out the 23 sets of chromosomes, the ideograms, and then had next to [each] where a gene had been identified, [McKusick] said, 'that's all well and good, but you can often map a phenotype [like Marfan syndrome] to a specific site on a chromosome before you know what the cause is' (Interview with Reed Pyeritz, April 18, 2012).

In effect, the morbid anatomy diagrams acted to breakdown the visual divide between laboratory and clinical knowledge: the anatomical markers of clinical disorders could also be located and observed within the human genome, at the visual level of chromosomes. As McKusick saw it, "this is what a geneticist does" (Interview with Reed Pyeritz, April 18, 2012). Just as Morgagni had associated clinical disorders with the anatomy of particular organs during the 18th century, McKusick felt that a major goal of 20th century geneticists should be to give human diseases a neo-Vesalian basis by locating them in discrete, visible regions within the genome.

Updated versions of the morbid anatomy of the human genome appeared frequently in print. For instance, morbid anatomy maps were included in the 1983 and 1986 editions of MIM (McKusick 1983; 1986c). New editions of the morbid anatomy of the human genome were also included in various medical genetics texts during the 1980s including, *The Metabolic Basis of Inherited Disease* (Scriver et al, 1989) and *Genetics in Medicine* (Thompson and Thompson, 1986). In addition, the morbid anatomy was published along with an interview of McKusick in a 1984 issue of the *Journal of the*

*American Medical Association*, and in a four part series appearing in the journal *Medicine* between 1986 and 1988 (McKusick 1984; 1986a; 1987a; 1987b; 1988). Indeed, during the mid-1980s, this "neo-Vesalian" depiction of the human genome had made its way more widely into the biomedical literature. With each new map, McKusick captured the ongoing "dissection" of the human genome, while presenting a particular way of seeing and thinking about the genome to his fellow geneticists and clinicians, who, by the mid-to-late-1980s, appear to have widely adopted it in their own texts (McKusick 1982, 88).

Like the anatomical prints in Vesalius' *Fabrica*, McKusick's human gene map and 'morbid anatomy' of the human genome map were artistic depictions of the genome's anatomy. These maps were based upon idealized representations of each chromosome, as captured by chromosomal ideograms. In addition, the gene locations presented by them were quite crude, with little or no indication of the distances between individual genes. More than anything, the genome map was meant as a database for collecting and depicting existing information, which would be used as a basis for future research. As McKusick put it in his 1986 report on "The Morbid Anatomy of the Human Genome" published in *Medicine*, "Just as [Vesalius'] *de corporis humani Fabrica* (1543) was the basis for the physiology of Harvey (1628) and the pathology of Morgagni (1761), the chromosome information is the foundation for our understanding and management of genetic disease in man" (McKusick, 1986a, 2).

**The Human Genome Goes Full Circle**

In this chapter, I have demonstrated how standardized depictions of the human chromosome set evolved during the 1970s and 1980s, just as conceptions of the human genome were also shifting among medical professionals. Between 1971 and 1981, chromosomal ideograms became increasingly linearized and densely packed with over 800 distinct bands, providing a standardized visual language for dividing up and identifying discrete locations on each human chromosome. At the same time, beginning around 1980, various clinically oriented geneticists, such as McKusick and Scriver, began talking about and depicting the anatomical aspects of the genome. This way of thinking about the human genome, as both observable and embodied, led to a noticeable shift in how the genome was discussed in medical texts between the 1970s and the 1980s. The genome was no longer referred to abstractly as 'all of the human genes'. Rather, definitions and representations of the genome became increasingly embedded in and bounded by visual depictions of the human chromosome set.

In the 1987 edition of Harrison's *Principles of Internal Medicine*, a new figure was added to the text, which perfectly captures the simultaneous evolution of standardized chromosomal ideograms and embodied conceptions of the human genome. This image combines selected elements of McKusick's human gene map and 'morbid anatomy' of the human genome, but in a new way. Previously, the chromosomal ideograms in these figures had been organized like a karyotype: with chromosomes lined up side by side, often in order by size. In this figure however, the chromosomal

ideograms were lined up end-to-end in a circle, from chromosome 1 to 22, followed by Y and X, with "THE HUMAN GENOME" printed in the middle (Brauwald et al, 318).

This way of depicting the human genome is interesting for a number of reasons. First, and most importantly, it takes the ongoing process of linearizing the chromosomal ideograms to make them more 'genomic' one step further: the human genome was now depicted as a continuous linear arrangement of all 24 human chromosomes, placed end-to-end. While each chromosome remains physically distinct, this image offers a particular view of the genome that allows it to be seen and mapped as a continuous whole, instead of in 24 distinct parts. Another fascinating result of this way of illustrating the human genome is how similarly it is in setup to the standard depiction of bacterial and viral genomes. Instead of being broken down into chromosomes, these genomes are comprised of one undivided loop of genetic material. Hence, such genomes can be sequenced or mapped continuously, beginning and ending at any point. Depicting the human genome, which is anatomically divided into 24 pieces, in this way, suggests a similar (conceptual) continuity. The human genome becomes a single, bounded entity that can be broken down visually into a continuous series of chromosomal bands, instead of into 24 individual chromosomes, each having their own unique banding pattern.

Indeed, "THE HUMAN GENOME" captures in one image, the various conceptual shifts that I trace in this chapter. In being physically comprised by the human chromosome set, the genome was presented as a component of the human anatomy. As part of becoming embedded in the chromosomes however, the human genome's presumed linearity clearly was not lost. Rather, as I have described, presumptions of

linearity literally reshaped how standardized chromosomal ideograms were drawn. The arrangement of these chromosomal ideograms from end-to-end into a closed circle demonstrates dual understandings: the human genome is at once physically embodied by discrete entities, and yet conceptually continuous and linear. In this image therefore, we see the human genome presented as a discrete scientific object: one that could be understood, described, and visually observed as a physically bounded whole.

**Conclusion**

Throughout this chapter, I have argued that, during the 1970s and 1980s, the human genome became increasingly embedded – conceptually, physically, and visually – within standardized depictions of the human chromosome set. After decades of being regarded and referred to in abstract terms, such as 'all of the human genetic material or genes' the genome began to be understood and analyzed in ways that made it increasingly tangible and anatomical, and thereby more relevant to the interests and daily practices of clinical researchers and medical geneticists. In addition to providing an anatomical basis for conceptions of the human genome, its association of with human chromosomal nomenclature has also helped to establish the genome as a scientific object. Indeed, the standardized visual nomenclature of the human chromosome set provides a universal language not only for identifying and communicating about chromosomal attributes, but genomic locations as well.

The establishment of the human genome as a scientific object was an iterative process: one which involved shifting definitions of the term 'genome', along with

evolving standardized depictions of human chromosomes. Additionally, the development of the genome as a scientific object involved new ways of thinking about, and visually representing, how human disease can be identified, observed, mapped, and potentially understood at the chromosomal level. While the human genome has frequently been situated primarily within the informationally oriented domain of molecular and computer-based biology, this study demonstrates that, during the 1970s and 1980s, the genome was conceptually re-appropriated as a tangible and valuable object of study among the more visually oriented practitioners of human and medical genetics. Just as Scriver has put it, the human genome could be seen as "a mosaic" in the 1980s, that was growing ever more densely filled in as the process of gene and disease mapping continued (Interview with Charles Scriver, May 30, 2012).[13]

Indeed, McKusick's 'morbid anatomy' diagrams helped to establish the human genome as an object amenable for broad-based biomedical research, by taking an idealized representation of the biologically 'normal' human chromosome set, and using it to map the genomic location of clinically-defined 'pathological' disorders. This seamless alignment of the normal and the pathological – the known biological and medical characteristics of the human genome – forms an important basis of contemporary biomedical research.[14] Therefore, along with molecular biology and its informational

[13] This statement is reminiscent of Daston (2008, 110) on scientific observation, "Science depends crucially on its own ontologies, so very different from commonsense ontologies, painstakingly assembled from diverse shards of evidence as a mosaic is assembled from tiny stones of diverse color and shape. It is observation, grounded in trained, collective, cultivated habit, that fuses these bits and pieces into a picture– often a literal picture crafted by the techniques of scientific visualization."

[14] This concept I draw from: Keating and Cambrosio (2003,72).

approaches, the observational analysis of chromosomes is deeply embedded in biomedicine's postwar development. With this in mind, continued recognition and analysis of observational approaches in postwar human genetics is likely to facilitate at once a broader and more nuanced understanding of the mid-to-late-20th century birth of biomedicine.

In upcoming chapters, I continue to trace the development and use of banded chromosomal analysis for improving the delineation, diagnosis, and understanding of genetic disorders. Throughout the 1970s and 1980s, the visual scanning of banded chromosomes provided new ways of seeing and identifying clinically characterized genetic disorders. Throughout this era, human and medical geneticists attempted to apply chromosomal analysis to identifying new, more exact techniques of delineating disorders. These novel methods were based in the association of clinical outcomes with particular genomic locations and visual genetic aberrations, rather than the more variable and confusing presentation of disease in human bodies.

As I explore, chromosomal methods for delineating human disease were at times quite successful, while, in other instances, they produced confusing and frustrating results. In working through these complications however, human and medical geneticists increasingly came to conceptualize the human genome in ways directly shaped by the visual analysis of chromosomes. As I argue, in the decades before the Human Genome Project began in earnest, chromosomal analysis provided an unexpectedly valuable experimental system for both the clinical delineation and mechanistic understanding of genetic disorders. In the course of this, chromosomal analysis also facilitated the

development of new, increasingly complex understandings of the structure and function of the human genome more broadly.

## CHAPTER 2
## Interpreting an '*In Vitro*' Phenomenon: The Delineation, Diagnosis, Prevention, and Treatment of Fragile X syndrome

In this chapter, I look at the role of chromosomal analysis in the history of a particular form of inherited intellectual disability, now known as Fragile X syndrome. Fragile X syndrome is believed to be the second most common cause of inborn intellectual disability (Smith and Berry, 1983; Sutherland and Hecht, 1985; Nussbaum and Ledbetter, 1986; McKusick, 1987; Kaufmann et al, 2002). Aside from intellectual disability however, patients affected by Fragile X syndrome have been regarded as being quite 'normal' in clinical appearance (Turner, 1983). Fragile X syndrome is one among many forms of 'non-specific' X-linked intellectual disability, referring to its lack of other highly relevant clinical features (Gerald, 1981). The delineation of Fragile X syndrome was facilitated by the identification of two associated visual markers in the 1970s, one of which – the fragile X site – became the disease's namesake during the next decade.

The fragile X site is one of more than 100 'fragile' sites that have been identified in the human genome, based on observational, chromosomal analysis. Fragile sites are believed to reflect specific structural characteristics of the human genome. Because of their wide distribution throughout the genome, they have also proven to be valuable visual markers for mapping various locations. The fragile X site discussed in this chapter is unique among these genomic features in that it is associated with a clinical disorder (Hecht, 1988). As I describe here, the correlation between the fragile X site and Fragile X syndrome was, for decades, both highly confusing for, and of significant interest to, clinicians and genetics researchers. Indeed, while it proved to be a valuable

chromosomal marker of Fragile X syndrome when visible, the inheritance pattern of the fragile X site, and its associated form of intellectual disability, did not fit neatly with existing assumptions about Mendelian traits and genomic stability.

The laboratory history of the fragile X site parallels the broader history of chromosomal analysis between the late-1960s and early-1990s in revealing ways. Indeed, this marker was identified before chromosomal banding was developed (see previous chapter) and was integrated into the new 'genomic' understanding of the human chromosome set during the 1970s and 1980s. Likewise, the clinical history of Fragile X syndrome offers a window into thinking about intellectual disability, more broadly, during the postwar period. By the late-1970s, it was widely accepted that Fragile X syndrome was 'X-linked' in two related ways. Because the disorder only seemed to cause significant intellectual disability in males, it was assumed to be caused by a recessive trait inherited on the X chromosome, of which females possess two copies, while males have just one. In addition to this pattern of clinical expression, Fragile X syndrome is also 'linked' to a microscopically visible "lesion" on the X chromosome (Pembrey et al, 1985, 713).

During the postwar period, clinicians and genetics researchers looked for the visible markers of disease both within the clinically visible body and the chromosomally visible human genome. Fragile X syndrome offers an exemplary historical case study of a genetic disorder that was delineated, diagnosed, and understood based on visible markers from both the laboratory and the clinic. Since the late-1950s, the human chromosome set has increasingly been understood as a part of the human anatomy, where

the visible markers and mechanistic causes of genetic disease intermingle. This has had implications for how clinicians and researchers think about and study the etiological causes of, and potential treatments for, Fragile X syndrome and other genetic disorders. In this chapter, and throughout this dissertation, I explore the impact that evolving understandings of the relationship between the human chromosome set and genome have had on the thinking and practices of postwar human and medical genetics.

## Mid-20th Century Perspectives on X-linked Intellectual Disability

In 1943, British physician James Purdon Martin and human geneticist Julia Bell, both at the National Hospital in London, published a report on a family showing an inherited form of what appeared to be X-linked intellectual disability. X-linked disorders often only affect males, because the causative genetic trait is located on the X chromosome, of which females have two copies, and males have only one. If a female inherits one aberrant X-linked genetic trait, its negative effects may be overridden by a normal copy of that genetic entity on her other X chromosome. However, since males have only one copy of the X chromosome, if they inherit a mutant genetic trait on it, they generally are affected by it clinically, because they have no normal copy to potentially override or mitigate these effects.

Among two generations of the family described by Martin and Bell (1943), eleven males were affected by intellectual disability, along with two females, though their symptoms were much milder. Unlike other forms of intellectual disability, such as Down syndrome and phenylkeoluria (PKU), no additional clinical manifestations were noted as

part of this inherited form of intellectual disability, which was described as involving an extremely limited vocabulary and not progressing past the mental capacity of a young child (Martin and Bell, 1943). At the time, these researchers regarded the family pedigree they described as being an isolated case of X-linked intellectual disability. Referring to the Colchester Study report (Penrose, 1938), the authors noted that, among 1280 cases of intellectual disability studied, British geneticist Lionel Penrose, "found insufficient evidence to support the view that sex-linked genes played a significant part in the etiology of mental defect – a testimony to the rarity of such a history as the one now described" (Martin and Bell, 1943, 157). Though the Colchester Study demonstrated that there was a higher incidence of intellectual disability among males, Penrose concluded that this did not have a simple genetic basis, but was the result of several factors (Kevles, 1985, 162). With this in mind, and given the lack of other similar pedigrees in the published literature, Martin and Bell concluded that the family they described was a rare case, instead of a more widely representative one.

Over the next thirty years, several additional reports of other families affected by similarly 'non-specific' forms of X-linked intellectual disability were published (Allan at al, 1944; Renpenning et al, 1962; Dunn et al, 1963; Roboz and Pitt, 1969). In 1965, physician John M. Opitz and colleagues reported on an extended family with 20 intellectually disabled males. This family became the basis of a Ph.D. thesis done by Robert Lehrke in the years thereafter on the genetic basis intellectual disability. Lehrke concluded that there existed one or more X-linked genes that were the cause of the higher incidence of intellectual disability in males (Lehrke, 1972). This was a controversial

conclusion at the time, both among Lehrke's doctoral examiners, and other human geneticists (Turner and Opitz, 1980; Turner, 1983). After all, as Lionel Penrose (1963) suggested, given that humans have 22 other chromosomes, why should the X chromosome in particular play an important role in intellectual disability?

Lehrke however, was not alone in arguing for the significant impact X-linked genes on intellectual disability. During the early-1970s, an English study with similar results was published (Davison, 1973), as were reports from Australian researchers Gillian and Brian Turner (Turner et al, 1970; Turner et al, 1971; Turner et al, 1972). Gillian Turner was working at this time, in a clinic for the intellectually handicapped, and therefore was exposed to a large and diverse number of cases, the genetic basis of which intrigued her. One day, Turner began going through a number of photographs of intellectually disabled males, to look for common physical traits, and noticed to her surprise, that many were quite 'normal' looking. As Turner later put it, "We gradually woke up to the fact that to be 'normal looking' in a moderately mentally retarded population was relatively abnormal" (Turner, 1983, 10). In these cases, the absence of any additional clinical effects beyond intellectual disability was regarded as so unusual as to be a visual marker of a unique disorder.

Turner noticed that the majority of 'normal' looking males with intellectual disability had other males in their family that were similarly affected. This was not the case for most intellectually disabled males that had more distinctive clinical features (Turner, 1983). Multiple reports were published based on this finding (Turner et al, 1970; Turner et al, 1971), in which it was suggested that these normal looking males had

a previously described X-linked disorder, 'Renpenning Syndrome' (Renpenning et al, 1962). Turner and her colleagues published an even larger study the next year, with similar results (Turner et al, 1972). As she continued to examine sets of brothers with non-specific intellectual disabilities in the Australian state of New South Wales, Turner eventually came across an interesting clinical finding: in some cases the affected brothers possessed unusually large testicles (also known as macro-orchidism) (Turner, 1983).

Testicle size is a feature that may be easily overlooked in the clinic; and, even when noticed, is difficult to accurately measure. In 1966, Andrea Prader (one of the physicians who first identified Prader-Willi syndrome, the topic of chapter 3) developed an 'orchidometer' for the measurement of testicles, which was comprised of a series of egg-shaped standards of known volume to be used for comparison (Prader, 1966). What could be considered a 'normal' testicle size was not yet well established in the early-1970s, as volume varied greatly by age and, some hypothesized, among different races (Turner, 1983). Turner et al (1975) reported on two families in which all of the males affected by an X-linked form of intellectual disability were also found to have testicles that were approximately twice the normal volume, as established by Zachmann et al (1974). In this paper, Turner and colleagues also noted a previous study that had mentioned the presence of enlarged male genitals in a family affected by X-linked intellectual disability, which was published earlier in the decade by a Ph.D. candidate in Sao Paulo, Brazil (Escalante, 1971). Multiple follow-up studies reported similar findings over the next few years, and established that the macro-orchidism in these cases did not

result from an independent hormonal abnormality (Cantu et al, 1976; Biederman et al, 1977; Ruvalcaba et al, 1977).

Was this clinical feature part of the disorder that caused X-linked intellectual disability? A patient with macro-orchidism who was not intellectually disabled was knonwn in the medical literature (Nisula et al, 1974), demonstrating that the two features did not always occur together. However, as Bowen et al (1978) pointed out, the consistent occurrence of macro-orchidism in families with otherwise non-specific X-linked intellectual disability was strong evidence that these two clinical outcomes were related. What clinicians and researchers needed in the mid-1970s was a third marker, common to this population, that could dispel doubts that the group was clinically distinct. As it happened, another researcher had already identified such a marker based on observational, chromosomal analysis in the late-1960s. However, as I describe in the next section, various technical complications delayed the demonstration of its clinical significance.

**A Chromosomal Marker for X-linked Intellectual Disability**

In 1969, Yale University School of Medicine physician Herbert Lubs reported on the discovery of a new type of chromosomal abnormality. He described it as "an unusual secondary constriction . . . seen at the ends of the long arm of a group C chromosome [referring to chromosomes 6-12 and X]". The aberration "gave the appearance of large satellites", greater in size than any of the satellites that normally appeared on certain other chromosomes (Lubs, 1969, 234). Lubs had discovered this 'secondary constriction'

while studying the chromosomes of a boy affected by a severe form of intellectual disability. Often times, when chromosomal aberrations are identified in patients with clinical abnormalities, the chromosomes of one or both of their parents are also analyzed, so as to determine whether or not the abnormality is inherited. If the same aberration is found in a parent who is not similarly affected clinically, it is generally assumed that it represents a benign form of 'normal' genetic variation.

In this case, after finding the secondary constriction in the intellectually disabled boy, Lubs analyzed the chromosomes of the boy's intellectually normal mother and his similarly affected brother. These two family members also showed the exact same chromosomal aberration. In response to this finding, Lubs noted, "Initially, it appeared that the secondary constriction was not clinically significant since it was present both in a normal mother and her abnormal son" (Lubs, 1969, 241). Upon further analysis however, the same secondary constriction was also found in multiple individuals in the patient's extended family, some of who showed a similar form of intellectual disability. It was noticed, in fact, that only males who possessed this secondary constriction were affected by intellectual disability. Females with the marker were reported as showing no clinical effects (Lubs, 1969).

Touting the importance of this new chromosomal marker to the future of clinical cytogenetics, Lubs suggested that such secondary constrictions might "prove to be the most important group of cytogenetic abnormalities both because they are common and because they may permit prevention of clinical disease" (Lubs, 1969, 231). Unlike other chromosomal abnormalities that had been identified in the previous decade, such as

trisomy 21 in Down syndrome patients, Lubs' marker was heritable, and thereby could be tracked in families. With this in mind, Lubs suggested that, "descriptive human cytogenetics is entering a new and important phase." Indeed, identifying smaller, heritable chromosomal anomalies, such as this one, was significant because, "they may permit prevention of clinical disease by identifying high-risk marriages and allowing subsequent amniocentesis and abortion of abnormal fetuses if requested by the family" (Lubs, 1969, 231).

Lubs could see that this secondary constriction occurred on a 'C group' chromosome, a classificatory unit that is comprised of chromosomes 6-12 and X. As I described in the previous chapter, it was very difficult to visually discriminate each C group chromosome at this time, as they are all similar in size and shape. The specific chromosome upon which the secondary constriction occurred therefore, could not be easily determined visually. However, since the marker was associated with intellectual disability only when present in males, and never when seen in female family members, Lubs inferred that it was likely an X-linked trait. Indeed, while Lubs used other laboratory methods to aid in demonstrating that the secondary construction occurred on the X chromosome,[15] it was the inheritance pattern of the related clinical disorder that first suggested that this would be the case. It was not yet clear how the secondary constriction caused intellectual disability, but since this clinical outcome was only seen in males, the genetic trait itself seemed to be located on the X chromosome (Lubs, 1969).

[15] This included a more exacting measurement of the chromosome's length and width, as well as an analysis of when during the cell reproductive process it was replicated (Lubs, 1969).

Lubs' 'Marker X' chromosome was one of three secondary constrictions that were independently reported by various cytogeneticists between 1968 and 1970. A year earlier, Jerome Lejeune in France had identified a similar heritable marker on chromosome 2 (Lejeune et al, 1968). And, in 1970, another was reported on chromosome 16 by American cytogeneticists Ellen Magenis and Frederick Hecht (Magenis et al, 1970). Unlike the Marker X however, neither of these visible chromosomal anomalies were associated with abnormal clinical outcomes. In their 1970 report, Magenis and Hecht referred to the chromosomal abnormality they had identified as a 'fragile site', a term that was widely adopted by geneticists thereafter.[16]

As it turned out, the immediate impact of Lubs' fragile site, in both in the laboratory and clinic, was minimal. No other researchers reported a similar X chromosome fragile site again for seven years (Giraud et al, 1976; Harvey et al, 1977). In fact, discussion of fragile sites largely disappeared from the scientific and medical literature during the first half of the 1970s. This delay had not occurred because Lubs' paper had gone unnoticed: it was published in the *American Journal of Human Genetics*, and cited when the fragile X site was once again identified in the mid-1970s. Indeed, as Gillian Turner later noted, she, and likely others, had been in pursuit of finding the Marker X chromosome in patients ever since Lubs' report (Turner, 1983, 12). Rather, researchers stopped reporting on fragile sites in the early-1970s quite literally because they stopped seeing them under their microscopes.

[16] Lubs however, continued to prefer the use of "Marker X chromosome" (Lubs, et al, 1984).

The disappearance of fragile sites did not occur because of an existing need for some technological advance to improve visibility: in fact, quite the opposite was the case. While fragile sites were understood to be visual representations of certain heritable structural characteristics of the human genome, it was ultimately determined that they could only be seen in the laboratory under certain chemical conditions. Australian geneticist Grant Sutherland eventually explained the disappearance of fragile sites from chromosomes in 1977, by demonstrating that a change in cell culture media by many cytogenetics laboratories around 1970 had inadvertently masked the sites (Sutherland, 1977; Gerald, 1981).

Following further experimentation, Sutherland determined that it was the presence of higher concentrations of folic acid in the new media that had led to the absence of visible fragile sites in the early-1970s.[17] After this was widely reported, folic acid deficient media was once again adopted for studying fragile sites, along with the new protocols recommended by Sutherland (1979) (Interview with Loris McGavran, August 20, 2012).[18] At this point, research on fragile sites, as well as their clinical associations, began anew (Sutherland, 1979; Sutherland and Hecht, 1985). Indeed, as it turned out, a technical change that was intended to improve chromosomal analysis (by enhancing the ability to cells to reproduce in culture) inadvertently disrupted the study of the fragile X site, at a key moment in the investigation of its associated genetic disease. As I describe

[17] Other cell culture factors such as pH also played an important role (Sutherland, 1979).
[18] Loris McGavran is a Ph.D. cytogeneticist who played an important role in bringing Fragile X testing to the University of Colorado Children's Hospital in Denver during the early-1980s.

next, by the time that fragile sites began to appear again, the practices of cytogenetics had been evolved significantly.

**The Fragile X Marker in a New Era of Cytogenetics**

Much had changed between the Lubs' report in the late-1960s and Sutherland's in the late-1970s. Cytogenetics had emerged from its "doldrums" into the revolutionary era of chromosomal banding (McKusick, 1997, 8) (For more on this, see the previous chapter). Each human chromosome could now be visually differentiated based on its unique banding pattern. As a result, standardized depictions of the human chromosome set also had changed quite significantly. Additionally, chromosomal banding provided a visual set of physical landmarks throughout the human genome, which could be used for the purposes of mapping. Genomic locations were now identified based upon a standardized visual nomenclature, built around the bands on each chromosome, which had been developed in 1971 (Paris Conference (1971), 1972).

Based on this visual nomenclature, various genetic elements were now being associated with particular genomic "addresses". For instance, a number of human genes and disease etiologies that had been visibly located in the human genome based on chromosomal analysis, among other techniques, were identified based on the chromosomal band in which they had been found (Scriver, 1982; McKusick, 1983). Lubs' X-linked fragile site, along with the twelve others known by 1982, was also associated with distinct a genomic location. In many cases, these fragile sites were named after the chromosomal band at which they appeared. For instance, the fragile site

on chromosome two, initially identified by Lejeune was named after the band 2q11 and the fragile X site was referred to as Xq27-8 (Sutherland, 1979; Hecht, 1982).[19]

With the rediscovery of the fragile X site, and its association with the genomic location Xq27-8, Gillian Turner and her colleagues began reexamining patients affected by X-linked intellectual disability and macro-orchidism cytogenetically. Among 16 families initially examined, Turner et al (1978) reported six families in which affected males showed both macro-orchidism and the fragile X site, and ten families in which intellectually disabled boys expressed neither. In four of the six families, female carriers of the fragile X site were also visibly identified under the microscope, suggesting the potential for offering prenatal diagnosis (Turner et al, 1978). In a number of families then, cases of 'non-specific' X-linked intellectual disability had now been associated with two visible markers, one clinical and the other chromosomal.

Around this time, discussions began concerning the appropriate name for this newly delineated disorder. Previously, eponyms such as Renpenning and Martin-Bell syndrome had been applied based on early reports of families with non-specific X-linked intellectual disability (Richards, 1970; Turner et al, 1970). Since, researchers had returned to the original family studied by Renpenning et al (1962), and found that males showed neither macro-orchidism nor the fragile X site, suggesting that this was a distinct form of intellectual disability (Fox et al, 1980). Turner and Opitz (1980) suggested a new designation, describing the distinguishing features of intellectual disability associated with the fragile X site and calling the disorder 'Macro-orchidism Marker X syndrome'

[19] The fragile X site was seen as being right at the border between bands Xq27 and Xq28 (Turner et al, 1978). For more on this cytogenetic naming system see: chapter 2.

(MOMX). They noted that it was unknown whether the family initially described by Martin and Bell (1943) possessed either of these delineating markers, making this eponym inappropriate. Other clinicians however, opposed the designation MOMX, because it made no reference to the most important feature in affected patients, intellectual disability (Kaiser-McCaw et al, 1980; Richards, 1981).

In 1980, Randi Hagerman, a pediatrician at the University of Colorado, Denver who had read Gillian Turner's 1980 paper associating intellectual disability with macro-orchidism and the Xq27-8 fragile site, became aware of a male patient being cared for locally, who had intellectual disability and abnormally large testicles. At the time, chromosomal analysis for the fragile X site was not available in Denver. Hagerman worked with University of Colorado cytogeneticist Loris McGavran to make Fragile X cytogenetic testing available for this and other patients in the area. The first test eventually came back positive for the fragile site. As McGavran recounted to me, "It was pretty thrilling to get our first positive and start down that road" (Interview with Loris McGavran, August 20, 2012). And indeed, over the next 18 months, Hagerman and McGavran identified about 25 additional similarly affected patients and diagnosed them both clinically and cytogeneticially with Fragile X syndrome (Interview with Randi Hagerman, March 2, 2012).[20]

Noting that there was very little US literature at the time on this disorder, Hagerman and her colleagues decided to collectively write a book on the topic. The book

[20] Randi Hagerman is a physician who specializes in child development and behavior. She began her career at the University of Colorado, Denver and is now at the University of California, Davis, where she is part of the MIND institute.

was ultimately published in 1983 under the title *The Fragile X Syndrome: Diagnosis, Biochemistry, Intervention* (Hagerman and McBogg, 1983). This term 'Fragile X syndrome' had already appeared in the published literature occasionally in the early-1980s (Fox, 1980; Gerald, 1980; Jacobs, 1980). Hagerman and her colleagues however, seem to be the first researchers to have fully embraced 'Fragile X syndrome' as the name for this disorder. As Hagerman put it,

> "We decided to use the name Fragile X syndrome, because Marker X wasn't interesting . . . and we were very struck with the fragile site. So we said, 'let's call it Fragile X syndrome' . . . There was a lot of confusion about what its name was, and we decided to use Fragile X syndrome consistently" (Interview with Randi Hagerman, March 2, 2012).

A few years before this, Richards et al (1981) had demonstrated cytogenetically that family members from the Martin and Bell (1943) study did indeed possess the Xq27-28 fragile site. With this in mind, some clinicians maintained the use of 'Martin-Bell syndrome' during the mid-1980s (Opitz and Sutherland, 1984). The name 'Escalante syndrome' was also suggested (Vianna-Morgante, 1982) during this time, but was opposed by some, who noted that Esclanate did not mention macro-orchidism in his original 1969 description of the disorder (and thereby did not have precedence over Lubs). As a result, the term Escalante syndrome has not been widely adopted outside of Brazil (Turner, 1983; Opitz and Sutherland, 1984). Indeed, while there were many

claims on the name of this disorder throughout the early-1980s, by the middle of the decade, the term Fragile X syndrome seems to have won out.

Despite its importance in the clinical delineation and naming of Fragile X syndrome however, the association between the fragile X site and intellectual disability remained unclear in the mid-1980s. Indeed, of the 17 fragile sites known at this time, only the one Lubs had identified on the X chromosome was associated with a genetic disease (Sutherland and Hecht, 1985). Clinicians and researchers continued to ponder over whether these fragile sites were a form of 'normal' genetic variation, or if they might be associated with some sort of 'pathological' mechanism. Whether the fragile X site itself somehow caused this clinical syndrome, or was just closely linked to a causative gene remained unclear (Hecht, 1982). Indeed, while much about the mechanistic role of the fragile site in Fragile X syndrome remained unknown, clinicians and researchers were not hesitant to use their existing knowledge of the fragile site in attempting to better understand, and even treat this disorder. In the next section, I explore how conceptions of the fragile X site based on observational, laboratory examination were applied in the course of searching for potential clinical treatments.

**The Fragile X Site as an *In Vitro* and *In Vivo* Phenomenon**

Chromosomal analysis, unlike other methods used for identifying the visual markers of human disease, takes place apart from the body (Landecker, 2007). Cells are cultured from the skin, blood, or other patient tissue, and manipulated both chemically and physically in a variety of ways, so as to make chromosomes visible and analyzable

(Rapp, 2000, de Chadarevian, 2010). When the human chromosomes are seen under laboratory conditions, it is anticipated that they look and act in much the same way as they do in the bodies of patients. Indeed, to a significant degree, the veracity of clinical cytogenetics is based on the assumption that chromosomes can pass from the body into the laboratory without the loss, or significant alteration, of their physical characteristics. This said, clinicians are well aware of the fact that, inside the body and out, chromosomes are fluid entities that exist physically only for brief moments in the life of a cell, during its reproductive cycle, and that they are continuously moving through varying stages of condensation, organization, and genetic activity.

Even with all this in mind however, fragile sites are particularly vexing. As Sutherland (1979) demonstrated, most fragile sites only can be seen in cell media that lacks folic acid, meaning that specialized laboratory conditions are necessary to make certain chromosomes appear 'fragile'. The fragile X site has thus been referred to as, "an *in vitro* phenomenon": one that is only made visible by laboratory manipulations (McGavran and Maxwell, 1983, 57). Is the fragile X site actually 'fragile' when it is in the body? Researchers do not have a good answer to this question, because they cannot see the X chromosome under normal bodily conditions. However, as Loris McGavran, a cytogeneticist at the University of Colorado, Denver, put it,

> I don't think that we ever had this concept that it [the fragile X site] would be manifest *in vivo* the same way, because then you would be wandering around with a lot of chromosome X deletions . . . we really thought that it

was an *in vitro* phenomenon (Interview with Loris McGavran, August 20, 2012).

Indeed, it was assumed that if the X chromosome acted in the same way *in vivo* as it appeared to *in vitro*, more severe clinical problems would have resulted. Adding to this, it was shown that the cells of Fragile X patients were not themselves deficient in folic acid (Popovich et al, 1983).

What implications did this have for the role of the fragile X site, as it exists *in vivo*, in causing intellectual disability? While this remained unclear in the 1980s, some clinicians attempted to apply the laboratory understandings of the fragile X site to their thinking and trials aimed at finding a clinical treatment for Fragile X syndrome. If folic acid in cell culture prevents the visible expression of the fragile X chromosomal "lesion" (Pembrey et al, 1985, 713), which is associated with intellectual disability, does this mean that treating patients with additional folic acid could in fact reverse the clinical effects of the Fragile X syndrome in the body?

French physician Jerome Lejeune posed this very hypothesis in a 1982 letter to the *Lancet* (Lejeune, 1982). Lejeune is famous for being the clinician who first identified the correlation between trisomy 21 and Down syndrome. By the late-1970s, identification of trisomy 21 had become the primary indication for prenatal testing, and offered the opportunity for these pregnancies to be terminated if Down syndrome was diagnosed. Lejeune however, being a devout Catholic, was publically very unhappy

about this application of his 1959 discovery (Cowan, 2008; Interview with Kurt Hirschhorn, January 26, 2012).

In his 1982 letter to the *Lancet*, Lejeune lamented the fact that cytogenetic findings were being used for terminating pregnancies instead of finding cures, saying, "Interest in the in utero detection of the fragile X chromosome for the purpose of aborting affected fetuses seems to have blurred the real prospect open to research" (Lejeune, 1982, 273). Based on the existing cytogenetic knowledge about Fragile X syndrome, Lejeune saw hope for a cure. He reported on a trial that he had conducted in which eight children clinically diagnosed with Fragile X syndrome were treated with high doses of folic acid. The results of this uncontrolled trial were very encouraging, with noticeable clinical improvement in seven of these children within the course of just a few weeks. Lejeune also suggested that high doses of folic acid, when given to a pregnant female carrier, might prevent the disease from developing in utero (Lejeune, 1982).

Multiple trials involving the treatment of Fragile X-affected individuals with high doses of folic acid were conducted soon thereafter, at first with some positive results being seen (Carpenter et al, 1983; Brown et al, 1984; Gustavason et al, 1985). Ultimately however, it was determined that folic acid was not a cure for Fragile X syndrome. The fragile X site was seen less often in the cells of patients that had been treated with high doses of folic acid. However, when a known fragile site inducing agent called 5-fluorodeoxyuridine was added to cell culture, the fragile X site appeared just as prominently as it had been before folic acid treatment (Brown et al, 1986; Fisch et al, 1988; Neri et al, 1988).

Additionally, since folic acid is water soluble, meaning that much of every dose is immediately excreted, it would have been impossible for large enough amounts of folic acid to build up in the body, and especially the brain, to have clinical effects within a few weeks, as the Lejeune (1982) trial suggested (Opitz and Sutherland, 1984, 55). While folic acid did not prove to be a preventative treatment or cure for Fragile X syndrome however, debate continues among clinicians over whether folic acid treatment for affected patients does at least improve attention span, hyperactivity, and behavior (Berry-Kravis and Potanos, 2004; Interview with Randi Hagerman, March 2, 2012). Indeedn, attempts to treat Fragile X syndrome with folic acid offer valuable insight into how clinicians and researchers interpreted the potential clinical implications of the laboratory finding that fragile sites are only visible in folic acid deficient media. The fragile X site represented, for clinicians, a physical "lesion" (Pembrey et al, 1985, 713) in the chromosomes (and thereby in the human body and the genome, see chapter 1) of their patients. As McGavran explained to me, "We're used to thinking about chromosome mutations as themselves sort of a phenotypic marker" (Interview with Loris McGavran, August 20, 2012). Indeed, to think of the fragile site like other visible bodily lesions fit with existing conceptions of the human genome, as at once anatomical and genetic.

By chance, clinicians knew of a way to make the fragile X site disappear, at least in cell culture. Based on this, they seemingly hoped that 'fixing' the fragile X site would somehow correct the genomic defect that they assumed caused Fragile X syndrome. This came, despite the fact that clinicians had little knowledge about the etiological mechanism that they were trying to repair. In the next section of this chapter, I further

explore attempts to use the fragile X site as a chromosomal marker for tracking Fragile X syndrome through a family. During the 1980s, the fragile site continued to pose challenges to clinicians and researchers. While frustrating, the variable expression of the fragile site did suggest new ways of thinking about this disorder.

**Expression of the Fragile X Site in Affected Families**

Despite its widespread association with a particular form of intellectual disability, some clinicians expressed doubts about the reliability of the fragile X site as a chromosomal marker for Fragile X syndrome. Daker (1981), for instance, had identified two brothers who expressed the fragile X site, but did not have either macro-orchidism or intellectual disability. Another paper published the same year raised doubts over whether the fragile X site was exclusive to just one distinct form of intellectual disability (Proops and Webb, 1981). Additional studies performed in the early-1980s also found patients that had X-linked intellectual disability and macro-orchidism, but showed no sign of the fragile X site (Jennings et al, 1980; Herbst et al, 1981; Fishburn et al, 1983). Indeed, the fragile X site did not prove to be as distinctive and reliable of a chromosomal marker as, for instance, trisomy 21 had for Down syndrome.[21]

Another major complication inherent to the fragile X site, going back to Lubs' initial identification of it in 1969, was that it rarely appeared in more than one-third of examined cells (usually out of 100-200 counted), even in severely-affected patients. This

[21] Though some complications also arose with the identification of trisomy 21 in some patients clinically diagnosed with Down syndrome as well (Gaudilliere, 2001; Santesmases, 2010).

variable expression of the fragile site occurred independently of the need for a folic acid-deficient medium worked out by Sutherland (1979). While the fragile X site was almost always visible, to some extent, in clinically affected males, in carrier females it was usually present in less than 10% of cells, and often not seen at all (Turner, 1983).[22] The inconsistent expression of the fragile X site greatly complicated attempts to determine the carrier status of clinically normal females within Fragile X families, who were potentially at risk for having affected children (Fryns, 1986; Hogan, 2012).

Going back to the first clinical description of (what was later determined to be) Fragile X syndrome (Richards et al, 1981), Martin and Bell (1943) recognized that this form of intellectual disability did not exactly follow the normal inheritance pattern of an X-linked recessive trait. Indeed, some female family members were affected by intellectual disability as well, though more mildly than their male relatives. One possible explanation for this was that the genetic trait causing intellectual disability was in fact 'dominant': meaning that a normal copy of this genetic entity could not fully overcome an aberrant copy, and prevent any sort of clinical expression.

Another hypothesis for why females are sometimes mildly affected by X-linked intellectual disability involves the process known as X-inactivation or 'Lyonization', named after English geneticist Mary Lyon. While females possess two copies of the X chromosome, only one of them is actively expressed in each of the body's cells (Lyon, 1962). Which of the two X-chromosomes is expressed in each cell is usually the result of random chance, meaning that on average about half of the body's cells express one X

[22] Clinicians and researchers expected, for instance, to see the fragile X site in mothers, 'obligate carriers' who had sons affected by Fragile X syndrome.

chromosome, while in the rest the other is active. However, sometimes due to chance or genetic effects, one of the two X chromosomes is expressed more often in the body's cells than the other. In these cases, if an X chromosome with a certain mutation is more often expressed, its clinical effects may be seen in that patient (Puck and Willard, 1998). Some females then, will show clinical effects of an X-linked disorder that generally only affects males, because it is an X-linked recessive trait.[23]

The mild expression of Fragile X syndrome in female heterozygotes (those who have one X chromosome with the fragile X site and one that appears normal) was studied cytogenetically throughout the 1980s. It was estimated at this time that anywhere from one-third to one-half of all females who possessed the fragile X site on one of their two X chromosomes were affected by mild intellectual disability (Turner et al, 1980; Fishburn et al, 1983). Such figures however, were complicated by the fact that the fragile X marker was not visible in all of the females who assumedly possessed it. Therefore, it was often females who already had sons clinically affected by Fragile X syndrome (making them 'obligate' carriers) who were analyzed in order to determine the clinical presentation of fragile X heterozygotes (Fishburn et al, 1983).

Multiple studies found that the clinical impact of Fragile X syndrome on female heterozygotes was correlated with the percentage of their cells that expressed the fragile X site (Jacobs et al, 1980; Fishburn et al, 1983; Fryns et al, 1986). As one set of researchers noted, "We found the proportion of cells with the fragile X to be strongly correlated with the mental status and to be inversely correlated to with age in

[23] An example of this has been described in the case of Duchenne muscular dystrophy, which is an X-linked recessive disorder normally only seen in males (Pena et al, 1987).

heterozygous females" (Jacobs et al, 1980, 487). In males, similar trends were identified. Parents with two sons affected by Fragile X syndrome were asked to identify which one seemed to be 'brighter'. These qualitative results were then compared to the percentage of cells in each boy expressing the fragile X site. It was found that the more intellectually capable brother of the two siblings often showed comparatively lower fragile X expression (Turner and Partington, 1988).

The presence or absence of the fragile site in Fragile X syndrome was not a black-and-white marker of disease, as would have been clinically desired. While correlations seemed to exist between the degree of fragile X expression and clinical outcomes, these studies did not offer clinicians and researchers with a reliable option for carrier identification or prenatal diagnosis. Though frustrating from one perspective, these findings continued to offer clues about the link between the chromosomal expression of the fragile site, and the clinical manifestation of this disorder. During the 1980s, observational, chromosomal analysis gave clinicians and researchers an increasingly nuanced understanding of Fragile X syndrome, particularly in terms of the range of intellectual disability it caused in both males and females. Additional chromosome level studies would eventually help researchers to better understand how the visible fragile X site was associated with the genomic cause of Fragile X syndrome.

**Tracing the Fragile X site Through Family Pedigrees**

To this point, I have primarily focused on the fragile X site as a diagnostically useful chromosomal marker for Fragile X syndrome in the clinical setting. The fragile

site however, is also a genetic entity: one that moves through families, passing from one generation to the next. When a relatively rare visible genetic abnormality, such as the fragile X site, is identified in multiple cousins within an extended family, clinicians and researchers generally assume that these individuals must have inherited the marker from a common ancestor. This suggests that the abnormality should be visible in that person's chromosomes as well. In addition, when an inherited chromosomal aberration is associated with a particular set of clinical outcomes in a younger generation, clinicians expect to see the same symptoms in previous generations. The fragile X site and Fragile X syndrome however, often did not meet these expectations, which left clinicians and geneticists perplexed about how this genetic trait moved through families.

Many genetic disorders are caused by *de novo* mutations, which occur randomly during the reproductive process, rather than being inherited from a parent. Patients clinically affected with Fragile X syndrome however, almost never have a *de novo* mutation (Brown et al, 1986). Instead the disorder occurs in families over multiple generations. For instance, cousins in multiple branches of the family that Martin and Bell first described in 1943 were affected by Fragile X syndrome, clearly suggesting that the causative trait had been passed down through a common relative. As it turned out however, neither clinical symptoms of Fragile X syndrome, nor the fragile site itself could be traced back through the generations (Pembrey et al, 1985).

Throughout the 1980s, similarly perplexing Fragile X pedigrees were reported (Fryns and Van den Berghe, 1982; Gardener et al, 1983; Froster-Iskenius et al, 1984). In each of these cases, Fragile X syndrome suddenly appeared in multiple branches of a

family, having never been seen previously (Sherman et al, 1985). Brown et al (1986) analyzed two pedigrees of Fragile X-affected families, in which both the parents and grandparents of the affected patients showed neither the clinical symptoms of Fragile X syndrome, or the fragile X site. In each case, the syndrome had appeared in grandchildren across multiple branches of the family, who did suffer from intellectual disability and showed the fragile site. However, those who had clearly passed the trait down showed no clinical or chromosomal signs of it (Brown, 1986).

The fragile X site, and its associated clinical effects, did not appear to be following the expected patterns of Mendelian inheritance. In Fragile X families, clinicians were finding a tight correlation between seeing this chromosomal marker and intellectual disability in younger generations, but when they traced backwards in the family tree, both the fragile site and intellectual disability disappeared from view. (Froster-Iskenius, 1984; Sherman et al, 1985; Brown et al, 1986; Nussbaum and Ledbetter, 1986). Indeed, the causative genetic factor for Fragile X syndrome seemed to always pass through multiple generations of a family before it was clinically expressed. As a result, this genetic trait was distributed throughout an extended family without any warning for decades before its clinical effects became apparent (Figure 5).

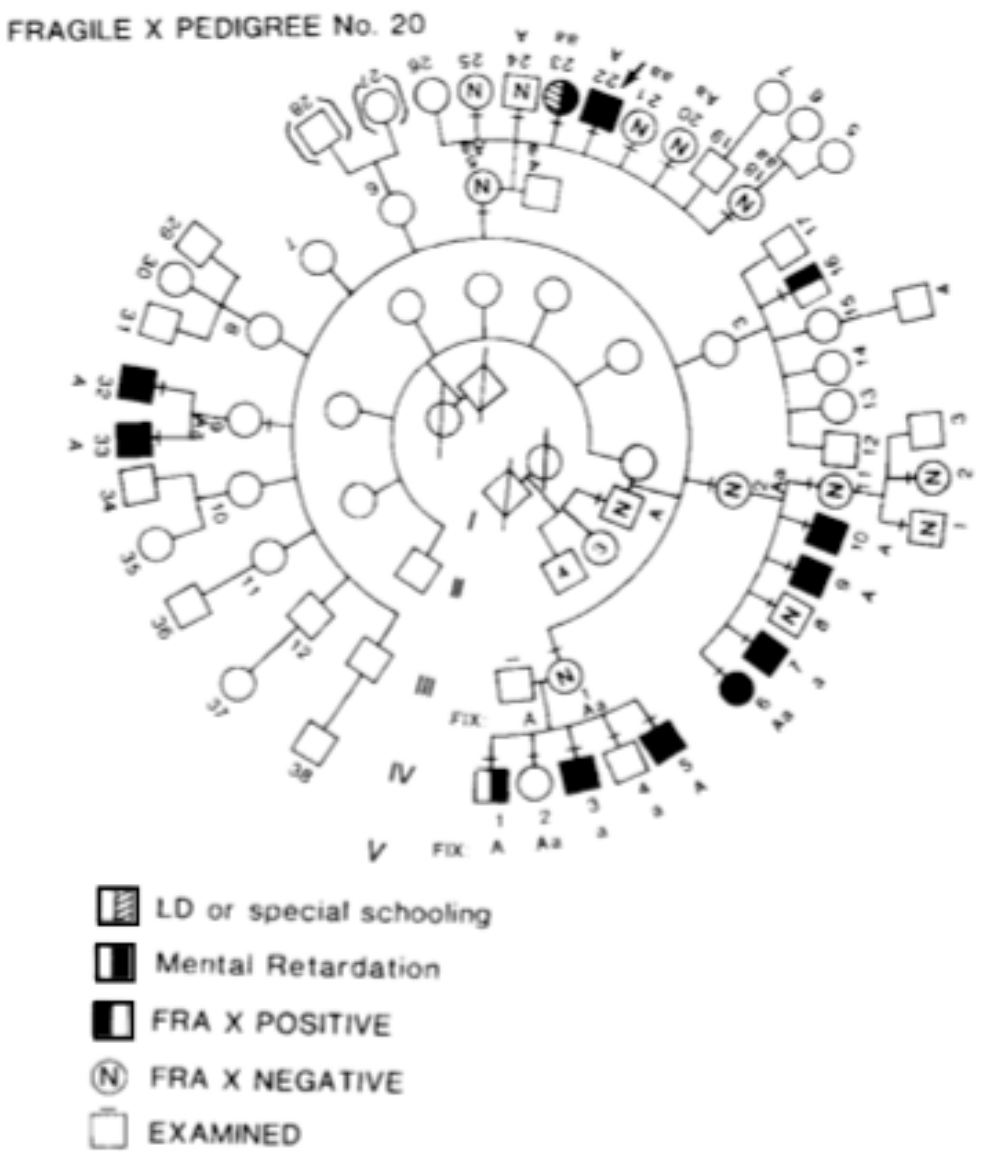


**Figure 5** Pedigree from a family impacted by Fragile X syndrome. Note that only individuals in younger generations are affected by intellectual disability (Brown, 1986). Reprinted with permission from John Wiley & Sons, Inc.

Various mechanistic theories for the unusual inheritance pattern of Fragile X syndrome were proposed in the mid-1980s. Some researchers suggested that the insertion of a transposable element might somehow be involved in the sudden occurrence

of Fragile X syndrome (Friedman et al, 1986; Hoegerman et al, 1986). Others proposed that the maternal uterine environment could play a role in the usual inheritance pattern of the disorder (Van Dyke et al, 1986). Another set of theories was derived from an idea developed earlier in the decade by John M. Opitz. He had previously proposed that a 'pre-mutation' might be responsible for the inheritance pattern of another genetic disorder: achondroplasia, which also had been seen to appear suddenly in distant relatives (Opitz, 1981, 1984). A pre-mutation is a genetic abnormality, which is benign in the patients who carry it, but may develop in future generations into a mutation with significant clinical implications.

Pembrey et al (1985) applied Opitz's theory to Fragile X syndrome, hypothesizing that a pre-mutation might be responsible for its unusual inheritance pattern. As they put it, the idea that Fragile X syndrome "is inherited in a regular X-linked fashion is becoming untenable with the increasing number of reports of transmission through phenotypically normal males." Instead, these clinical researchers proposed, "an inherited sub-microscopic chromosome rearrangement involving the Xq27/8 region that causes no ill effect per se, but generates a significant genetic imbalance when involved in a recombination event with the other X chromosome" (Pembrey et al, 1985, 709).

This hypothesis was meant to address multiple unique aspects of Fragile X syndrome transmission as observed in family pedigrees. How was the causative genetic factor passed through family members in earlier generations with no visible clinical effects? And why did the symptoms of Fragile X syndrome only appear when the genetic trait was passed from mother to child? Pembrey and colleagues envisioned a sub-

microscopic chromosomal event responsible for turning a pre-mutation into a causative mutation. Their assumption was that the visible fragile X site, and its subsequent clinical effects in men and women, must come about due to an uneven recombination event between two X chromosomes, which must happen during the production of eggs in females with a pre-mutation. Since males possess only one copy of the X chromosome, such a recombination event cannot occur during the production of sperm, thereby explaining why a pre-mutation can only be transformed into a causative mutation when this X-linked genetic abnormality passes from mother to child (Pembrey et al, 1985).

The model proposed by Pembrey and colleagues however, was purely theoretical. It was based on an analysis of family pedigrees, instead of laboratory experimentation. During the latter half of the 1980s, researchers were increasingly focused on attempting to characterize the fragile X site molecularly (Brown et al, 1988). Indeed, it was widely assumed that only molecular level analysis of this genomic region could uncover the mechanism that explained the unusual inheritance pattern of Fragile X syndrome (Turner et al, 1986, 53-54). This proved to be a multi-year challenge however, for various technical reasons (Interview with Robert Nicholls, April 5, 2012). In this interim however, chromosomal analysis continued to provide valuable, and even experimental, insights about the fragile X site, which helped clinicians and researchers to better understand its genomic nature, and role in causing Fragile X syndrome.

**Is X Chromosome Fragility Normal?**

One point of debate and uncertainty throughout the 1980s was over the threshold of fragile X expression sufficient to diagnose a carrier or affected individual. Patricia Jacobs and colleagues suggested in a 1980 paper that expression of the fragile X site in at least 4% of cells was necessary for a positive diagnosis. In a second paper published two years later, Jacobs suggested that 3% visibility was probably sufficient for the diagnosis of a female carrier (Jacobs et al, 1980; Rhoads et al, 1982). Another group suggested that fragile site expression in 1% or more of examined cells was sufficient to diagnose carrier status (Herbst et al, 1981). According to McGavran, the normal threshold for diagnosis in her laboratory was 5% fragile X site expression (Interview with Loris McGavran, August 20, 2012).

Edmund Jenkins, who was the first cytogeneticist to successfully diagnose Fragile X syndrome in a prenatal sample, has suggested to me that his laboratory was more conservative, at least when it came to identifying an affected fetus. He preferred to see 10% fragile X cells before offering a positive diagnosis (Jenkins et al, 1981; Interview with Edmund Jenkins, May 26, 2011). While Jenkins believed in the clinical value of the fragile X site, he felt that relatively high expression levels were necessary for accurate diagnosis of carriers and affected individuals, since the expression level of fragile X sites in the wider population of clinically normal individuals was not well established.

Based on this own laboratory experience, Jenkins told me, "We found some morphologically similar lesions, that looked like fragile sites, in control people at very low frequencies." Whether or not clinically normal individuals (aside from carriers)

could show any fragile X site expression was a matter of debate at the time. Jenkins recounted one instance to me from a conference where he was presenting on his laboratory experience with the fragile X site, "someone from the audience [another prominent cytogeneticist] said, 'if you see one fragile site, that's all you need' and I said, 'well what about baseline and controls?' and they said, 'that's just the way it is'" (Interview with Edmund Jenkins, May 26, 2011).[24]

If one fragile X site was seen among 100 examined cells in a fetus or a potential carrier, was this sufficient evidence to make a positive diagnosis? Researchers and clinicians clearly were adamant about developing the most sensitive test possible, so long as it was still reliable. As McGavran put it to me, "Reproducibility was one of our big deals" (Interview with Loris McGavran, August 20, 2012). While getting accurate results was the number of goal of cytogeneticists, a 1984 review article pointed out, under the section heading "A doctor's dilemma", "The early 1980s are witnessing a rush to entice the fragile X to express itself reliably in lymphocytes, fibroblasts, amniocytes, and fetal cells" (de Arce and Kearns, 1984, 88). In some cases, folic acid deficiency was not enough to induce sufficient fragile X visibility, so researchers supplemented the cell cultures with chemicals known to enhance fragile site expression. Like McGavran and Jenkins, the authors of this article encouraged diagnostic caution, noting that the correlation between the amount of fragile X site expression and the long-term clinical severity of Fragile X syndrome remained unclear (de Arce and Kearns, 1984).

[24] Edmund Jenkins is a Ph.D. cytogeneticist who has worked for over 30 years at the New York State Institute for Basic Research in Developmental Disabilities, in New York City.

Amongst all this, medical geneticists David Ledbetter of the Baylor college of Medicine, and Robert Nussbaum, at the University of Pennsylvania, began to wonder if it would be possible to chemically induce fragile X site expression in clinically normal individuals. They hypothesized that folic acid deficiency alone would probably not be sufficient. Chemicals such as flouorodeoxyuridine (FUdR) had previously been adopted to enhance fragile site expression, particularly for purposes of prenatal diagnosis (Tommerup et al, 1981; Jenkins et al, 1984). Ledbetter and Nussbaum used FUdR to increase fragile site visibility, but they also began adding caffeine into cell culture as well. This had a significant impact on fragile site expression (Ledbetter et al, 1986; Interview with David Ledbetter, March 21, 2012).[25]

As many geneticists have been known to do, Ledbetter and Nussbaum began testing their own cells for fragile X site expression, assuming that they were not carriers of the mutant trait.[26] With the addition of caffeine to cell culture, these researchers were able to detect very low-level expression of the fragile X site in their own cells and those of a chimpanzee (Interview with David Ledbetter, March 21, 2012). Additionally, Ledbetter and Nussbaum demonstrated that clinically normal 'transmitting' males from fragile X families showed an intermediate level of fragile site expression, which fell in

[25] David Ledbetter is a Ph.D. geneticist, who spent much of his career at the director of the Cytogenetics Laboratory at Baylor College of Medicine in Houston. He has also directed the Division of Medical Genetics at the Emory University School of Medicine in Atlanta, and is now Chief Scientific Officer for the Geisinger Health System in Danville, Pennsylvania.

[26] One example of this, involving Roger Donahue who karyotyped his own chromosomes during his training (as many geneticists do), is discussed in the previous chapter. Another well-known example is Craig Venter's use of his own DNA for sequencing the human genome.

between that of clinically affected individuals and their own normal cells. With caffeine induction, affected males showed fragile X site expression in 30-40% of their cells, normal transmitting males had 12% expression, and the two researcher's cells showed 4-5% fragile X site expression (Ledbetter et al, 1986).[27]

As the observational cytogenetic analysis of Ledbetter and Nussbaum demonstrated, fragile site expression was not all-or-nothing, but always a matter of degrees: levels of fragile X expression could be seen to progressively increase from normal individuals, to clinically unaffected transmitting males, to affected patients (Ledbetter et al, 1986; Nussbaum and Ledbetter, 1986). Everyone's X chromosome is (at least) a little bit fragile. This finding implied a dynamic continuum that had mechanistic implications for how the fragile site variably appeared in families and caused Fragile X syndrome.

Indeed, Ledbetter and Nussbaum's findings were the first experimental demonstration of the existing 'pre-mutation' theoretical model based on pedigree analysis. The fragile X site was shown to progress from normal, to predisposed for mutation, to pathological over the generations of certain families. The researchers suggested, based on these results – and in line with Pembrey et al (1985) – that a series of chromosomal recombinations involving the fragile X site region might initiate the development of a predisposed 'carrier' male or female, and then (in a later generation) the production of a causative mutation. Interpreting their cytogenetic results to propose a DNA level theory, the researchers concluded, "Thus, our data suggest that a normal DNA

[27] Fragile X site expression in chimpanzees was seen in 1.6% of cells, suggesting that this fragile site has a long evolutionary history (Ledbetter et al, 1986).

sequence at Xq27 [the fragile X site] may be altered to produce a continuous quantitative variation in fragile site DNA resulting in varying degrees of cytogenetic expression and a threshold for clinical manifestation of a mutation" (Ledbetter et al, 1986, 163).

This finding came at an important time for clinicians and researchers interested in Fragile X syndrome. Ultimately, it would take five more years for the fragile X site to be fully characterized molecularly. When this did finally occur, the findings of Ledbetter and Nussbaum were largely verified. Molecular analysis allowed for a DNA level explanation of the mechanism behind fragile X syndrome. However, Ledbetter and Nussbaum's chromosome level demonstration that the fragile site became increasingly prominent between clinically normal transmitters of Fragile X syndrome and clinically affected individuals offered significant insight into the complex relationship between this cytogenetic marker and its associated clinical disorder. In addition, this observational experiment provided a new perspective on the impact of visible variations in genomic structure on human disease.

**A Molecular Genetic Explanation of Fragile X Syndrome**

Throughout the late-1980s and into the early-1990s, clinicians and researchers attempted to locate, at the DNA sequence level, a gene, mutation, or abnormality that could account for the fragile X site as well as the clinical expression of Fragile X syndrome (Brown et al, 1988; Heilig et al, 1988; Nguyen et al, 1988; Dahl et al, 1989; Oostra, 1990). In 1991, the exact location of the causative genetic trait for Fragile X syndrome was identified, and found in close proximity to the fragile site. As Ledbetter

and Nussbaum's earlier experiment had suggested, the structure of this genomic region seemed to change over the generations of a family.

The molecular explanation of Fragile X syndrome was an international accomplishment (Interview with Randi Hagerman, March 2, 2012). In 1991, papers from research teams in Australia, the Netherlands, France, and the United States were all significant contributions to the molecular characterization of the fragile site and its role in Fragile X syndrome. Vincent et al (1991) offered evidence that the Fragile X region is abnormally methylated in affected individuals (more on this below). Yu et al (1991) molecularly demonstrated the instability of the fragile X site, showing that it sometimes grew significantly in size when passed down from mother to child. Verkeck et al (1991) were able to sequence the DNA in this region, finding that it contained a gene, which these researchers named FMR-1 (fragile X mental retardation-1), as well as a long string of CGG trinucleotide repeats. Fu et al (1991) found that the number of consecutive CGG repeats in the FMR-1 area was directly related with clinical outcome in Fragile X families. Normal individuals appeared to have less than 52 CGG repeats in this region, while those with a 'pre-mutation' had between 52 and 200. Pre-mutations were at risk for expanding further when passed from mother to child, with Fragile X-affected children having 200 or more CGG repeats, which became known as a 'full mutation'.

Further analysis of Fragile X families suggested that there were no exact boundaries among normal individuals, those with a pre-mutation, or the presence of a full mutation. In general, if one has less than 55 CGG repeats in the FMR-1 region, it is unlikely that there will be an expansion into a pre-mutation. What defines pre-mutations

is instability: the likelihood of CGG expansion from one generation to the next, though only when passed from mother to child. A full mutation begins at about 200 CGG repeats, but is actually defined by its direct affect on FMR-1 gene activity. When 200 or more CGG repeats are present, this region often becomes 'methylated'. Methylation involves the addition of a small molecule onto certain nucleotides in the region, which in effect turns 'off' the FMR-1 gene. The lack of FMR-1 gene product, (or its reduced activity, which occurs in females who possess a second, normal FMR-1 gene), is what causes the Fragile X syndrome clinical outcome (Oberle et al, 1991; Nolin et al, 1996).

This sequence-level analysis of the FMR-1 gene region helped to resolve many of the abnormal characteristics of Fragile X syndrome inheritance. The pre-mutation theory turned out to be quite accurate, with molecular genetics providing an explanation for what defined a pre-mutation, how it became a full mutation over the generations of a family, and how a full mutation causes Fragile X syndrome. It was also demonstrated that the prevalence of the visible fragile X site under the microscope is directly related to the presence, and relative size (in term of CGG repeats) of a full mutation (de Vries et al, 1993). This means that the fragile site was a visible effect of Fragile X syndrome, rather than its etiological cause. Such a finding did not come as much of a surprise given the large number of other clinically benign fragile sites that had been identified by the late-1980s (Hecht, 1988).

Once the molecular techniques now used for Fragile X diagnosis were clinically proven in the mid-1990s, the era of using chromosomal analysis for the diagnosis of Fragile X syndrome was declared over (Jenkins et al, 1995). Individuals possessing a

Fragile X pre-mutation or full mutation are now diagnosed using molecular genetic techniques. This has allowed for those women at risk for having a Fragile X syndrome-affected child to be much more accurately identified, and for more reliable prenatal diagnosis of the disorder. Additionally, the ability to identify pre-mutation carriers of Fragile X syndrome has led to the recognition that these individuals are themselves at risk for certain clinical effects. Females with a Fragile X pre-mutation are at risk for pre-mature ovarian failure (Allingham-Hawkins et al, 1999), and many pre-mutation males experience Parkinsonian tremors and ataxia late in life (Hagerman and Hagerman, 2004; Hogan, 2012). As a result, Fragile X pre-mutation status has also become an important indicator of potential clinical outcomes.

The association of Fragile X syndrome with an expanded trinucleotide repeat also led researchers to hypothesize that other clinical disorders that seemed to grow worse over the generations of a family (a phenomenon also referred as genetic 'anticipation') might have a similar cause (Sutherland et al, 1991; Harper et al, 1992; Friedman, 2011). Over the next two years, both Huntington's disease and Myotonic Dystrohy were demonstrated molecularly to also involve the expansion of trinucleotide repeats (MacDonald et al, 1993; Orr et al, 1993). Indeed, the multi-step mechanism of Fragile X syndrome quickly became an important exemplar for thinking about how genomic abnormalities might play a role in other, similarly inherited genetic diseases. While the identification of the CGG trinucleotide repeat in 1991 was the lynchpin in demonstrating this novel mechanism of disease development, the important contribution of observational chromosomal analysis should not be overlooked. After all, it was the

cytogenetic analysis of Ledbetter and Nussbaum that first demonstrated to researchers that genomic abnormalities are not necessarily all-or-nothing, but rather that they may also be observed and thought of as occurring over a dynamic continuum from normal to pathological.

**Conclusion**

In his 1982 paper, "Window Panes of Eternity. Health, Disease, and Inherited Risk", geneticist Charles Scriver discussed heritable fragile sites in the human genome as, "Another example [that] illustrates how neo-Vesalian anatomy can be put to use" (Scriver, 1982, 498).[28] Scriver highlighted the role that the Xq27-8 fragile site had in the clinical delineation of a particular, and prominent, form of intellectual disability. He also noted the potential uses of this chromosomal marker for carrier and prenatal screening. Indeed, during the 1980s, the fragile X site came to be one exemplar of a novel way of naming, diagnosing, and understanding human disease: based on the visible association of a genetic disorder with a particular genomic location.

When Lubs first identified the Marker X chromosome in 1969, he recognized its diagnostic, and preventative, value within families impacted by inherited intellectual disability. As this chapter traces however, by the time the fragile X site was re-identified in the late-1970s, its potential stretched beyond just clinical diagnosis. At this point, the fragile X site became integrated into a newly developing conceptual framework, based on the standardization of the human chromosome set and evolving conceptions of the human

[28] For more on Scriver's concept of genome mapping as a "neo-Vesalian" anatomy, see the previous chapter.

genome as an object of clinical research. This visual abnormality was now understood to be the Xq27-8 fragile site, a unique genomic characteristic, which had potential implications for understanding novel structural and functional characteristics of the human genome, and their role in causing disease.[29]

As I suggest throughout this dissertation, the human chromosome set (and the human genome) is as a place in the body where the visual markers and etiological causes of disease are understood to intermingle. This way of thinking among human and medical geneticists can be seen in various attempts to correlate the relative expression of the fragile X site with the severity of its clinical impacts. It is also made particularly apparent by various attempts to treat Fragile X syndrome in the clinic through the application laboratory knowledge about how the fragile X chromosome "lesion" could be fixed by adding folic acid to cell culture.

While the mechanistic correlations between the visible fragile X marker and intellectual disability remained unexplained by clinicians and researchers during the 1980s, these individuals continued to assume that they could come to better understand Fragile X syndrome, and identify potential treatments, with the help of knowledge collected from sustained observational, chromosomal analysis. As Loris McGavran put it to me,

[29] Indeed, I have been told that Lubs has said in jest that the implications of the Marker X chromosome have become so complex that sometimes he wishes he had never discovered it (Phone Interview with Charles A. Williams, March 16, 2012).

> Not knowing the mechanism [of Fragile X syndrome], we were just so fascinated by what it could be. And, living for almost 10 years not knowing was pretty interesting. It allowed us to ask questions that probably were not very smart, but still interesting (Interview with Loris McGavran, August 20, 2012).

Indeed, the clinicians and researchers who examined Fragile X syndrome cytogenetically did not always know what they were looking at (or for) under the microscope. Nonetheless, observational analysis of the fragile X site produced new and interesting research questions, and novel directions for improving the clinical understanding of Fragile X syndrome, and simultaneously, the functional role of the human genome in genetic disease more broadly.

This historical case study of Fragile X syndrome highlights the importance and influence of chromosomal analysis in postwar biomedicine. While the desire to develop molecular level understandings of disease certainly was prevalent among clinicians and geneticists during this era, the central role and contributions of 'observational' cytogenetics should not be overlooked. Indeed, seeing the fragile X site under the microscope was the original and definitive basis for delineating and naming Fragile X syndrome. In addition, the localization of the fragile X site pointed geneticists toward a particular genomic "address" for continued chromosomal and molecular research. As the experimental cytogenetic work of Ledbetter and Nussbaum and the clinical trials of Lejeune, among other examples, demonstrates researchers and clinicians were not sitting

on the sideline waiting for a molecular characterization of this disease. Rather, they took full advantage of existing knowledge and opportunities, based on chromosome level analysis, to further examine the etiology and potential treatment options for Fragile X syndrome.

While Fragile X syndrome may be thought of today as a molecular disease, the history of its delineation and diagnosis during the 1970s and 1980s highlights the visual culture of postwar biomedicine. Along with many other genetic disorders, Fragile X syndrome was integrated into a larger visual framework of human disease during this time, which highlighted the unique genomic location and nature of individual disorders. Following its observational localization in the genome, Fragile X syndrome gained both an anatomical "neo-Vesalian basis" and a likely genetic etiology. It, along with Prader-Willi syndrome, which I discuss in the next chapter, was an exemplar of a new way of locating diseases in the human body. Based on its genomic location at Xq27-8, Fragile X syndrome could be further examined, leading to new mechanistic understandings of its cause, and of the human genome's functionality in disease more broadly.

## CHAPTER 3
## Establishing and Reimagining a Genomic Exemplar: Prader-Willi Syndrome and its Unanticipated Relationship with Angelman Syndrome

This chapter examines the history of two clinically distinct diseases: Prader-Willi and Angelman syndromes. Unlike Fragile X syndrome, each of these disorders was clinically delineated and diagnosed decades before being associated with a visible chromosomal marker or genomic location. Indeed, until the late-1980s, there was no reason to believe that the historical trajectories of these two syndromes would ever intersect. This changed in 1987 however, when multiple clinical teams reported that the same chromosomal aberration had been identified in patients clinically diagnosed with each disorder. Suddenly, these two distinct diseases, with their independent histories, social interest groups, and clinical identities became chromosomally, and genomically, related. In this chapter, I explore the lead up to this finding, as well as its long-term implications for biomedical conceptions of the human genome, and its structural, as well as functional, role in disease.

As I described in Chapter 1, medical genetics introduced a new nosological system to clinical diagnosis during the postwar period, based on the idea that many human diseases could be associated with discrete locations in the human genome. Cytogenetic analysis, and in particular the development of chromosomal banding in the 1970s, offered clinicians and researchers the opportunity to identify visible abnormalities in the human chromosome set, and specify their standardized genomic location. This system was further enhanced later in the decade by the development of high-resolution chromosomal analysis. As Victor McKusick put it in a 1988 grant application to

establish a center for genomic analysis at Johns Hopkins, "The refinement of high resolution cytogenetics of extended chromosomes increased the precision of mapping and revealed small deletions and other changes that were important initial clues to the location of mendelian disorders . . ." (McKusick Papers, Box 2010-081-53, "Program Project Grant-Mapping the Chromosomes of Man", 1988 Grant Application Folder, 157). Indeed, the human genome, seen at the microscopically visible level of chromosomes, had become an entity that clinicians and researchers increasingly turned to in order to 'locate' new markers of disease.

During the early-1980s, Prader-Willi syndrome was a regularly cited example of this ability to chromosomally identify genetic diseases in the human genome (McKusick, 1981, 79; McKusick, 1982, 17; Scriver, 1982, 498). As I describe presently, this came about after the association of Prader-Willi syndrome with a small, but still microscopically visible, deletion of genetic material on chromosome 15. Over time, it became apparent that this deletion could not be seen in the genome of all individuals clinically diagnosed with Prader-Willi syndrome, but it was present often enough to suggest that the aberration played a role in the disorder's etiology.

Similar to Fragile X syndrome, the association between Prader-Willi syndrome and a visible chromosomal abnormality did not prove to be as straightforward or reliable as the correlation between Down syndrome and trisomy 21. The deletion's not infrequent absence in clinically diagnosed Prader-Willi patients kept clinicians and geneticists guessing about the nature of this chromosomal marker and its role in causing the disease. However, it was not until the late-1980s, when this deletion was also identified in patients

clinically diagnosed with Angelman syndrome, that clinicians and researchers began to recognize that Prader-Willi syndrome's relationship with the chromosome 15 deletion was more complex than previously anticipated. This finding forced clinicians to rethink the apparent ease with which, in the early-1980s, most cases of Prader-Willi syndrome could be cytogenetically delineated and diagnosed.

As I describe in this chapter, medical geneticists never seriously discussed or debated the possibility, during the late-1980s, that Prader-Willi and Angelman syndrome were in fact two historically distinct forms of the same genetic disease. These two disorders were simply too different in their clinical expression to possibly have the exact same genetic cause. Indeed, at this time, most clinicians and researchers felt that there was something still unseen in the human genome that could account for such different clinical outcomes being caused by the same chromosomal deletion. As evidence mounted that the chromosome 15 deletions in both Prader-Willi and Angelman patients were the same, in terms of their genomic location and size, researchers began to consider novel conceptions of human genome functionality that could account for what they saw in the clinic.

This chapter offers another example of the dual role of chromosomal analysis as both a diagnostic tool and experimental system in postwar biomedicine. As in the case of Fragile X syndrome, the simple presence or absence of a visual cytogenetic marker alone was not sufficient to reliably diagnose Prader-Willi syndrome. Ultimately however, the complications incurred in course of chromosomally analyzing Prader-Willi, and later Angelman, patients led to long-term improvements in diagnosis, as well as a better

understanding of the human genome and its role in disease. This historical instance, I argue, is representative of the important part that the human genome has played as a conceptual space in the development of contemporary biomedicine (Hogan, 2013a). As I describe, the chromosomally examined and depicted human genome became a location where the conventions, questions, and interests of clinical and basic genetics continue to intersect and intermingle.

**The Clinical Delineation of Prader-Willi Syndrome**

In 1956, Swiss clinicians Andrea Prader, Alexis Labhart, and Heinrich Willi reported on nine children affected by obesity, short statue, abnormally small genitals (cryptorchidism), intellectual disability, and muscle weakness during infancy (Prader et al, 1956). In the five years following this initial report, other clinicians also described children with similar attributes (Jenab et al, 1959; Dunn et al, 1961, Laurance, 1961). Prader and Willi alone published a follow-up paper five years later, in which they reported on five additional affected children (Prader and Willi, 1961). In a paper on delineating different forms and causes of muscle weakness (hypotonia) early in life, Zellweger et al (1962, 599) noted that, "'Floppy' or 'limp' infants and children are encountered frequently", in the clinical setting. Among those cases he and his colleagues had seen, ten were similar to the syndrome that Prader, Labhart, and Willi had described seven years earlier (Zellweger et al, 1962).

Prader-Willi syndrome was clinically differentiated from other forms of infant hypotonia by the sudden onset of obesity in children around the age of three. Evans

(1964, 207) described the early childhood progression of Prader-Willi syndrome in this way, "They remain feeble and emaciated for a time; then the wasting gives way to obesity." Indeed, Prader-Willi syndrome was defined by this uniquely cruel twist, in which very weak, poorly feeding infants, just as they seem to be getting better, suddenly developed a voracious appetite. Without careful, and difficult dietary regulation, these children quickly grew to be dangerously obese.

Some clinicians referred to Prader-Willi syndrome, and other conditions like it, as "Pickwickian-like syndrome" in reference to the Charles Dickens character (Jenab et al, 1959, 23; Zellweger and Schneider, 1968, 597; Hawkey and Smithies, 1976, 155). It has also been suggested that a short and overweight child depicted in Velazquez's famous painting *Las Meninas* was affected by Prader-Willi syndrome (Hawkey and Smithies, 1976, 152). Some reports of Prader-Willi syndrome during the 1960s identified individuals who were likely affected, but not fully obese. These young male patients however, were overweight and showed distinctly abnormal fat distribution, described as resembling that of an older woman, with accumulation in the buttocks and thighs (Forssman and Hagberg, 1964). Another report referred to the fat distribution in Prader-Willi patients as being "feminine", (Juul and Dupont, 1967, 19).

It was quickly recognized that Prader-Willi patients were at great risk for developing debilitating adult-onset diabetes by their teenage years due to their obesity (Evans, 1964). Unfortunately, these patients not only showed a ravenous appetite for food, but also seemed to have a slower than normal metabolism. Evans (1964) recounted a case in which a 500-calorie daily diet was necessary to help a 16 year-old male Prader-

Willi patient to lose weight and overcome diabetic symptoms (a normal daily diet for this individual would be 2500 calories). In some early cases, such diets were quite successful for treating patients. This however, did not change their voracious appetite, so maintaining a healthy weight was a challenge (Evans, 1964).

Prader-Willi patients were also recognized as being intellectually disabled (Buhler et al, 1963; Engel and Hogenhuis, 1965). Evans (1964) described eight Prader-Willi patients who were developmentally delayed and had IQs between 41 and 87. Some of these individuals seemed to improve intellectually as they grew older, though only one patient was able to attend a normal school. Engel and Hogenhuis (1965) described three additional affected individuals as having an IQ between 60 and 80, a level regarded as below normal, but still 'mild' in terms of intellectual disability. In comparison, the majority of males affected by Fragile X syndrome have 'moderate' intellectual disability, with IQ scores between 35 and 49 (Hagerman et al, 1983, 41). Most children affected by Prader-Willi syndrome required special education, but only a few were regarded as 'ineducatable' in the 1960s. Indeed, some showed improved IQ scores and acuity with age (Evans, 1964; Laurance, 1967).

Discussions of what to call this distinct clinical syndrome began appearing in the medical literature during the mid-1960s. Engel and Hogenhuis (1965) suggested "H2O syndrome" referencing the disorder's primary distinguishing features of hypotonia in infancy, hypomentia (intellectual disability), and obesity beginning around age three. Another set of researchers suggested that a third H should be added to the description to account for the common presence of hypogonadism (small or hidden testicles), making it

"HHHO syndrome" (Zellweger and Schneider, 1968). In 1968, a letter to the *Lancet* described three additional cases of this disorder under the title "Prader-Willi syndrome" (Spencer, 1968, 571). It is unclear why Labhart's name was excluded from this designation (perhaps it was because he had not been a co-author on the Prader and Willi (1961) follow-up report). The eponym Prader-Willi syndrome has been widely used ever since, though the disorder has also occasionally been referred to as Prader-Labhart-Willi syndrome as well (Cassidy et al, 1984; Magenis et al, 1990).[30]

During the 1960s, the disorder that eventually became known as Prader-Willi syndrome featured a rather unique clinical signature. What set the disorder apart from others like it was its distinct two-stage natural history, from 'floppy' infants to overweight young children. Seeing these two stages in progression within a single patient made for an easier clinical diagnosis. However, when viewed in isolation, either the weakness or obesity could easily be mistaken for another disorder. From a treatment perspective, preventing obesity before it took hold was the most promising strategy. Ideally, clinicians would be able to make the diagnosis of Prader-Willi syndrome before the transition to overeating began. But, based on clinical presentation alone, this was often difficult to do, especially in such physically weak and poor feeding infants. As I discuss in the next sections, the identification of another visual sign of Prader-Willi syndrome, this time a chromosomal marker, promised to improve both diagnosis and treatment.

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[30] Some have suggested that Langdon Down, the British clinician best known for identifying Down syndrome in the mid-19th century, was also the first person to describe Prader-Willi syndrome, in 1887 (Kousseff and Douglass, 1982; Bonuccelli et al, 1982; McKusick, 1987)

**Associating Prader-Willi Syndrome with a Chromosomal Marker**

Clinicians and geneticists who examined early cases of Prader-Willi syndrome frequently performed chromosomal analysis on their patients, with a few findings of interest. While many patients appeared to have normal karyotypes (Laurence, 1961; Dubowitz, 1967; Juul and Dupont, 1967; Cohen and Gorlin, 1969), Dunn et al (1961) identified an extra G group (21, 22, Y) chromosome in one patient with Prader-Willi features. Two years later, a translocation involving two D group (13, 14, 15) chromosomes was seen in one individual who appeared clinically to have Prader-Willi syndrome. Similar D group translocations had previously been seen in normal individuals by other clinicians though, calling into question whether this aberration in fact played a role in the patient's disorder (Buhler et al, 1963). In 1969, a woman was reported who also had a D group translocation, and appeared clinically normal, but had experienced multiple miscarriages (Lucas, 1969). Ridler et al (1971) identified an extra chromosome in a clinically diagnosed Prader-Willi patient, though it appeared smaller than any of the normal human chromosomes. Based on its visible structure, the chromosome was reported to be an extra, duplicated fragment from either the D or G group.

As in the case of Fragile X syndrome, a major problem with these early reports of abnormalities in Prader-Willi patients was that the exact identity of the chromosomal aberrations was unknown. The extra chromosomes reported by Dunn et al (1961) and Ridler et al (1971) may have represented the duplication of a G group chromosome (as

happens in Down syndrome), or the presence of an extra small piece of another chromosome. Likewise, the D group translocations reported by other clinicians might have involved any of three different chromosomes (13, 14, 15). Once again, it was the introduction of banding techniques in the early-1970s that helped to resolve these issues of uncertainty. Cytogenetic banding offered clinicians a more definite knowledge of which chromosomes were involved in the abnormalities they observed, and what portions, if any, of these chromosomes were lost or duplicated.

In 1976, with the aid of chromosomal banding, an individual clinically diagnosed with Prader-Willi syndrome was seen to have an unbalanced chromosomal translocation involving the fusion of both copies of chromosome 15 (Figure 6). The researchers who saw this translocation microscopically, inferred that at least the short arm, and perhaps a small portion of the long arm of one copy of chromosome 15 had been deleted (Hawkey and Smities, 1976). Such a specific description of this translocation would not have been possible five years earlier, before the introduction of new chromosomal banding techniques (Hirschhorn et al, 1973). In response to their finding, the authors stated, "it would be tempting to speculate that the number 15 chromosome is involved in this pathogenesis [Prader-Willi syndrome]. However, as the great majority of cases had normal karyotypes it may be that the chromosomal abnormality in our patient was unrelated to the clinical condition" (Hawkey and Smithies, 1976, 156).

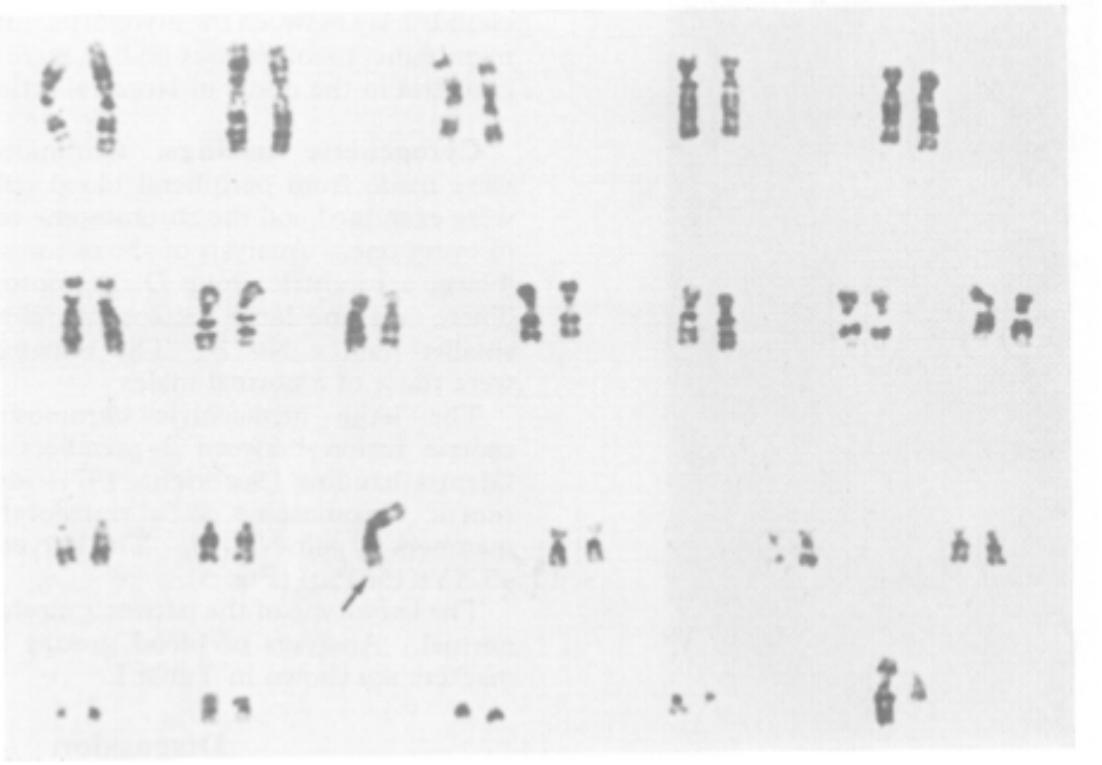

Fig. 5. Karyotype. 45,XY,t(15q;15q). Abnormal meta centric chromosome is arrowed.

**Figure 6** Karyotype from a Prader-Willi patient showing a translocation involving both copies of chromosome 15. Reproduced from *Journal of Medical Genetics*, Hawkey and Smithies, vol. 13, pp. 152-163, 1976, with permission from BMJ Publication Group Ltd.

A second report of an unbalanced translocation also involving chromosome 15 in two Prader-Willi syndrome patients came a year later. Based on these findings, Fraccaro et al (1977) suggested that all individuals clinically diagnosed with Prader-Willi syndrome should be evaluated for anomalies of chromosome 15. This publication was followed by multiple other reports over the next four years of Prader-Willi syndrome patients showing chromosome 15 translocations (Zuffardi et al, 1978; Fleischnik et al, 1979, Wisniewski et al, 1980). In some instances, the researchers were able to more specifically identify what portions of chromosome 15 had been affected by the

translocation. For instance, Zuffardi et al (1978) noted that the visible chromosomal region 15q11-15 had been lost in one of the translocations they observed in a Prader-Willi patient. By the end of 1980, it was quite clear to clinicians that Prader-Willi syndrome was in some way associated with a genetic abnormality on chromosome 15.

While this series of reports suggested that Prader-Willi syndrome had something to do with chromosome 15, a translocation involving this chromosome could not be considered as either a necessary or sufficient cause of the syndrome. Translocations were only seen in a few affected patients, and family members of these patients sometimes appeared to have the same translocation, but showed no clinical signs of Prader-Willi syndrome (Smith and Noel, 1980). As one group of researchers put it, "Normal karyotypes have been found in many cases of Prader-Willi syndrome. These could be assumed to be due to a deletion of 15p undetectable by present cytogenetic techniques" (Zuffardi et al, 1978).

It was thought that the chromosome 15 translocations, which clinicians were seeing in certain patients affected by Prader-Willi syndrome, likely involved some loss of genomic material. As I describe in the next section, the further improvement of cytogenetic methods in the late-1970s helped to provide geneticists with the visual resolution necessary to identify a common chromosomal deletion in many Prader-Willi patients. Based on these techniques, Prader-Willi syndrome became associated with particular chromosomal bands, and a specific genomic location, in the early-1980s. As a result, the disorder came to be an exemplar of the promise of using cytogenetic analysis in describing the 'morbid anatomy' of the human genome.

**The Application of High Resolution Chromosomal Analysis to Prader-Willi Syndrome**

Throughout the 1970s, human and medical geneticists were working on various methods to improve the visual resolution of chromosomal analysis. Functionally, chromosomes are a highly condensed form of DNA, the compaction of which varies greatly during the process of cell reproduction. This makes chromosomes look different under the microscope at each stage. During the late-1970s, the promise of 'high-density' cytogenetic analysis was recognized. This technique involves arresting cellular reproduction at a stage when chromosomes are less condensed (and hence more stretched out) than usual, meaning that smaller aberrations might be visible. In effect, staining chromosomes when they are less condensed means that more bands are visible, thereby improving the resolution of analysis (Yunis et al, 1978).[31]

In the late-1970s, Ph.D. student David Ledbetter, having read the Hawkey and Smithies (1976) report of a chromosome 15 translocation in Prader-Willi syndrome, began to wonder if high-resolution chromosomal banding might reveal a deleted chromosomal region in affected patients. At the time, Ledbetter was a member of the Baylor College of Medicine cytogenetics laboratory, run by clinical geneticist Vincent Riccardi. Ledbetter was actually working in this laboratory part time as a technician, in order to make money as he pursued his dissertation research on primate chromosomal evolution in the nearby laboratory of T.C. Hsu (Interview with David Ledbetter, March

[31] For more on the impacts of 'high-density' chromosomal banding on standardized depictions of the human chromosome set, see chapter 1.

21, 2012).[32] Riccardi's laboratory was involved, at the time, in applying high-resolution techniques to diagnose a form of kidney cancer, the Wilms' tumor, based on a visible deletion on chromosome 11 (Riccardi et al, 1978; Riccardi et al, 1980).

According to Ledbetter, a sample from a Prader-Willi patient came into the Baylor cytogenetics laboratory one day, thus giving him the opportunity to see if high-resolution chromosomal analysis would reveal a chromosome 15 aberration. Ledbetter was already quite experienced in identifying small deletions in patients with Wilms tumor, and so when he saw the karyotype of the Prader-Willi patient, he was able to see that one copy of chromosome 15 looked smaller than the other, suggesting a small deletion. To verify this finding, a new sample was acquired from the patient, and Riccardi set up blinded analysis. Ledbetter was still able to pick out the deletion in this one patient. Based on this, samples from five additional patients, also blinded, were acquired from a Prader-Willi researcher in Boston, and Ledbetter was once again able to identify the same chromosome 15 deletion in the affected patients. This suggested a reliable, and potentially informative, visual correlation between the chromosome 15 aberration and Prader-Willi syndrome (Interview with David Ledbetter, March 21, 2012).

In a 1981 *New England Journal of Medicine* article, Ledbetter et al (1981) reported that they were able to identify the same deletion on the long arm of chromosome 15 in the karyotypes of four out of five patients clinically diagnosed with Prader-Willi syndrome. The specific genomic location of this deletion, based on visual chromosomal nomenclature, was 15q11-13 (Figure 7). Ledbetter followed-up on his initial report with

[32] For more on Ledbetter, see chapter 2.

a larger cytogenetic study of PWS patients published the next year. He found the very same 15q11-13 deletion in many more PWS patients, though only 19 of the 40 analyzed (Ledbetter et al, 1982). The same year, another group of researchers published similar results, 14 Prader-Willi syndrome patients had been cytogenetically analyzed, and seven showed a 15q11-13 deletion (Butler et al, 1982).

Clearly this deletion was associated with Prader-Willi syndrome, but why was it only visible in about half of all patients? Were there multiple causes of this disorder, or was the deletion simply too small to be seen microscopically in many patients (Bonnucelli et al, 1982)? With possible clinical implications in mind, Ledbetter et al (1982) suggested that the two cytogenetic sub-populations of Prader-Willi syndrome patients, those with and without a visible 15q11-13 deletion, should be examined for variability in their clinical outcomes. Studies of larger cohorts of PWS patients in the following years continued to find that almost half showed no 15q11-13 deletion. Very little in the way of clinical variations were found however, among the deletion and non-deletion groups (Reed and Butler, 1984; Butler et al, 1986). Differences that were identified included lighter skin tone and hair color, along with increased sun sensitivity, in Prader-Willi patients with a visible 15q11-13 deletion. Individuals with this deletion also showed greater homogeneity of fingerprint patterns (Butler et al, 1986).

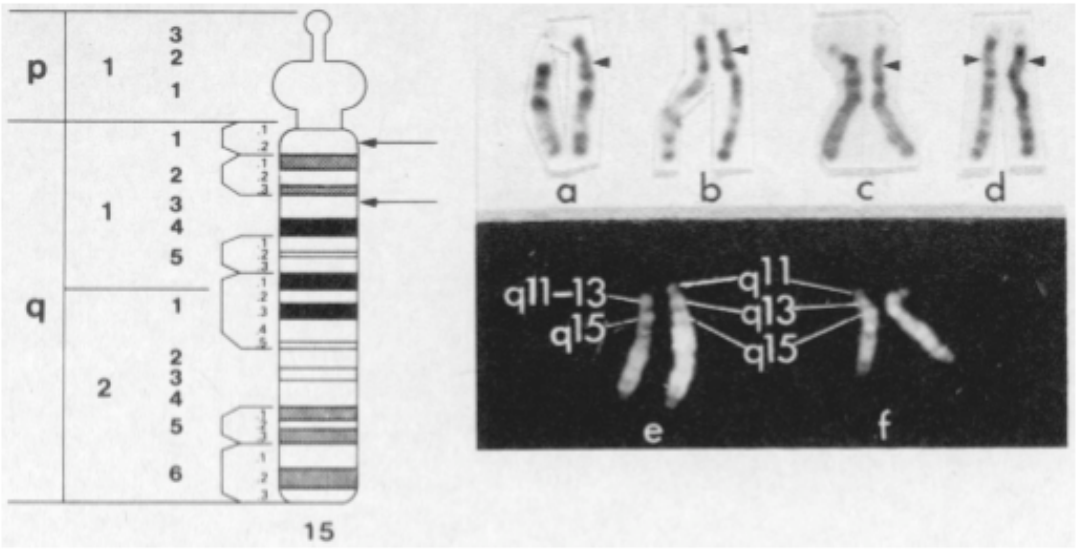


Figure 1. Ideogram of Chromosome 15 (Adapted from Francke and Oliver[23]), Showing Breakpoints in q11 and q13 of Cases 1 through 4 (Upper Panel) and Partial Banded Karyotypes (Lower Panel).

G-banded karyotype a is that of Case 1, b is that of Case 2, c is that of Case 4, and d is that of Case 5; arrowheads indicate the presence of band q12 in the normal homologue (on the right) of Cases 1, 2, and 4 and in both homologues of Case 5. Partial R-banded karyotype e is that of Case 3 (the twin of Case 2), and f is that of Case 5. The deleted chromosome is on the left in Case 3.

**Figure 7** Chromosome 15 ideogram showing the location of the visible deletion in Prader-Willi patients. Also, comparative images of chromosomes with and without the deletion. Reproduced with permission from *New Englad Journal of Medicine,* Ledbetter et al, vol. 304, pp. 325-329, 1981, Copyright Massachusetts Medical Society.

During the mid-1980s, clinicians widely agreed that Prader-Willi syndrome was somehow associated with chromosome 15 anomalies. How these visually observed cytogenetic abnormalities were involved in the clinical expression of Prader-Willi syndrome however, remained unclear. In 1986, clinicial cytogeneticist Arabella Smith noted, "While an association between chromosome 15 abnormality and the Prader-Willi syndrome is clearly apparent and undisputed, there is debate as to whether this relationship is causal" (Smith, 1986, 278). Indeed, while the visual correlation seemed

undeniable, one team of researchers surmised that the chromosome 15 deletion seen in many Prader-Willi patients might be a chromosomal effect of the disorder, rather than its etiological cause (Kousseff and Douglass, 1982).

Uncertainty about the functional role of chromosome 15 aberrations in Prader-Willi syndrome did not stop clinicians and researchers from touting the importance of these visual markers in the clinic. McKusick noted in 1981 that the association of Prader-Willi syndrome with chromosome 15 anomalies helped to distinguish it from other conditions characterized as eating disorders, thus giving it a genetic basis. As McKusick put it, "Like other problems lumped together as eating disorders, this has often been viewed as a psychiatric state and the organic basis revealed by the chromosomal aberration has been in my experience a relief to families of the afflicted. This finding adds another stone to the foundations of an organic basis of morbid obesity" (McKusick, 1981, 80). Indeed, even if Prader-Willi syndrome's visual association with chromosome 15 did not explain the exact nature of its etiology, it did offer families peace of mind, knowing that the cause of obesity was genetic, rather than psychological.

The next year, Charles Scriver pointed to the value of being able to associate clinical disorders with genomic locations, noting that even though not all patients with a particular syndrome may show the associated chromosomal marker, knowledge of the link could still be used to identify the genes and biochemical pathways etiologically involved. In his paper, Scriver suggested, "Careful study of these and other syndromes [Wilms' tumor, retinoblastoma, and Prader-Willi] will determine whether enzyme phenotypes can be used systematically to diagnose the chromosomal phenotype

prospectively. This represents an interesting development in genetic counseling and a novel application of gene mapping and cytogenetics" (Scriver, 1982, 498). While uncertainty remained concerning the relationship between visible chromosome 15 aberrations and Prader-Willi syndrome, the marker could still be used in the laboratory and the clinical in attempts to improve the treatment and understanding of the disorder.

Indeed, during the early-1980s, the microscopically visible 15q11-13 deletion became an important exemplar of a new way of doing medical genetics. With the example of cytogenetic analysis of Prader-Willi patients specifically in mind, Scriver suggested that high-resolution chromosome banding was a "technology clearly capable of further refining the morbid anatomy of human disease" (Scriver, 1982, 496). This statement was followed by a full-page figure depicting McKusick's 'morbid anatomy' of the human genome diagram. New cytogenetic capabilities had been successfully used to associate Prader-Willi syndrome with a unique, and visible, anatomical location in the human genome. Just as the 15q11-13 deletion had helped to give Prader-Willi a genetic basis, and distinguish it from Froelich syndrome and other clinical disorders, high-resolution chromosomal seemed to promise improvements in the delineation and diagnosis of other diseases. Later in the 1980s however, an unanticipated complication arose: the same 15q11-13 deletion was identified in multiple patients diagnosed with a very different clinical disorder. In the next section, I offer a clinical history of this distinct disease.

**The Clinical Delineation of Angelman Syndrome**

In 1965, British pediatrician Harry Angelman reported on three cases of what he called 'Puppet' children. These children all suffered from inborn intellectual disability, and showed similar physical abnormalities. Of their clinical attributes Angelman noted, "Their flat heads, jerky movements, protruding tongues and bouts of laughter give them a superficial resemblance to puppets, an unscientific name but one which may provide easy identification" (Angelman, 1965, 681). These children were developmentally delayed, severely disabled intellectually, and suffered from frequent epileptic seizures (Angelman, 1965). Angelman later recounted that what convinced him to publish on these three patients, as being affected by a unique and distinct clinical syndrome, was coming across the painting "Boy with a Puppet" by Giovanni Caroto while on vacation in Italy. He said of this moment, "The boy's laughing face and the fact that my patients exhibited jerky movements gave me the idea of writing an article about the three children with a title of Puppet Children. It was not a name that pleased all parents but it served as a means of combining the three little patients into a single group" (Williams, 2011). The delineation of this syndrome was entirely visible and impressionistic. Though Angelman's name for the syndrome was understandably offensive to affected families, it ultimately helped other clinicians to see the disorder in their own patients.

Another group of clinicians reported on two additional similarly affected patients in 1967, referring to this disorder as 'Happy Puppet' syndrome. These clinicians said of Angelman's initial account of this syndrome, "It was immediately apparent to us that two patients whom we had studied for several years conformed to his description" (Bower

and Jeavons, 1967, 298). Clearly, Angelman had succeeded in capturing the gestalt of this syndrome through its name, thereby making it more likely that other clinicians would see it. A third account of a patient with severe intellectual disability and similar clinical characteristics appeared in the literature again five years later. The individual was diagnosed as having "Angelman's ('Happy Puppet') Syndrome" (Berg and Pakula, 1972). Many additional reports of the syndrome were published over the next decade, all based on Angelman's original clinical description and name (Mayo et al, 1973; Elian, 1975; Kuroki et al, 1980; Dooley et al, 1981).

In 1982, U.S. clinicians Charles Williams and Jamie Frias noted six additional cases of the disorder, and suggested that this syndrome might be less rare than had been assumed ten years earlier (Berg, 1972). At the end of their report, the clinicians put forward a new potential name for the disorder remarking, "We feel that the term 'Happy Puppet' is inappropriate as the patient's family may feel the term is derisive and derogatory. For this reason, and despite the limitation of eponymic designations, we propose the name of this disorder should be Angelman syndrome" (Williams and Frias, 1982, 460). Another group of clinicians made a similar call at about the same time for a new "less imaginative, eponymous designation" (Dooley et al, 1981, 624). As other historical syndrome case studies in this dissertation also demonstrate, clinicians are often hesitant to move away from an existing name for a syndrome when it plainly describes the disorder's symptoms. In this instance though, it was argued that despite its descriptive accuracy, a name like 'Happy Puppet' syndrome was both offensive and not in keeping with medical precedent (Dooley et al, 1981; Williams and Frias, 1982). No

less, while Angelman syndrome was widely adopted in the mid-1980s, the 'Happy Puppet' designation has not fully disappeared (Willems et al, 1987; Brown and Consedine, 2004; Sarkar et al, 2011).

Many of the early studies on this syndrome included chromosomal analysis, but none of them identified any microscopically visible abnormalities (Angelman, 1965; Bower and Jeavons, 1967; Berg, 1972; Dooley et al, 1981). Williams and Frias (1982) identified an inversion within chromosome 3 in an Angelman syndrome patient, but suspected that it was benign, since no genomic material appeared to have been lost as a result. As Charles Williams later noted to me, "We had no idea of what causation was. I think if any anything we presumed it was possibly a single gene disorder, although we didn't know that either . . . there was some recurrence in families" (Interview with Charles Williams, March 16, 2012).[33]

For the most part, Angelman syndrome was seen to occur sporadically, suggesting that the causative mutation, if there was one, happened *de novo*. As Williams alluded to, the disorder was occasionally identified in siblings (Kuroki et al, 1980; Pashayan et al, 1982; Willems et al, 1987); however, this was not seen frequently enough to infer that Angelman syndrome was inherited (Willems et al, 1987). Indeed, well into the 1980s, the disorder's etiological basis and genetic characteristics (if any) remained unknown to the clinical community. All of this would change in 1987 however, with two independent reports of a small chromosome 15 deletion in multiple Angelman syndrome patients.

[33] Charles Williams is a Pediatrician and Medical Geneticist at the University of Florida. He has spent much of his career doing research pertaining to Angelman syndrome, and played a central role in the founding of the Angelman Syndrome Foundation in the United States.

This event brought about a previously unanticipated chromosomal relationship with Prader-Willi syndrome, and forced clinicians and researchers to reassess their assumptions about the anatomical and etiological relationship between visible genomic locations and abnormalities, and clinical disease.

**Prader-Willi and Angelman Syndrome As Chromosomally Related**

In 1987, Prader-Willi syndrome researchers became aware of a set of vexing cytogenetic findings: in two clinical reports, the 15q11-13 chromosomal deletion was seen in multiple patients, who by clinical analysis, were affected by a disorder that was not Prader-Willi syndrome. Lawrence Kaplan and colleagues reported on three patients with this chromosomal aberration, but heterogeneous clinical outcomes. One patient showed what could be interpreted as a mild case of Prader-Willi syndrome, another was diagnosed with Angelman syndrome, and a third patient was identified as having Williams syndrome, which shares some similar attributes with Angelman syndrome, including intellectual disability, abnormal facial features, and a happy demeanor (Kaplan et al, 1987). Another publication that year by Ellen Magenis and colleagues identified two additional, unrelated, patients with the 15q11-13 deletion, who were not affected by Prader-Willi syndrome. Magenis had initially presented these cases at a 1987 national Prader-Willi syndrome meeting in Houston, Texas, not knowing what clinical disorder these patients had. A clinical geneticist in the audience named Charlotte Lafer, recognized the patients as having Angelman syndrome (Interview with Charles Williams, March 16, 2012; Magenis et al, 1987).

As I outlined in previous sections, Prader-Willi and Angelman syndrome are two very different disorders clinically. Therefore, it was quite confusing for clinicians and researchers to hear that they had been associated with the same chromosomal aberration. Some suggested that the cytogenetic analysis done of Prader-Willi and Angelman patients was not yet high-resolution enough to identify the subtle differences in the deletions that caused Prader-Willi and Angelman syndromes. Kaplan et al (1987, 45) suggested in their paper, "It is proposed that different molecular abnormalities involving specific points or segments along the long arm of chromosome 15 might account for the clinical diversity seen among these and other patients". This position was echoed by Magenis et al (1987, 837), "Further resolution of these syndrome and their clinical characteristics will likely be at the molecular level".

These clinical researchers were suggesting that what could not be seen at the level of chromosomes would be resolved at the level of molecular markers. In making this argument, Magenis et al (1987) cited the work of Donlon et al (1986), who had been working on developing molecular 'probes' for this region of chromosome 15. Such probes were able to interact specifically with particular areas of a chromosome at the DNA level, while still being visible under the microscope because of an attached radioactive trace. It was assumed that the chromosomal deletions that cause Prader-Willi and Angelman syndrome would be differentiated using this technique. In this case, it was expected that one probe would not be able to attach to chromosome 15 in Prader-Willi patients, and another probe would not be able to affix itself in Angelman patients. This would indicate that adjacent, but distinct (though perhaps overlapping) regions were

differentially deleted in these patients. Donlon (1988) however, found this not to be the case. Even at the molecular level, the deletions in patients with Prader-Willi and Angelman syndrome seemed to be the same.

At about the same time Robert Nicholls, a postdoctoral fellow at Harvard, was also using molecular techniques to study the chromosome 15 deletion in Prader-Willi and Angelman patients. He found similar results. According to Nicholls, "We found first that the deletions, using the DNA probes that we had access to, were actually the same size, and so that was kind of unexpected in the field" (Interview with Robert Nicholls, April 5, 2012). Indeed, existing assumptions about the human genome, and its role in disease, could not account for how two very distinct clinical disorders might be caused by a deletion in the same genomic location. Williams described the confusing situation to me in this way, "We had a good two to three years where people thought it must be the same gene, which causes the two syndromes . . . it took a while to sort that out" (Interview with Charles Williams, March 16, 2012).

When asked if these genomic findings suggested to clinicians and researchers that Prader-Willi and Angelman syndrome were in fact historically distinct forms of the same genetic syndrome, Williams remarked to me, "They were totally different . . . Back then there wasn't any sense that these are very similar disorders, they are quite different" (Interview with Charles Williams, March 16, 2012). Indeed, it was assumed that some functional distinction must exist, which would explain how one genomic aberration could cause two clinical disorders. While this finding complicated the cytogenetic diagnosis of Prader-Willi syndrome, it also opened the door to new ways of thinking about the human

genome and its role in disease. As David Ledbetter put it to me, "There was a lot of frustration and curiosity . . . it was pretty puzzling" (Interview with David Ledbetter, March 21, 2012). In the late-1980s, various theories and experiments were proposed to explain this unanticipated genomic link.

With his molecular findings in mind, Donlon (1988) offered one hypothesis for the different clinical outcomes. He suggested that instead of being caused by 'dominant' chromosomal deletions, Prader-Willi and Angelman syndromes might instead each represent, " a compound recessive disorder . . . involving more than one gene" (Donlon, 1988, 326). Since the deletion associated with these two syndromes was so large, and likely contained 10-30 genes (in Donlon's estimation), various combinations of recessive genes on the non-deleted chromosome 15 might lead to differing clinical outcomes. When there is no deletion on the other copy of chromosome 15, these clinical outcomes are prevented. However, with the 15q11-13 genetic material on one chromosome missing, the remaining recessive genes are fully expressed, leading in some cases to either Prader-Willi or Angelman syndrome. In additional, Donlon (1988) suggested that other syndromes with overlapping clinical outcomes might also be associated with such deletions and compound recessives.

In 1989, Robert Nicholls, then at Harvard University, made an important observation: in patients with Prader-Willi syndrome, the 15q11-13 deletion, if present, was always inherited on the copy of chromosome 15 inherited from their father, who did not himself have the mutation (Nicholls et al, 1989). Humans possess two copies of each of their chromosomes: one copy comes from their father and one from their mother.

Throughout the 1980s, clinicians were able to visually distinguish between different copies of chromosome 15 based on subtle variations on its short arm (Wachtler and Musil, 1980). Interestingly, the observation reported by Nicholls et al (1989) had already been published on six years earlier by a different team of clinical researchers (Butler and Palmer, 1983). However, Butler and Palmer (1983) had not inferred that this occurrence was unique to Prader-Willi syndrome. Rather, they suggested that such *de novo* deletions might occur more often on paternally inherited chromosomes.

At the time, Nicholls was a postdoctoral fellow in the laboratory of Samuel Latt, who had died in August 1988. The team of researchers he had assembled however, proved to be highly productive in the year after his passing. Also in 1989, cytogeneticist Joan Knoll, a colleague of Nicholls' in Latt's laboratory, reported on multiple cases in which patients with Angelman syndrome had inherited the 15q11-13 deletion on their maternal copy of chromosome 15 (Knoll et al, 1989). This provided further evidence that differential inheritance indeed did play a role in clinical outcome for these syndromes. As Williams later put it, "Once there was recognition that the maternal deletion has a different syndrome, then everything caught fire" (Interview with Charles Williams, March 16, 2012).

Closely related to Knoll's report, Nicholls' 1989 paper had discussed an interesting case of a patient affected by Prader-Willi syndrome who had no visible 15q11-13 deletion.[34] This individual did however, possess a different variety of chromosome 15 abnormality: he had inherited two maternal copies of chromosome 15,

[34] Nicholls was second author on Knoll's 1989 paper, and Knoll was second author on Nicholls'.

but no paternal copy, a condition referred to as 'uniparental disomy'. This finding provided even more evidence that there existed some difference between the maternally and paternally inherited copy of chromosome 15, leading to either Prader-Willi or Angelman syndrome in the clinic (Nicholls et al, 1989). The difference, researchers knew, was most likely not in the DNA sequence itself, as chromosomes (aside from the Y) are not themselves gendered: a chromosome inherited paternally in one generation may have been passed down maternally in the previous generation.

Ultimately, these findings also provided an explanation for why the 15q11-13 deletion was not always visible in Prader-Willi syndrome patients. Prader-Willi was caused by a paternally inherited deletion of 15q11-13, but the disease also occurred in instances when an individual inherited two normal maternal copies of chromosome 15 and no paternal copy. Angelman syndrome was clinically seen in the opposite case, when two paternal, but no maternal copy of chromosome 15 was inherited (Nicholls et al, 1989). These findings suggested a novel functionality of the human genome, called 'imprinting' that had only previously been described in mice. As Ledbetter has put it to me, "When Rob Nicholls published his paper, we all kicked ourselves for not figuring it out, because we should have been able to based on the mouse literature. If any of us had paid attention to imprinting in the mouse, we should have been able to predict this" (Interview with David Ledbetter, March 21, 2012). Genomic imprinting led to the expression of certain genes on one parental copy of a chromosome, but not the other. Indeed, the differential clinical outcomes in Prader-Willi and Angelman syndromes demonstrated that in humans, just as in mice, certain portions of the genome are always

'turned off' on one member of a chromosome pair (Knoll et al, 1989; Nicholls et al, 1989).

**Further Analysis of Prader-Willi and Angelman Syndrome in the 1990s**

During the early-1990s, clinicians worked to better resolve the genetic basis of Prader-Willi and Angelman syndrome, and to further explain the previous decades' often confusing chromosomal observations. When abnormal clinical outcomes were initially noted in patients with a 15q11-13 deletion in 1987, it was suggested that those with non-Prader-Willi phenotypes, eventually determined to be Angelman syndrome, might have a larger deletion than classic Prader-Willi patients (Greenberg et al, 1987). By the early-1990s however, it had become widely accepted, following the 1989 papers from Samuel Latt's laboratory by Robert Nicholls, Joan Knoll, and their colleagues, showing that differential parental inheritance led to the vastly different clinical outcomes. Based on the clinical and cytogenetic analysis of 17 Prader-Willi and Angelman patients, Ellen Magenis and colleagues noted that, on average, the chromsome 15 deletion in Angelman patients appeared under the microscope to be larger than in those with Prader-Willi syndrome (Figure 8). Among the patients with either Prader-Willi or Angelman syndrome however, the visual size of the deletion did not seem to correlate with the severity of clinical effects. Magenis and colleagues also suggested the possible role of uniparental disomy in these two disorders, citing the work of clinical researcher Judith Hall (Magenis et al, 1990).

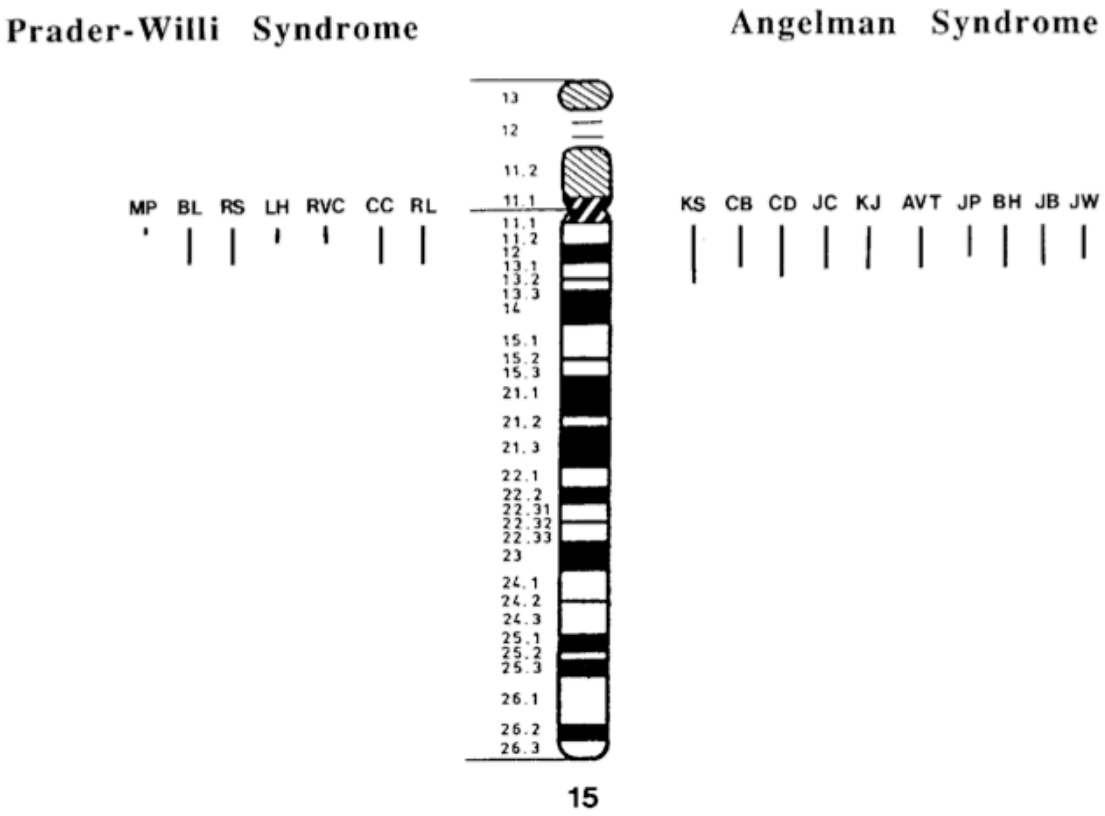


Fig. 11. Summary of deletions present in the 17 patients. The extent of the deletion in each patient is indicated on the diagram as a bar. Note that, in general, the PWS deletions are smaller than those of AS. The largest PWS deletion we studied overlapped those found in some AS patients.

**Figure 8** Chromosome 15 ideogram depicting the relative cytogenetic size of 15q11-13 deletions seen in Prader-Willi (left) and Angelman syndrome (right) patients (Magenis et al, 1990). Reprinted with permission from John Wiley & Sons, Inc.

In the early-1990s, clinicians and researchers continued to ponder how many of the cases of Prader-Willi and Angelman syndrome having no visible cytogenetic deletion were caused by uniparental disomy, versus a deletion that was too small to see microscopically. Among 30 patients who were clinically diagnosed with Prader-Willi syndrome, but did not have a microscopically visible 15q11-13 deletion, Mascari et al (1992) found 18 had uniparental disomy of the maternal copy of chromosome 15, and another eight patients who had a deletion that was only detectable using molecular

techniques. Four individuals showed no sign of a deletion or uniparental disomy, but each of these patients had clinically abnormal forms of Prader-Willi syndrome. From this, it was inferred that about 20% of all cases of Prader-Willi syndrome was caused by uniparental disomy (Mascari et al, 1992).

Uniparental disomy proved to be much less common in Angelman syndrome, only occurring 2-5% of the time. Researchers believed that this was related to the relative prevalence of non-disjunction in female reproductive cells (when an ovum ends up with two copies of a certain chromosome instead of just one), which is the cause of other chromosomal disorders, such as Down syndrome. It is suspected that the rapid increase in Down syndrome cases in women over age 35 is an effect of the increase in non-disjunction with maternal age. A similar effect was demonstrated with Prader-Willi syndrome (Robinson et al, 1991). It was further surmised that uniparental disomy often results from what is called a 'trisomic rescue': when a fertilized egg begins with three copies of chromosome 15, but then loses one early on in the development process. If the embryo has received two maternal copies of chromosome 15 and one paternal, and the paternal copy is lost in this 'rescue', the embryo will develop with uniparental disomy. This is more commonly the case in Prader-Willi than Angelman syndrome simply because non-disjunction (and hence the presence of two maternal copies of chromosome 15) is more prevalent in women. Therefore, maternal uniparental disomy, leading to Prader-Willi syndrome, occurs much more frequently than Angelman syndrome due to paternal uniparental disomy (Nicholls, 1993).

Very much like in the case of Fragile X syndrome, it was suggested in the early-1990s that DNA methylation might also play a role in the differential expression of Prader-Willi and Angelman syndrome. As I have already described, methylation involves the addition of a small molecule to certain DNA nucleotides in particular genetic regions, in effect turning them off.[35] In Fragile X syndrome, methylation was determined to be the result of a causative genetic aberration (more than 200 CGG trinucleotide repeats) (Yu et al, 1992). Prader-Willi and Angelman syndrome, on the other hand, appeared to be associated with the normal pattern of methylation (due to 'imprinting') on chromosomes. Driscoll et al (1992) demonstrated that the 15q11-13 chromosomal region is differentially methylated based on its maternal or paternal origin, and that this methylation pattern can be used as a diagnostic test for Prader-Willi and Angelman syndrome. These clinicians identified a gene, DN34, that was differentially methylated in the 15q11-13 region based on parental origin, thereby suggesting that it might be involved in the clinical expression of one or both of these disorders (Driscoll et al, 1992).

Over time, additional candidate genes for causing Prader-Willi and Angelman syndromes have been identified. Ozcelik et al (1992) identified the SNRPN gene, which is located within the smallest deleted genomic region known in Prader-Willi patients. SNRPN was shown to be maternally methylated in mice, so clinicians assumed that it probably would be in humans as well. Additionally, it was found that the SNRPN gene was specifically disrupted by chromosomal translocations in multiple Prader-Willi patients (Nicholls and Knepper, 2001). Angelman syndrome is now thought to be cause

[35] For more on this, see chapter 2.

by a specific gene, UBE3A. This result is based on the finding that Angelman syndrome can be caused by a mutation in this gene alone (Matsuura et al, 1997; Kishino et al, 1997). This is not believed to be the case in Prader-Willi syndrome, which has never been associated with a specific mutation in any one gene alone, suggesting that it is likely caused by multiple maternally imprinted genes (Buiting, 2010).

Indeed, clinical and laboratory conceptions and understanding have evolved significantly over the past 30 years. During the 1980s, Prader-Willi syndrome went from being representative of the ability to visually and discretely 'locate' a disease in the human genome using chromosomal analysis, to an exemplar (along with Angelman syndrome) of an entirely new form of genomic functionality. As I discuss in the next section, clinicians and researchers looked to this new phenomenon, genomic 'imprinting', during the early-1990s as a possible explanation for a number of other complex genetic diseases. It appears that experience with Prader-Willi and Angelman syndrome taught human and medical geneticists to think differently about the human genome and how its various dimensions of functionality impact clinical disease.

**Examining a New Exemplar of Genomic Functionality**

What was the relevance of genomic imprinting to human and medical genetics more broadly? This question was addressed by clinical geneticist Judith Hall in a series of papers published during the early-1990s. Hall targeted multiple audiences with these reports, which appeared in the *American Journal of Human Genetics*, *Development*, *Archives of Disease in Childhood*, Current *Opinion in Genetics & Development*, and *The*

*New England Journal of Medicine* (Hall, 1990a, 1990b, 1990c, 1991, 1992). At about the time that Robert Nicholls' 1989 report on imprinting in Prader-Willi and Angelman syndromes first appeared, Hall was just returning from a Sabbatical year in the UK, where she had been working with mouse geneticists studying imprinting in mammals. Based on this experience, Hall already recognized the potential importance of imprinting in human and medical genetics (Interview with David Ledbetter, March 21, 2012). The emerging Prader-Willi and Angelman story offered an excellent forum to present imprinting to the broader biomedical community.

In her 1990 *American Journal of Human Genetics* paper, Hall noted the potential value of genomic imprinting in explaining diseases that did follow normal inheritance patterns,

> One of the important challenges of contemporary genetics is to explain those traits and conditions that do not mendelize [show Mendelian patterns of inheriance]. It is in that regard that the concept of genomic 'imprinting' has assumed increasing importance, because it may provide an explanation for a remarkably diverse set of observations on conditions whose genetic transmission and expression does not conform to the predictions of single-gene inheritance (Hall, 1990a, 857).

Imprinting, suggested Hall, offered a new way of thinking about genomic functionality, and one that could open up new avenues of biomedical thought and research, "Genomic

imprinting appears to be a form of regulation, allowing another level of flexibility within the control and expression of the mammalian genome, and may explain why mutations in some parts of the mammalian genome function differently depending on whether they come from the father of the mother" (Hall, 1990a, 857). It was no longer sufficient to consider just what genes a person possessed, but also from whom they were inherited.

Within each of her papers on genomic imprinting, Hall discussed Prader-Willi and Angelman syndromes as a prime example of how imprinting impacted the human genome and its role in genetic expression. Based on what researchers had learned about the impacts of uniparental disomy in Prader-Willi and Angelman patients, and what had been observed in mice more generally, Hall suggested other human diseases in which imprinting might play a role. Researchers had found that in mice uniparental disomy was often associated with disorders that showed behavioral rather than structural abnormalities. With this in mind, Hall pointed to other disorders, beyond Prader-Willi and Angleman syndrome, that might be caused by uniparental disomy based on clinical traits, "If one reflects on common human syndromes that are as yet unexplained, such as Rubinstein-Taybi syndrome, Cornelia de Lange syndrome, Williams syndrome, Russel-Silver syndrome, etc. the possibility that they represent uniparental disomy for other chromosomes must be explored" (Hall, 1991, 144).

Targeting clinicians more broadly, Hall attempted in her 1992 *New England Journal of Medicine* article to use the example of Prader-Willi and Angelman syndrome to demonstrate why and how genomic imprinting could matter to them, "What do rare conditions such as the Prader-Willi and Angelman syndromes have to do with the real

world of the busy practitioner? They seem esoteric and exotic, and yet these rare syndromes are windows into the world of a newly recognized phenomenon of inheritance called genomic imprinting" (Hall, 1992, 827). Hall explained that imprinting was particularly relevant in the context of prenatal testing, when there was little else to rely on than the analysis of chromosomes, "There is urgent need to determine whether uniparental disomy will be a problem in the case of each of the other chromosomes, since there may be vary real consequences for prenatal diagnosis" (Hall, 1992, 828).

Indeed, the role of imprinting and uniparental disomy in Prader-Willi and Angelman syndromes made it particularly clear that seemingly 'normal' karyotypes may in fact be aberrant in clinically significant ways. Two cautionary reports were independently published on this in 1992 (Cassidy et al, 1992; Purvis-Smith et al, 1992). Each involved prenatal testing based on chorionic villus sampling (CVS), a technique that samples from the placenta rather than the amniotic fluid as in amniocentesis (Hogan, 2013b). In both cases, CVS results suggested trisomy 15, though the fetus appeared to be normal under ultrasound. Not infrequently, trisomies are confined to the placenta, but do not affect the fetus due to the occurrence of a 'trisomic rescue' described in the previous section. In each of these cases, amniocentesis later suggested that each fetus had a normal karyotype, and the pregnancies were continued to term.

When born, each of the children showed muscle weakness, and eventually progressed to reveal signs of Prader-Willi syndrome in early childhood. It turned out that each individual had uniparental disomy of the maternal copy of chromosome 15, thus causing Prader-Willi syndrome (Purvis-Smith et al, 1992; Cassidy et al, 1992). Hall

(1992) highlighted the case uncovered by Cassidy and colleagues in making her argument for why widespread awareness about genomic imprinting was so important for the medical community. Referencing the new era of laboratory and clinical thinking that Prader-Willi and Angelman syndrome had helped to bring about, Hall noted, "The concept of genomic imprinting is a difficult one for many physicians and scientists who were not trained to ask whether the sex of the transmitting parent makes a difference to the outcome. But from now on we must ask this question about biological phenomena and all disease processes that may have a genetic component" (Hall, 1992, 828-9). Indeed, clinicians and researchers had to be on the look out, because even karyotypes that appeared 'normal' at first glance might in fact possess significant chromosomal abnormalities.

In the next section, I discuss another interesting and unexpected occurrence, involving the at times confusing 'look' of Prader-Willi patients. While the disorder's association with chromosome 15 abnormalities improved its delineation and diagnosis during the 1980s, clinicians sometimes continued to see Prader-Willi syndrome when, genomically, it was not there. Whether the term 'Prader-Willi' should be used exclusively to designate a specific genetic syndrome, or if it had value for describing a broader clinical presentation, was a matter of debate in the 1990s. As I describe in the next section, the names of genetic disorders sometimes move out beyond the boundaries set by their discrete genomic localization.

**'Prader-Willi-like' as a Clinical Category**

In 1981, McKusick suggested that the association of Prader-Willi syndrome with chromosome 15 aberrations made it a genetic disease, thus distinguishing it from a broader array of clinically diagnosed dietary problems (McKusick, 1981). Despite this however, the 'look' of Prader-Willi, in one interesting case involving Fragile X syndrome, has been identified as a clinical category, even in the absence of chromosome 15 abnormalities. The first instance of this was a report by J.P. Fryns and colleagues in 1987. Four patients clinically and cytogenetically diagnosed with Fragile X syndrome, also appeared to have 'Prader-Willi-like' characteristics, most notably, showing short stature and extreme obesity as young children (Fryns et al, 1987).

J.P. Fryns, Bert de Vries, and colleagues reported on additional five patients in 1993, clinically and cytogenetically diagnosed with Fragile X syndrome, but who once again showed distinct 'Prader-Willi-like' features. Each of these individuals expressed the fragile X site and showed no sign of the 15q11-13 deletion. Among family members also affected by Fragile syndrome, most did not show similar Prader-Willi characteristics. Unlike most Prader-Willi patients, these individuals did not show hypotonia as infants, nor was their obesity caused by a sudden change in eating habits. Also, most patients showed more severe intellectual disability than is common to Prader-Willi syndrome. Many of these patients did however, have small testicles, a common feature of Prader-Willi syndrome, rather than abnormally larger ones, as is frequently seen in Fragile X syndrome (de Vries et al, 1993).

One group of clinicians criticized the use of 'Prader-Willi-like' by de Vries et al (1993), arguing that obesity and intellectual disability are common in many disorders, and that their association with Prader-Willi syndrome would only confuse its clinical diagnosis (Gillessen-Kaesbach and Horsthemke, 1994). De Vries and Niermeijer (1994) however, responded to this criticism by noting that this special sub-category of patients showed many features common to Prader-Willi syndrome, but not Fragile X patients, like a round face and small testicles. Since two of their Fragile X patients had previously been misdiagnosed with Prader-Willi syndrome, these clinicians felt broader clinical knowledge of this sub-type of Fragile X syndrome would improve diagnosis, rather than confuse it (de Vries and Niermeijer, 1994).

An additional report of a patient diagnosed with Fragile X syndrome, but demonstrating Prader-Willi-like symptoms came, once again from the Fryns group, in 1994. The patient showed fragile X site expression, early onset childhood obesity, and small genitals, but no 15q11-13 deletion. Hormonal studies on this patient did not suggest any abnormalities that could explain his obesity (Schrander-Stumpel et al, 1994). Another more recent report has also identified 13 additional cases of this Prader-Willi phenotype of Fragile X Syndrome. Nowicki et al (2007) noted overeating, leading to obesity, and more severe behavioral problems than what is seen in most Fragile X patients. These researchers also pointed to a gene, CYFIP1, in the 15q11-13 region, which appeared to interact with the FMR-1 protein, as a possible molecular explanation for this overlapping clinical phenotype. Indeed, it has been found that patients with

'Prader-Willi-like' Fragile X syndrome often show lower than normal expression of CYFIP1 (Nowicki, 2007; Hagerman et al, 2010).

While interesting to this dissertation in a coincidental sense, the Prader-Willi phenotype of Fragile X syndrome also offers a valuable opportunity to reflect on how researchers and clinicians name genetic diseases and clinical categories. The name Fragile X syndrome comes from its chromosomal marker, whereas the term Prader-Willi syndrome seems to evoke a particular clinical expression. Is classifying certain patients with Fragile X syndrome as being 'Prader-Willi-like' clarifying or confusing for other clinicians? Those who opposed the designation felt that it would mislead physicians, and add to an existing over diagnosis problem (Gillessen-Kaesbach and Horsthemke, 1994). On the other hand, the term reminded clinicians that individuals with Fragile X syndrome could 'look' as much like Prader-Willi patients as they did Fragile X patients, meaning that clinical expression in these individuals often included significant Prader-Willi features like obesity and very small genitalia, while excluding macro-orchidism and facial characteristics common to Fragile X syndrome. Indeed, it is important to keep in mind that what clinicians call a disorder may be as much about defining how it should be classified: 'Prader-Willi-like' Fragile X syndrome, as how it should not: Prader-Willi syndrome.

**Conclusion**

This chapter examines the history of Prader-Willi syndrome, and its unanticipated genomic and clinical overlaps with Angelman, as well as Fragile X syndromes. As I have described, during the early-1980s, Prader-Willi syndrome came to exemplify the

promises of a new 'genomic' nomenclature in postwar biomedicine. The association of Prader-Willi syndrome with a visible deletion at the chromosomally defined genomic location 15q11-13, even if the aberration was not seen in all patients, offered a representative example of how chromosomal analysis could aid human and medical geneticists in their attempts to better to delineate, diagnose, understand, prevent, and treat human diseases. Indeed, Prader-Willi syndrome was pointed to as an important example of biomedicine's growing knowledge of the 'morbid anatomy' of the human genome, and its central role in disease.

With the unanticipated identification of the chromosomally visible 15q11-13 deletion in patients not expressing the classical clinical features of Prader-Willi syndrome, the exemplary status of the disorder was called into question, and ultimately reconceived. The nosological system linking genetic diseases to discrete genomic locations had shown great success throughout the 1980s (Hogan, 2013a). Nonetheless, the visual association of both Prader-Willi and Angelman syndrome with the same genomic location was not regarded as evidence that they were in fact historically distinct forms of the same genetic disorder. Indeed, clinical expression unequivocally trumped genomic localization as far as classification was concerned.

The genomic overlap between Prader-Willi and Angelman syndromes posed complications for its accurate diagnosis and understanding in the clinic. As I demonstrate throughout this dissertation however, findings such as this one effectively turned human chromosomal analysis into unexpectedly productive experimental system. Hans-Jorg Rheinberger's description of experimental practices in biomedicine highlights

the concept that the most productive experimental systems are those that continuously produce unanticipated outcomes (Rheinberger, 1997). Such a situation, while of great value in basic laboratory research, is seemingly at odds with the goals of clinical analysis, which seeks to provide consistent and reliable results. In the long-term however, unanticipated findings like the one described in this chapter may improve the clinical diagnosis and understanding of disease. Central to the resolution of how Prader-Willi and Angelman syndrome could be caused by the same genomic deletion, was the application of knowledge about genomic functionality in mice to research on human disease. This intersection, I suggest, was facilitated in part by the late-20$^{th}$ century 'genomic' conception of human disease, which placed disease analysis within the realm of both basic biological and clinically targeted research. Indeed, during this era, the human genome became a location where the conventions, questions, and interests of clinical and basic genetics intersect and intermingle.

What does the chromosomal relationship between Prader-Willi and Angelman syndrome mean for the researchers who study them and the families who are affected by them? Pedagogically and genomically these two disorders likely will always be linked. Indeed, clinicians still need to know about their common genetic deletion and differential clinical outcomes, lest they should be misdiagnosed. Also, as the papers of Judith Hall demonstrate, Prader-Willi and Angelman syndrome are an important teaching point more generally, both as an example of the role of uniparental disomy and methylation in human disease, and well as of the complex functionality of the genome more broadly. Institutionally however, these two disorders remain quite distinct. As Charles Williams

noted, "Years ago we thought about, should we have a joint meeting [between the Prader-Willi and Angelman syndrome foundations] . . . really it never quite had a dynamic, it just didn't make sense" (Interview with Charles Williams, March 16, 2012). Though these two disorders are linked chromosomally and genomically, for those affected by them they are remain distinct. Indeed, while genomic overlaps like the one described in this chapter are interesting and valuable findings in both the laboratory and the clinic, they do not necessarily change the day-to-day experience of living with a genetic disorder.

In the next chapter, I explore another instance of genomic overlap in the recent history of human and medical genetics. While the two genetic syndromes examined were clinically and historically distinct for more than a decade, the finding that they were associated with the same chromosomal deletion was pointed to, in this instance, as evidence that they were in fact two versions of the same genetic disorder. As in this chapter, I will examine the implications of this finding for the naming, diagnosis, understanding, and institutional organization of these two disorders in the years after they became genomically associated. The contrasts between these two stories are meant in part to highlight the ongoing power and influence of both laboratory and clinical observation in the thinking and practices of postwar biomedicine.

## CHAPTER 4
## A Single 'Elephant' in the Room: How DiGeorge and Velo-Cardio-Facial Syndromes Become One Genomic Disorder

In this chapter, I focus on another instance of genomic overlap, though in this case, one with a very different outcome. Just like Prader-Willi and Angelman syndromes, DiGeorge and Velo-cardio-facial (VCF) syndromes were each initially identified and historically diagnosed independently based upon clinical observations alone. In the early-1990s however, it was demonstrated that many patients clinically affected by DiGeorge and VCF syndromes possessed the same deletion in the chromosomal band 22q11. This finding was not quite as surprising for clinicians as the overlap between Prader-Willi and Angelman syndromes had been a few years earlier. Indeed, going back to the mid-1980s, some clinicians had noted similarities between DiGeorge and VCF syndrome patients. However, despite these commonalities, the natural and clinical histories of these two disorders did not frequently overlap.

The established 'look' of a disease may be significantly impacted by its clinical severity. If a disorder is generally lethal during early infancy, conceptions of clinical appearance will tend to focus on its most visually obvious characteristics: like structural birth defects or highly deadly attributes. In cases where patients are not so severely or noticeably impacted at birth, and are expected to live for years or decades after diagnosis, more subtle characteristics of the disease become relevant to its diagnosis and treatment, as well as to the experiences of patients and their families. Into the 1980s, the differing 'looks' of DiGeorge and VCF syndrome were closely related to the age of patients when first diagnosed. DiGeorge syndrome was diagnosed at birth and almost always proved

deadly by age two. VCF syndrome, on the other hand, was generally only identified in school-aged children based on non-life threatening symptoms.

In this chapter, I trace the various visual signs that were used to independently identify DiGeorge and VCF syndrome, and which over time brought them together as one clinical entity. The convergent histories of these two disorders highlight the problem of 'ascertainment bias' in genetics research. As many scholars have previously noted, there is nothing certain or obvious when it comes to identifying disease categories. Indeed, nosological categories in medicine are affected by the variable expression of disorders as well as the institutional and professional infrastructures that divide clinical specialties and assumptions. Thus, in analyzing clinical nosology, we must focus the collective ways in which clinicians and researchers learn and agree to see, name, and standardize their objects of study (Fleck, 1979; Daston, 2008).

Throughout this dissertation, I explore how the introduction of visual genetic evidence has impacted the clinical categorization of diseases. As I show in this chapter, while a common genetic marker has the power, in some cases, to make two diseases one from etiological and ontological perspectives, the existing names and institutional affiliations associated with the disorders will not always immediately follow suit. In fact, to this day, a common name has not been universally accepted for describing DiGeorge and VCF syndromes. It has not been the bodily presentation of disease that has gotten in the way in this case, but rather the importance of maintaining certain disease classifications, which expand beyond medical textbooks and diagnostic interactions to the identity of research grants, foundations, institutions, and careers.

The association of DiGeorge syndrome with a particular genomic location occurred, as with Prader-Willi syndrome, through observational chromosomal analysis. Translocations, and small deletions, involving the long arm of chromosome 22 eventually came to be understood as an additional genomic and anatomical markers for DiGeorge syndrome, as well as key pieces of genetic evidence linking the disorder to VCF syndrome. Indeed, over time, a deletion in the chromosomal region 22q11 came to be understood as the most significant and reliable indicator of the presence of this genetic disease, now widely referred to as '22q11 deletion syndrome'. Unlike DiGeorge syndrome, which was historically associated with severe birth defects and early infant death, 22q11 deletion syndrome is now traced in family pedigrees, with parents being retroactively identified as having a mild case of the disorder after their children are clinically diagnosed. The natural history of DiGeorge, reconceived as 22q11 deletion syndrome, now spans entire lifetimes, rather than being primary seen in infants and young children. Indeed, the 'look' of 22q11 deletion syndrome is broader, and in some cases may be quite distinct from, the severe clinical attributes initially associated with DiGeorge syndrome.

This chapter traces the intersecting clinical and laboratory histories of DiGeorge and VCF syndrome. As I have suggested throughout this dissertation, during the postwar period, clinicians and geneticists increasingly looked to the human genome, at the microscopically visible level of chromosomes, for new anatomical markers of disease. In previous chapters, I have examined the use of genomic markers for the nosological "splitting" off of unique clinical disorders. The converging histories of DiGeorge and

VCF syndrome instead, offer an equally valuable example of how visible chromosomal analysis has facilitated the categorical "lumping" of disorders as well. Indeed, in postwar biomedicine, nosological categories have, in some cases, been broken down, rebuilt, and even made more clinically variable, based on the association of existing disorders with particular genomic locations (McKusick, 1969).

**The Clinical Characterization of DiGeorge Syndrome**

In 1965, Angelo DiGeorge, an endocrinologist at St. Christopher's Hospital for Children in Philadelphia, commented on a series of three newborns he had recently observed who had been born without a thymus. The thymus is an organ located above the heart, which plays an important role in the development of the immune response in early infancy. DiGeorge noted that these children suffered from continuous infections due to their lack of a thymus, among other problems, and died within the first two years of life (DiGeorge, 1965; 1968). The infants had initially come to the attention of clinicians because they also lacked or had under-developed parathyroid glands, which play a key role in calcium regulation in the body. Absence of the thymus was actually only recognized in these three patients upon autopsy. Indeed, as DiGeorge (1965) points out, the finding that infants born with under-developed parathyroid glands also lack a thymus is not all that surprising given that these two organs develop from the same primordial structures, known as the 3$^{rd}$ and 4$^{th}$ pharyngeal pouches (Kretschmer et al, 1968).

Two years later, DiGeorge and two of his colleagues in Philadelphia reported on a similarly affected child. This child suffered from multiple infections due to an inadequate immune response, and died at 17 months. As DiGeorge anticipated, upon autopsy, the child was found to lack a thymus, as well as parathyroid glands. At the time, there was no treatment for this condition. Indeed, DiGeorge was largely interested in these children from a research perspective: they offered a unique opportunity to study the nature of the human immune response (Lischner et al, 1967). The affected children also had significant cardiac problems, due to occasional inborn heart defects, and because their under-developed parathyroid glands were not able to regulate calcium levels, thus disrupting proper heart function (DiGeorge, 1968).

DiGeorge inferred that these symptoms taken together likely represented a unique clinical disorder. Whether or not this disorder was of genetic origin remained unclear. A similar clinical presentation had previously been seen in mice in which it was inherited in a recessive manner. However, none of the siblings or relatives of affected patients were known to have similar clinical outcomes, suggesting that the disease occurred sporadically in humans. By 1968, congenital lack of the thymus, and its clinical effects, was already been referred to by some clinicians as 'DiGeorge syndrome', following the suggestion of immunologist Robert Good. DiGeorge himself "demurred" though, noting that such cases had previously been reported, as early as the first half of the 19$^{th}$ century (DiGeorge, 1968; Lammer and Opitz, 1986, 114). The term DiGeorge syndrome, nonetheless, was widely adopted by the clinical community soon thereafter (Kretschmer et al, 1968; Dodson et al, 1969; Harvey et al, 1970).

Many additional patients who appeared to be affected by DiGeorge syndrome were identified in the published medical literature during the late-1960s and early-1970s. These individuals showed previously unreported symptoms including seizures, palate abnormalities, and developmental delay, as well as heart defects or murmurs (Kretschmer et al, 1968; Dodson et al, 1969, Harvey et al, 1970, Freedom et al, 1972). Among the few patients who lived past infancy, mild to moderate intellectual disability was seen (Conley et al, 1979). At the time, clinicians were interested in the potential to treat DiGeorge patients by using thymus tissue transplants to restore immune function. One group reported doing so, with apparent success, using human fetal thymus tissue in 1968 (Cleveland et al, 1968). Steele et al (1972) also attempted such a transplant, which seemed to be somewhat effective in restoring immune response. The long-term impact of this treatment however, remained unknown, because this patient died of pneumonia nine days later.

While thymus tissue transplants offered hope for DiGeorge patients, it was becoming increasingly apparent during the 1970s that heart defects were also a common cause of infant death in this population. Finley (1977) reported on seven DiGeorge patients, all but one of whom died in the first weeks of life due to cardiac problems. As these authors put it, "diagnosis of DiGeorge syndrome should be possible in the newborn. The important features are not, however, related to immune deficiency, but rather to severe congenital cardiovascular disease" (Finley, 1977, 637). While clinicians continued to consider an underdeveloped or absent thymus to be the most important anatomical marker of DiGeorge syndrome during the 1970s, the majority of DiGeorge

patients at this time first came to medical attention, and were most likely to die form, congenital cardiac problems (Conley et al, 1979). Indeed, two prominent, and deadly, features defined the morbid anatomy of DiGeorge syndrome in the 1970s. As I describe in the next section, during the 1980s, additional anatomical features came to be associated with DiGeorge syndrome. Perhaps most important among these were visible cytogenetic aberrations, often involving chromosome 22. Based on this chromosomal marker, DiGeorge syndrome was associated with a particular chromosomal 'address', and was listed in the earliest editions of Victor McKusick's 'morbid anatomy' of the human genome (McKusick, 1982).

**Cytogenetic Analysis of DiGeorge Syndrome**

Medical geneticist Albert de la Chapelle and his Finnish colleagues reported in 1981 on an extended family affected by DiGeorge syndrome. Four children of one father, and a cousin of theirs on the same side of the family, had died of DiGeorge syndrome as infants. The adult brother and sister, who were separately the parents of these affected children, were both found to possess the same balanced translocation involving chromosomes 20 and 22. The balanced translocation in these siblings was passed down in an unbalanced manner, meaning that some genetic material was lost or gained among individuals the next generation who were affected by DiGeorge syndrome. Each of the children in this family who died of DiGeorge syndrome had a similar unbalanced translocation, while healthy family members either had a balanced translocation or normal chromosomes. The chromosomal rearrangement could not be

traced back further however, because older family members refused to provide tissue samples for this study, citing religious reasons (de la Chapelle et al, 1981).

The unbalanced translocation seen in affected family members caused a partial trisomy of chromosome 20 and a partial monosomy of chromosome 22. This included the deletion of the entire short arm of chromosome 22, as well as a portion of the long arm (de la Chapelle et al, 1981). The clinical effects of trisomy 20 had previously been described by Francke (1977), and did not resemble those of DiGeorge syndrome. From this, it was inferred that the deletion of a portion of chromosome 22 was more likely to play a causative role in the disorder. Existing evidence on such abnormalities was mixed however, with a previous report of one patient with monosomy 22 appearing to show DiGeorge syndrome features (Rosenthal et al, 1972), while another patient lacking one copy of chromosome 22 did not (DeCicco et al, 1973).

Despite these confusing previous findings, de la Chapelle et al (1981) theorized that DiGeorge syndrome in these cases was most likely associated with the deleted portion of chromosome 22 in the patients his group had described. Previous individuals who lacked the short arm of chromosome 22 had not shown similar clinical characteristics. Therefore, de la Chapelle and colleagues pointed to the deleted portion on the long arm of chromosome 22, noting, "the most likely location of the gene is at 22q11" (de la Chapelle et al, 1981, 255). These researchers however, could only account for the familial patients that they had studied. DiGeorge syndrome was known to occur both sporadically and in families, but no consistent chromosomal anomalies had previously been reported (Steele et al, 1972; Raatikka et al, 1981).

Summing up their report, the authors admitted, "As of yet we have no clue to the nature of the postulated gene. Another point that remains to be clarified is the part played by the remaining gene on the structurally normal chromosome 22 in our cases" (de la Chapelle et al, 1981, 255). Indeed, the idea that the loss of one copy of one particular gene played an etiological role in DiGeorge syndrome was purely hypothetical at this time. These clinicians could only see that their DiGeorge patients were missing a rather large portion of chromosome 22. How this visible anatomical aberration impacted the genome was a point of speculation.

The next year, clinical geneticists in Philadelphia identified an additional DiGeorge patient with chromosome 22 abnormalities. As Beverly Emanuel, a Ph.D. geneticist at the Children's Hospital of Philadelphia and University of Pennsylvania, described it to me,

> In that family [described by de la Chapelle], the children who wound up missing the proximal part of chromosome 22 all had DiGeorge syndrome, and it was almost like an 'ah ha ' moment. We have a child here [in Philadelphia] missing part of 22, who has DiGeorge, and they have a family, with three affected, missing part of 22, who have DiGeorge (Interview with Beverly Emanuel, November 9, 2011).

Clinicians at the Children's Hospital of Philadelphia immediately contacted DiGeorge and cytogenetist Hope Punnett across town at St. Christopher's Hospital to ask if they

had seen any similar chromosomal abnormalities in their own DiGeorge patients. As Children's Hospital of Philadelphia clinical geneticist Elaine Zackai described it, "We went back to DiGeorge [and asked], 'do you have any others that have chromosome abnormalities?' 'Yes' [he said]. Sure enough, it was the 22" (Interview with Elaine Zackai, November 10, 2011).

The case identified at the Children's Hospital of Philadelphia, along with two described by DiGeorge himself, were published in the *Journal of Pediatrics* in August 1982. These three unrelated DiGeorge patients were each found to have lost the all of the short arm of chromosome 22, as well as the 22q11 region on the long arm, due to translocations involving 22 and another chromosome (3, 10, and 20). Based on their own results, these authors agreed with de la Chapelle et al (1981) that the deletion of 22q11 was most likely involved in the clinical onset of DiGeorge syndrome (which the Philadelphia researchers referred to as 'DiGeorge anomalad', a difference in naming that I take up in the next section). Referring to the identification of a chromosomal deletion associated with Prader-Willi syndrome a year earlier (discussed in chapter 3), the researchers noted the possibility that DiGeorge syndrome may also be caused by a small deletion that could be made visible using high-resolution cytogenetics. Of more immediate importance, in two of the cases reported, the unbalanced translocation in affected children seemed to result from a balanced translocation in one of the parents. This suggested that there was a significant recurrence risk for DiGeorge syndrome in these families (Kelley et al, 1982).

An addendum to the Kelley et al (1982) report identified an additional family impacted by DiGeorge syndrome due to translocations involving chromosomes 22 and 4. A full report on this family was submitted the next year, and subsequently published in 1984. A sibling of this affected patient had previously died in infancy due to congenital heart problems, and was later found to lack a thymus as well. Chromosomal studies however, had not been performed. The authors even inferred that the mother likely had a mild form of DiGeorge syndrome herself, as her immune cell count was low, and because she showed the same unbalanced translocation as her affected son. Indeed, it was becoming clear that the same visible abnormality of chromosome 22 could lead to a wide range of clinical outcomes (Greenberg et al, 1984).

The extent and specifics of this potential causative link between chromosome 22 aberrations and DiGeorge syndrome remained unclear throughout the 1980s. Multiple cases where reported during this time in which, among family members who all appeared to have the same visible chromosome 22 translocation, only some were clinically affected with DiGeorge syndrome (Augusseau et al, 1986; Bowen et al, 1986). In addition, DiGeorge-like symptoms were also identified in a few patients with other visible abnormalities, such as deletions on chromosomes 10 and 18 (Greenberg et al, 1988). As Children's Hospital of Philadelphia genetic counselor Donna McDonald-McGinn summed it up to me, "They did chromosomes [on DiGeorge patients] and in 25% they could see a visible piece [of chromosome 22] missing. But from 1982 to 1992, they didn't know what to do with the other 75%" (Interview with Donna McDonald-McGinn, November 10, 2011). Indeed, like with the history of Prader-Willi syndrome described in

chapter 3, similar chromosomal abnormalities appeared frequently enough in DiGeorge patients to suggest a genomic location for the disorder. However, their visual presence or absence alone could not be relied upon diagnostically.

**Searching for the 'DiGeorge Gene(s)'**

How were chromosome 22 abnormalities mechanistically linked to DiGeorge syndrome? As Emanuel explained it to me, "At the time, and this is kind of interesting, the thinking in the field was, somewhere on 22 there's a DiGeorge gene, and probably the patients with the translocation were just putting us into the right region. The idea was they all involve 22. So, missing the 'DiGeorge gene', on chromosome 22 was going to give you the syndrome" (Interview with Beverley Emanuel, November 9, 2011). How the DiGeorge gene was actually disrupted in patients with chromosome 22 translocations, and why this led to such a range of clinical outcomes remained unclear. Nonetheless, the chromosomal band 22q11 had been widely adopted as the genomic address where the cause of DiGeorge syndrome resided.

In 1984, researcher Frank Greenberg and colleagues suggested that, "some cases of DiGeorge syndrome might have an interstitial deletion of 22q11, a situation analogous to that of Prader-Willi syndrome, and interstitial deletions of 15q11-12" (Greenberg et al, 1984, 318). Indeed, the 15q deletion associated with Prader-Willi syndrome identified by Ledbetter et al (1981) was seen as a potential model for other genetic disorders that were associated with chromosomal translocations, and thereby might be caused by small deletions. The authors however, admitted that even if an interstitial 22q11 deletion did

exist in many or all DiGeorge patients, it might well be too small to identify microscopically on banded chromosomes, even with the use of new high-resolution cytogenetic techniques. To this point, Greenberg and colleagues (including David Ledbetter) had never seen such an interstitial deletion in DiGeorge patients (Greenberg et al, 1984), nor had any other DiGeorge researchers (Rohn et al, 1984).

Clinical geneticist Roy Schmickel, of the University of Pennsylvania, included DiGeorge and Prader-Willi syndrome among what he called 'contiguous gene syndromes' in a 1986 publication in the *Journal of Pediatrics*. Contiguous gene syndromes are associated with a broad and variant array of clinical outcomes. Schmickel suggested that this might be the result of multiple adjacent genes being deleted in one genomic region. The size of the deletion in this region, and thereby the number of genes lost, suggested Schmickel, might be the basis for clinical variation. As Schmickel notes in his discussion, "these genes many be quite independent and no more related than apples and Appalachian Mountains; the loss of an encyclopedia page could remove both entries. The organization of genes may be as arbitrary as that of words" (Schmickel, 1986, 236). As a result, the loss of multiple genes in a genomic deletion may have wide ranging effects on the body, and thereby cause a diverse array of clinical outcomes.

Schmickel was a mentor of Emanuel at the University of Pennsylvania, and encouraged her to study the chromosomal basis of DiGeorge syndrome, "Roy Schmikel said, 'Bev, you know, you ought to study DiGeorge syndrome, I think there's something there' . . . And I said, 'but the reality is, so many of these kids with DiGeorge die in the neonatal period, how are we going to study them?' and he said, 'I think you're just going

to find out that it is really interesting' (Interview with Beverly Emanuel, November 9, 2011). As Emanuel noted, the natural history of DiGeorge syndrome remained difficult to define in the 1980s, since few affected individuals older than childhood were known. Indeed, only recently had surgical interventions for heart defects succeeded in allowing most patients to survive infancy. As Schmickel and Emanuel clearly recognized however, DiGeorge syndrome offered a unique opportunity to study the genomic impact of multiple genes being lost due to a deletion.

As Schmikel wrote his 1986 piece on contiguous gene syndromes however, the prevalence of 22q deletions in DiGeorge patients remained unclear. In 1988 Greenberg reported on the use of high-resolution chromosomal analysis in 27 DiGeorge patients. Chromosomal abnormalities were identified in five, including a visible 22q11 interstitial deletion in one. This was the first report of a visible cytogenetic deletion in a DiGeorge patient, which was seen without a chromosomal translocation. The parents of this individual showed no such deletion, suggesting that it occurred spontaneously during the reproductive process, on the maternally inherited copy of chromosome 22 (Greenberg et al, 1988). A second report of a microscopically visible 22q11 deletion in a DiGeorge patient came the next year (Mascarello et al, 1989).

The lack of visibly identifiable 22q11 deletions in DiGeorge patients suggested that most of these aberrations, if they existed, were likely too small to see, even using high-resolution cytogenetic techniques. During the late-1980s, molecular techniques began to be applied to the identification of such deletions. In a 1987 abstract conference abstract printed in the *American Journal of Human Genetics*, Emanuel and her colleague

Wendy Fibison, identified a molecular DNA probe specific to the 22q11 region that did not bind one copy of chromosome 22 in DiGeorge patients, but bound to both copies in normal individuals. This finding suggested that the molecular probe was specific to the DiGeorge deleted region (Fibison and Emanuel, 1987).

From here, additional molecular studies conducted over next five years helped to better define the size and prevalence of the 22q11 deletion in DiGeorge patients. The impacts of molecular analysis will be further discussed later in this chapter. Before returning to this narrative however, the next section explores broader discussions about the clinical nature and categorization of this disorder, which was named after DiGeorge and associated with chromosome 22 abnormalities. In addition, I describe the parallel clinical history of VCF syndrome, during the years before it was genetically linked to DiGeorge 'syndrome'.

**DiGeorge 'Syndrome'?**

Throughout the 1980s, clinicians and researchers raised various questions about the disorder named after DiGeorge. Was it a syndrome, or something else? Definitions of what defines a syndrome vary. Medical geneticist John M. Opitz and colleagues lamented in a 1979 account of the history of the term 'syndrome' that, "Through indiscriminate use, lately also by sociologists and political commentators, the word 'syndrome' has become so debased that few know how to use it correctly anymore" (Opitz et al, 1979, 98). Clinician and geneticist Kurt Hirschhorn has suggested to me that identifying syndromes is sometimes, "Tricky business," noting that, "The conception of a

syndrome is simply that, once it's described, you have the possibility of recognizing it and accepting the fact that the same syndrome can have manifestations that are absent or present" (Interview with Kurt Hirschhorn, January 26, 2012). Indeed, as Opitz et al (1979) note, ontological thinking is central to how clinicians conceive syndromes: the expression of syndromes may be variable within individuals, but nonetheless a syndrome is a real world entity, that can be delineated and diagnosed.

In a letter to the editor of the *Journal of Pediatrics*, medical geneticist John Carey called into question the designation of DiGeorge as a 'syndrome' (Carey, 1980). Noting that the disorder was regarded instead as an 'anomalad' in the 1976 edition of Smith's *Recognizable Patterns of Human Malformation* (Smith, 1976, 374), Carey suggested that its associated clinical features may represent a discrete developmental defect, rather than a syndrome. Carey offered the term 'DiGeorge malformation complex', to describe a set of related embryological defects that may be features of many different syndromes. He went on to note that, if additional expressions, such as abnormal facial features, were seen along the with heart and immune system defects associated with the DiGeorge 'malformation complex', then these visual characteristics taken together may constitute a DiGeorge 'syndrome'. In fact, the 2006 edition of Smith's *Recognizable Patterns of Human Malformation* has separate entries for the DiGeorge 'sequence' or 'malformation pattern', and what some call DiGeorge 'syndrome' (Jones, 2006, 298, 714).

During the 1980s, some clinicians and researchers used the terms DiGeorge 'malformation complex' and the DiGeorge 'anomalad' in their published papers (Kelley et al, 1982; Keppen et al, 1988). Goldberg et al (1985), perhaps in following with the

1982 edition of Smith's *Recognizable Patters of Human Malformation* (Smith, 1982, 470), referred to the disorder as 'DiGeorge sequence', as did Swiss medical geneticist Albert Schinzel in a 1988 paper on chromosomal syndromes. As Schinzel put it, "The DiGeorge sequence is a localised defect of development and therefore not a syndrome. It can occur as an isolated defect or as a component of a variety of syndromes" (Schinzel, 1988, 458).

A sequence is defined as a chain of physiological abnormalities resulting from one primary defect, which leads to a number of secondary and tertiary effects. In the case of DiGeorge syndrome the primary defect occurs during the embryonic development of the 3$^{rd}$ and 4$^{th}$ pharyngeal pouches and the 4$^{th}$ branchial arch, which develop into the parathyroid, thymus, and heart. On the other hand, a syndrome involves a number of symptoms that occur together, and usually have a common etiology, but are not the result of a chain reaction of events (Cohen, 1982). As a result, sequences are localized disorders (Schinzel, 1988), while syndromes more universally affect the body.

In a 1986 paper, Developmental specialist Edward J. Lammer, writing with Opitz, referred to this disorder as the 'DiGeorge anomaly' saying, "without detracting in the slightest from [Angelo] DiGeorge's discovery, a change in our conception of the condition is necessary because this so-called syndrome is not an etiologically unique 'syndrome' at all, but rather a causally non-specific and heterogeneous complex polytropic developmental field defect" (Lammer and Opitz, 1986, 115). By calling the disorder a 'developmental field defect' the authors implied that DiGeorge is associated with "a group of embryonic cells and primordial that share some morphogenic property

that causes them to develop abnormally together" (Lammer and Opitz, 1986, 116). The DiGeorge 'anomaly' is caused by an embryonic abnormality, Lammer and Opitz note, which may have various etiologies. Indeed, the disorder was associated with aberrations on chromosomes 1, 8, 10, and 22, and also linked to fetal alcohol exposure.

During the 1980s then, while many clinicians were seeking to associate DiGeorge syndrome with a specific chromosomal abnormality, multiple others doubted its status as a 'true' syndrome (Opitz et al, 1979) with a clear etiological cause. While translocations involving the long arm of chromosome 22, and even a small interstitial deletion at 22q11 (Greenberg et al, 1988; Mascarello et al, 1989) were considered to be relevant genomic markers associated with this disorder, in the majority of cases no chromosomal aberration was seen. The cause of DiGeorge syndrome remained unclear, as did its nosological status as a clinical syndrome. During the 1990s, the identification of a genetic link between DiGeorge and a previously distinct clinical disorder, VCF syndrome, would both further clarify and confuse its diagnosis and classification.

**The Clinical Identification of Velo-cardio-facial Syndrome**

In 1978, Robert Shprintzen and colleagues at the Center for Cranio-Facial Disorders of Montefiore Hospital in New York City reported on what they believed to be a newly identified clinical syndrome. Ten school-age children and two newborns had been referred to the center due to various palate problems, and showed "very similar patterns of symptoms" (Shprintzen et al, 1978, 56). Elaborating on these similarities, the authors noted, "Perhaps the most striking feature of these patients was the similar facies

of all twelve" (Shprintzen et al, 1978, 57). The children all had large, wide noses, flattened cheeks, narrow eyes, and a long face, among other features. Nine of the twelve patients had heart defects, some had been surgically corrected, eleven showed learning disabilities (the twelfth was an infant with developmental delay). IQ scores suggested borderline to mild intellectual disability (Shprintzen et al, 1978).

The most noticeable similarities among these patients were oral and nasal (velopharyngeal) abnormalities, heart defects, and distinct facial characteristics, which led to the name Velo-cardio-facial (VCF) syndrome for this disorder. Another syndrome had previously been called Cardio-facial syndrome (Yurchak and Fallon, 1976), characterized by 'elfin' facial features, heart defects, and intellectual disabilities. However, the authors of this paper suggest that the mouth and nasal features as well as the facial characteristics of patients with VCF syndrome were distinct from those with Cadrio-facial syndrome (Shprintzen et al, 1978).

The cause of VCF syndrome remained unclear at this time. There did not seem to be an environmental or genetic common denominator among all twelve patients. Among them however, there was one instance of familial transmission, with the mother and sibling of a patient showing similar clinical effects (Shprintzen et al, 1978). A 1981 follow-up report accounted for 39 patients with VCF syndrome. Most had been referred to clinicians because of their hypernasal speech, which is associated with palate clefting. Among these patients, four instances of familial transmission of VCF syndrome were noted. This suggested that the syndrome might be inherited in a Mendelian dominant

manner. Chromosomal analysis performed on these familial patients however, showed no visible abnormalities (Shprintzen et al, 1981).

In 1985, a conference abstract published in the journal *Clinical Genetics* by the Shprintzen group noted a phenotypic overlap between the DiGeorge 'sequence' and VCF syndrome. A patient diagnosed as having VCF syndrome suffered multiple infections, and was found to have the type of immune dysfunction common in DiGeorge patients. Upon review of other VCF syndrome patients, it was found that many were judged by their parents to have frequent infections, while others were shown to have specific immune dysfunction. In addition, clinically diagnosed DiGeorge patients were seen to share the 'look' of facial features similar to those affected by VCF syndrome. The disorders were also associated with the same developmental defect of the third and fourth branchial arches (Goldberg et al, 1985, A54).

Based on four instances of familial transmission, among 39 patients reported in early-1981, VCF syndrome was regarded by Shprintzen and colleagues as likely following an autosomal dominant inheritance pattern (Shprintzen et al, 1981). The 1982 edition of Smith's *Recognizable Patterns of Human Malformation*, lists this disorder as Shprintzen syndrome, and notes that it is a "Probable autosomal dominant" (Smith, 1982, 194). Familial transmission of DiGeorge syndrome had been noted previous in a number of cases, though the inheritance pattern remained unclear in the early-1980s (de la Chapelle et al, 1981; Raatikka et al, 1981). A 1984 paper, published in the *Journal of Pediatrics*, by researchers from Norfolk, Virginia and Philadelphia, reported on a family

that showed an autosomal dominant inheritance pattern for DiGeorge syndrome, with no visible chromosomal abnormalities (Rohn et al, 1984).

Among other similarities, this genetic inheritance pattern further strengthened suspicions that DiGeorge and VCF syndromes were related. As the Shprintzen group noted in their 1985 conference abstract, "VCF should be considered in any familial instance of DGS [DiGeorge sequence]" (Goldberg et al, 1985, A54). While the genomic cause of inherited DiGeorge in VCF syndromes remained unclear at this time, the presence of abnormalities in the chromosomal band 22q11, identified in a number of DiGeorge patients using observational cytogenetic analysis, suggested a viable starting place for molecular studies seeking to identify a genetic etiology.

**Molecular Analysis of DiGeorge Syndrome Patients**

In 1991, Peter Scambler, and colleagues in London and Stockholm, began to use molecular probes to examine DiGeorge patients whose chromosomes appeared normal under the microscope. A report of this research, published in the journal *Genomics* in 1991, noted that five of six individuals examined had submicroscopic deletions in the 22q11 region. Among these patients however, there was a wide variety of clinical outcomes, from mild to severe cases of DiGeorge syndrome. There did not seem to be a correlation between the size of the 22q11 deletion and the severity of the clinical outcome. The researchers therefore hypothesized that DiGeorge syndrome was likely caused by the loss of just one gene, common to all of the deletions found (Scambler et al,

1991), instead of being a 'contiguous gene syndrome', associated with the disruptions of multiple genes in the 22q11 region (Schmickel, 1986).

The next year, Deborah Driscoll and colleagues at the University of Pennsylvania, identified a 22q11 deletion in each of 14 DiGeorge patients tested using molecular probes. Five of these patients had microscopically visible 22q11 deletions as well. The deletions were determined to be both maternally and paternally inherited, ruling out instances of imprinting, as had been identified in the cases of Prader-Willi and Angelman syndromes. These findings provided further evidence that a 22q11 deletion was the etiological cause of DiGeorge syndrome in most clinical cases. The genomic deletions identified all involved the loss of at least 500,000 DNA base pairs (a large deletion on the molecular level, but much too small to be seen microscopically), suggesting that multiple genes were impacted (Driscoll et al, 1992).

Familiar with previous reports about the clinical overlaps between DiGeorge and VCF syndromes, Driscoll and Emanuel were interested in using their molecular probes to scan for the 22q11 deletion in patients diagnosed with the latter syndrome as well. As Emanuel recounted to me, "We were very eager to figure out whether there was a connection, because we thought there probably was, and we talked to people in our own cleft clinic here and at CHOP [Children's Hospital of Philadelphia] and asked, 'have you ever seen any of those patients here with VCFS?' . . . We made an arrangement to go into the cleft clinic to see if we could detect any of these, VCFS patients, and sure enough they were there" (Interview with Beverly Emanuel, November 9, 2011). Similarly, Driscoll told me that, once they were able to identify patients in the cleft clinic who were

likely affected with VCF syndrome, and test them for the 22q11 deletion, the link to DiGeorge syndrome became obvious, "We found that it is really essentially one and the same: it is the same disorder" (Interview with Deborah Driscoll, November 29, 2011).

Based on this additional research, near the end of their report, Driscoll and colleagues note, "Our observation of deletion of loci from within the DGCR [DiGeorge Critical Region] in several patients with velo-cardio-facial (Shprintzen) syndrome (authors' unpublished results) may explain the overlapping phenotypic features observed in DGS [DiGeorge syndrome] and velo-cardio-facial syndrome" (Driscoll et al, 1992, 931). This was the first time that VCF syndrome had been associated with a specific chromosomal abnormality or genomic location. Clearly, this genetic link expanded the awareness of DiGeorge and VCF syndrome among cleft palate clinics, while also creating the opportunity to improve laboratory diagnosis. Beyond this, Driscoll and colleagues hypothesized that further work might reveal the basis of the differential clinical outcomes associated with these historically distinct syndromes (Driscoll et al, 1992).

Peter Scambler and David Kelly in London reported similar findings in a *Lancet* article also published in 1992: deletions in the 22q11 region were found in five additional VCF syndrome patients. These authors interpreted their findings as evidence that DiGeorge and VCF syndromes were indeed etiologically related (Scambler and Kelly, 1992). A larger follow-up the next year by Driscoll and colleagues analyzed 76 patients diagnosed with either DiGeorge or VCF syndrome. Including their previous results, Driscoll et al (1993) reported that the 22q11 deletion could be identified molecularly in

88% of DiGeorge patients and 76% of VCF patients. Many of these individuals were referred by outside clinicians, suggesting that diagnoses may not have been consistent: some of these patients may not have been affected by DiGeorge or VCF syndrome. In this latter study, the Driscoll and colleagues used a new laboratory technique: fluorescence in situ hybridization (FISH). FISH uses fluorescently labeled DNA probes instead of radioactive ones, which allow for easier, quicker, and safer laboratory analysis.

Also in 1993, another paper from Scambler and Kelly, this time with Shprintzen and Rosalie Goldberg as co-authors, reported on 12 additional VCF syndrome patients who each possessed the 22q11 deletion. These clinicians noted that the deletions molecularly identified in both DiGeorge and VCF syndrome patients were very closely linked to one another, and indeed might be identical. It seemed as if the physical genomic deletion itself was not directly responsible for the somewhat different clinical manifestations associated with these two disorders. Rather the two syndromes, "Could be part of a spectrum of abnormalities which many be caused by monosomy [the deletion of one genomic copy of] 22q11. Chance events during morphogenesis could be responsible for much of the difference in phenotypes [clinical outcomes]" (Kelly et al, 1993, 311). Indeed, the molecular analysis of increasing numbers of patients suggested that DiGeorge and VCF syndrome were each associated with the exact same, or at least closely overlapping, genomic abnormality on chromosome 22.

In a retrospective look at the diagnosis of these two disorders, Greenberg said of the common deletion found in many DiGeorge and VCF syndrome patients, "This suggests that the two disorders represent a spectrum of the same gene defect . . . patients

with suspected or confirmed DGA [DiGeorge anomaly] should be evaluated for features of VCFS" (Greenberg, 1993, 806). Indeed, with the identification of 22q11 deletions in patients diagnosed with each disorder, clinicians had identified yet another visible bodily marker shared by DiGeorge and VCF syndromes. And, since this was a chromosomal marker, it was understood to have potential etiological and diagnostic implications. By the mid-1990s, based on their common genomic location and aberration, DiGeorge and VCF syndromes were increasingly understood as being two historically distinct forms of one genetic syndrome.

**Bringing Together DiGeorge and VCF Syndrome**

As noted at the beginning of this chapter, the association of DiGeorge and VCF syndrome with the same genomic location, designated by the chromosome band 22q11, is in many ways similar to the overlap between Prader-Willi and Angelman syndromes described in chapter three and elsewhere (Hogan, 2013). In the historical case of Prader-Willi and Angelman syndromes, clinicians immediately discounted the hypothesis that the two disorders were one and the same. As I have described so far in this chapter, the historical trajectory of DiGeorge and VCF syndromes was quite the opposite. Indeed, clinicians took the common 22q11 deletion as definitive evidence that the two disorders were in fact historically distinct forms of one clinical syndrome. However, while the etiological sameness of these two disorders was widely accepted in the mid-1990s, discussions and disagreements were ongoing (and continue, as Navon and Shwed (2012) have recently described) concerning what to name this, now joint, disorder.

One early designation came from Wilson et al (1993), who suggested the acronym CATCH-22 syndrome. The name was meant to remind clinicians of the main features of DiGeorge and VCF syndromes: Cardiac defects, Abnormal facial features, lack of or an underdeveloped Thymus, Cleft palate, and Hypocalcemia (low calcium levels due to underdeveloped parathyroids), all associated with chromosome 22 abnormalities. The authors of this paper went on to note, "We think that these conditions are all part of one clinical spectrum and the diagnostic label depends upon the age of presentation and the predominant clinical manifestation" (Wilson et al, 1993, 865). Indeed, DiGeorge syndrome tended to be diagnosed primarily in infants with heart defects and immune deficiency, whereas VCF syndrome was most often identified in school age children with distinct speech anomalies and learning disabilities. The goal of the term CATCH-22 was not to replace these two historical designations, but to bring them together under one clinical and diagnostic heading (Wilson et al, 1993).

Clinician Judith Hall commented at this time that the designation CATCH-22 would prove helpful for remembering what symptoms tend to occur together with the 22q11 deletion. She also suggested, "CATCH 22 is a wonderful model for what is to come over the next 10 years of human genome work" (Hall, 1993, 802). Indeed, Hall recognized that this complicated situation, in which multiple clinically defined disorders were found to be associated with the same genomic location and aberration, was likely to become increasingly common in the coming years. Such situations would force clinical researchers to consider more complex explanations for why and how the same genomic

defect can produce a range of clinical outcomes. This would include various gene interactions, environmental inputs, and epigenetic effects (Hall, 1993).

A collaboration of clinical geneticists from the US and Australia, including Driscoll and Emanuel, responded to the suggestion of CATCH-22 in a letter to the editor of the *Journal of Medical Genetics*. These researchers pointed out that the designation CATCH-22 could be misleading for families, since those impacted by the 22q11 deletion often did not show all of the clinical features captured by the acronym. This was particularly relevant in familial cases where the diagnosis of a child with a mild form of the disorder also served as an indicator that future siblings could be more severely affected. If such a diagnosis was missed, that warning would be lost as well (Lipson et al, 1994). Indeed, this problem is central to why syndrome delineation and diagnosis is such, "Tricky business" (Interview with Kurt Hirschhorn, January 26, 2011). While a syndrome in general may be associated with a large set of outcomes, and an affected individual may only be impacted by some symptoms, and thus overlooked.

Julie Leana-Cox and colleagues at the University of Maryland also opposed the use of 'CATCH-22', but for a different reason. These clinicians saw the term as having negative connotations due to its association with the 1962 Joseph Heller book of the same name. Similar to the instance of 'Happy Puppet syndrome' (described in chapter 3), CATCH-22 was seen as, "inappropriate for use when counseling family members" (Leana-Cox et al, 1996, 315). Rather than CATCH-22 syndrome, Leana-Cox and colleagues supported simply combining the disorder's two historically distinct designations: DiGeorge/VCF syndrome, "It calls attention to the phenotypic spectrum

using historically familiar names" (Leana-Cox et al, 1996, 315). Still other clinicians during the mid-1990s suggested naming the syndrome after its common, and newly defining, genomic feature: the (sometimes microscopically visible) 22q11 deletion (McDonald-McGinn, 1997). For their part, the Leana-Cox group liked that the name '22q11 deletion syndrome' was neutral, when compared to the pejorative 'CATCH-22 syndrome'. Nonetheless, they opposed the designation because it failed to communicate any of the common the clinical features of the disorder (Leana-Cox et al, 1996; Wulfsberg et al, 1996).

**Just One Elephant in the Room?**

In a second 1996 paper, also published in the *American Journal of Medical Genetics,* Leana-Cox, writing with Eric Wulfsberg and an Italian colleague, once again addressed the issue of naming, this time making reference to the parable of the blind men and elephant. The parable, it turns out, comes up relatively often in the medical literature when clinicians make the argument to their colleagues that two previously distinct disorders should instead be thought of as one (Hirschhorn, 1975; Kassirer, 1986; Tobin; 1987). In this classic story, multiple blind men are asked to describe the characteristics of an elephant, based on touching it alone. Each man focuses on just one portion of the elephant, leading one to claim that an elephant is like a wall, and another to compare it to a fan, and a third to think of an elephant as tree-like. Being blind, none of the men can see the elephant for what it truly is, as one continuous whole. Instead, they continuously argue about the elephant's defining features, based on their own limited experience.

Using this parable, Leana-Cox and Wulfsberg suggested that a similar situation had played out over the clinical history of DiGeorge and VCF syndromes. Due to their own specialties and patient populations, clinicians had defined and diagnosed the same syndrome in different ways, and had failed to see that each of these designations was part of a greater whole. With the identification of the common 22q11 deletion in the early-1990s however, it became clear to clinicians that DiGeorge and VCF syndromes were indeed two historically distinct forms of just one genetic syndrome (Wulfsberg et al, 1996). Driscoll later put it to me this way,

> I think what we realized, is that so much of genetics is based on your ascertainment bias [what population of patients you see] . . . you can't always define syndromes based on phenotype. We came to appreciate that they are so highly variable. When we thought about DiGeorge syndrome, we thought immune deficiency, hypocalcaemia, and heart defects: that's it. Many of these were so severe they never survived. Whereas here [in patients initially diagnosed with VCF syndrome] we have this much milder phenotype, with mostly learning difficulties and cleft palate, and those were how those children were ascertained. Maybe they had a heart defect. What we realized is really they were all one and the same, and it was kind of an 'a-ha' moment that you have (Interview with Deborah Driscoll, November 29, 2011).

Indeed, this 'a-ha' moment was akin to a number of formerly blinded clinicians suddenly being made to see: the common presence of the 22q11 deletion had brought them all together.

McDonald-McGinn and colleagues at the Children's Hospital of Philadelphia made a similar argument in an editorial response to the Wulfsberg and Leana-Cox paper. The researchers even provided their own drawn image of 'the blind men and the elephant' to make their point (Figure 9). The depiction shows multiple clinicians, all focused on different portions of the same elephant, and failing to appreciate its singular presence. The elephant in their drawing is also shown wearing a banner with the number 22 on it, designating the common genomic location and aberration that ties DiGeorge and VCF syndromes together. Indeed, just as Wulfsberg and Leana-Cox had argued, the presence of 22q11 chromosomal deletions in patients with both of these syndromes represented convincing evidence for clinicians that there had been just one 'elephant' in the room all along.

## Velo-cardio-facial as the One True Syndrome

While clinicians widely agreed in the mid-1990s that DiGeorge and VCF syndrome were two historically distinct forms of one clinical syndrome, they continue, to the present day in fact, to disagree about what it should be called (Navon and Shwed, 2012). As Shprintzen (1998, 5) suggested, paraphrasing Cohen (1982, 158), "Geneticists would rather share their toothbrushes than their terminology." Indeed, Shprintzen remains one of the primary holdouts in the effort to agree on a common name for these two syndromes. One grouping of clinicians have agreed upon 22q11 deletion syndrome,

while Shprintzen and others maintain that VCF syndrome, a name that Shprintzen and his colleagues coined in their 1978 paper, represents the most descriptive and accurate name for this disorder.


**Figure 9** Drawing referencing the relevance of the parable of the blind men and the elephant to the case of DiGeorge and VCF syndromes (McDonald-McGinn et al, 1997). Reprinted with permission from John Wiley & Sons, Inc.

In a 1994 letter to the editor of the *Journal of Medical Genetics*, Shprintzen also pointed to the parable blind men and the elephant, noting that previously DiGeorge was primarily diagnosed in patients initially presenting with heart defects, and VCF syndrome in individuals with facial and palate abnormalities. In line with other clinicians and researchers (Carey, 1980; Lammer and Opitz, 1986; Schinzel, 1988, Shprintzen (1994, 1998) did not consider DiGeorge to be itself a syndrome, and instead acknowledged only the existence of the DiGeorge 'sequence'. He went on to suggest that VCF syndrome had

been associated with over 40 clinical features, and among them were those attributed to the DiGeorge sequence.

As a result, according to Shprintzen (1994), DiGeorge sequence should be thought of as a possible outcome of VCF syndrome. Its etiology however, was heterogeneous: the DiGeorge sequence had been associated with multiple chromosomal abnormalities. In response to the finding of Driscoll et al (1993) that the 22q11 deletion was only seen in 76% of VCF syndrome cases, Shprintzen argued that any patient thought to have VCF syndrome, who did not have the 22q11 deletion, was clinically misdiagnosed. He also noted that Scambler and colleagues identified a 22q11 deletion in all of the VCF syndrome patients they tested (Kelly et al, 1993). As opposed to DiGeorge sequence argues Shprintzen, "There is simply no valid evidence to suggest that velocardiofacial syndrome is aetiologically heterogeneous" (Shprintzen, 1994, 424). As Shprintzen saw it, while the DiGeorge sequence had multiple causes, VCF was the one 'true' syndrome because it was *always* associated with the 22q11 chromosomal deletion. Pointing to a singular genetic etiology helped to establish the ontological status of VCF as a 'syndrome'.

In a 1998 paper titled "The Name Game," Shprintzen further explicates his position, distinguishing between the meaning and medical implications of sequences and syndromes, and providing arguments for why 'VCF syndrome' remained most appropriate for naming the clinical disorder associated with 22q11 deletions. First, Shprintzen noted that he an his colleagues, in their 1978 paper, were the first to suggest that VCF represented a newly delineated clinical syndrome, an argument that Angelo

DiGeorge had never made in his own publications during the late-1960s. Second, no earlier studies of syndromes with similar features as VCF syndrome fully addressed all of its clinical manifestations. Third, while the DiGeorge sequence had been found to be of heterogeneous origin, no other cause of VCF syndrome besides 22q11 deletions had ever been identified (Shprintzen, 1998). Taken together, argued Shprintzen, the term VCF syndrome had priority of in terms of timing, clinical scope, and etiological clarity.

Shprintzen has since presented still more arguments for the designation VCF syndrome. In a 2008 history of VCF syndrome, he noted that his chosen name is, "descriptive, geographically nonspecific, free of eponyms, and much easier to write and say than 22q11.2 deletion syndrome" (Shprintzen and Golding-Kushner, 2008, 16).[36] Shprintzen also notes in the same paragraph that most other syndromes associated with such genomic abnormalities are not named after their chromosomal designations. In a second 2008 publication, Shprintzen noted, "VCFS is simply easier to say and write and communicate than any other labels and its use should therefore be encouraged" (Shprintzen, 2008, 4). Shprintzen's arguments for his chosen name therefore extend beyond issues of priority, to a consideration of communicative simplicity.

Clearly the name VCF syndrome is important to Shprintzen in a way that DiGeorge syndrome never was to the clinician after whom it was named. While Shprintzen has dedicated his career to VCF syndrome research, Angelo DiGeorge rather quickly moved on to other interests. Shprintzen has published regularly on VCF syndrome, especially after it became associated with 22q11 in the early-1990s.

[36] 22q11.2 represents an additional level of cytogenetic specificity made possible by high-resolution analysis.

DiGeorge, on the other hand, who died in 2008, did not publish on DiGeorge syndrome again after 1969, though he did remain interested in keeping up with ongoing research concerning the disorder (Interview with Beverly Emanuel, November 9, 2011). Indeed, Shprintzen's professional identity is much more closely tied to VCF syndrome, the disorder he named in 1979, than DiGeorge's ever was in the disorder named after him.

Perhaps as a result, to this day, VCF syndrome advocates maintain their own website, hold separate annual research conferences, and have a specific research institute, the Velo-Cardio-Facial International Center located at the State University of New York's Upstate Medical University in Syracuse. At the same time, the 22q11.2 Deletion Syndrome Foundation also has its own website, annual conferences, and institutions, such as the 22q Center located in the Children's Hospital of Philadelphia. The 22q foundation has an ongoing "Same Name Campaign" aimed at bringing together patients, families, advocates, and clinicians impacted by or interested these historically distinct syndromes, under one name (Interview with Donna McDonald-McGinn, November 10, 2011).

I have no interest in taking a side in this debate. However, I do think that this ongoing difference of opinion over naming demonstrates that the identification of a common genomic location, in some cases, does not provide sufficient force to meld together the existing social networks and institutions built around two historically distinct syndromes. For the remainder of this chapter, I will refer to this disorder as DiGeorge/VCF syndrome, a name that captures its historical duality, but one that proponents from both the Velo-Cardio-Facial International Center and 22q11.2 Deletion Syndrome Foundation would likely oppose.

**A Gene for DiGeorge/VCF Syndrome?**

Just like most cases of Prader-Willi and Angelman syndrome, DiGeorge/VCF syndrome is associated with the deletion of genetic material, specifically from chromosome 22. Since humans have two copies of chromosome 22, the genes deleted in the 22q11 region among DiGeorge/VCF patients continue to exist on the non-deleted copy of the chromosome. Unlike Prader-Willi and Angelman syndromes however, the deleted region of 22q11 is not affected by genetic imprinting in any way: all genes in this region are believed to be functional on the remaining single copy present in DiGeorge/VCF syndrome patients with the deletion (Driscoll et al, 1992). Over 35 genes are present within the genomic area that is most frequently deleted in DiGeorge/VCF patients (Kobrynski and Sullivan, 2007). One of more of these genes may play a role in the clinical expression of the disorder when a copy of it is lost due to a 22q11 deletion.

During the late 1990s, a number of 'candidate genes' were identified in the 22q11 region, which could be associated with the DiGeorge/VCF syndrome etiology (Budarf and Emanuel, 1997; Lindsay and Baldini, 1998). Based on studies in mice, in which targeted segments of the 22q11 region were deleted, Elizabeth Lindsay of the Baylor College of Medicine, along with American and British colleagues, highlighted the gene Tbx1 in 2001 (Lindsay et al, 2001). Researchers from Columbia University in New York also published on Tbx1 the same month (Jerome et al, 2001). The loss of Tbx1 in mice seemed to cause heart abnormalities, suggesting that it might also play a significant role in the clinical outcomes of DiGeorge/VCF patients. Researchers continued to believe

however, that Tbx1 alone probably is not entirely responsible for the wide-ranging clinical expression of this disorder (Kobrynski and Sullivan, 2007).

Others have similarly suggested that the clinical expression of DiGeorge/VCF syndrome is too variable for it to be explained by one gene mutation alone (Schinke and Izumo, 2001). In 2003, Hisato Yagi and colleagues in Japan reported in the *Lancet* on five patients diagnosed with DiGeorge/VCF syndrome who did not have a 22q11 deletion, but did have a mutation specific to the Tbx1 gene. The clinical expression of these patients remained variable, but most showed the major features of this disorder, including distinctive heart, thymus, parathyroid, palate, and facial abnormalities. These patients however, did not show the mild intellectual disability normally associated with the disorder. The authors of this paper also proposed that the variability of expression among the five patients they studied suggested that additional environmental and developmental factors likely influence clinical outcomes (Yagi et al, 2003).

Indeed, going back to the mid-1990s, clinicians and geneticists have been aware that individuals with the same exact 22q11 deletion can have quite variant clinical outcomes. This has been made particularly apparent by the identification of identical twins with widely variable expression of DiGeorge/VCF syndrome. For instance, in 1995 Goodship, Scambler, and colleagues reported on twins who had the same 22q11 deletion, and showed some similar features of this disorder. However, only one twin had the heart defect characteristics of DiGeorge/VCF syndrome (Goodship et al, 1995). In 1998, Yamagishi and colleagues in Japan reported a similar case. In this instance, the identical twins both had the same 22q11 deletion, and each showed the distinct facial

features of the DiGeorge/VCF syndrome, but only one twin expressed other clinical indicators of the disorder, including a heart defect (Yamagishi et al, 1998). As clinical geneticist Eli Hatchwell noted in a letter to the editor responding the report by Goodship et al (1995), a number of genetic mechanisms might lead to such an outcome. For instance, the deletion of 22q11 from one chromosome may uncover recessive mutations present on the other copy of 22q11. It is also possible that a second abnormality, somewhere else in the genome, may represent a 'second hit' facilitating certain clinical outcomes (Hatchwell, 1996).

As Emanuel has described to me, there are a number of factors, such as the genomic background and chance events embryonic during development that may impact clinical outcomes. In addition, she noted that, "We don't know for example, and we're trying to pick away at it: what about the non-deleted allele? There are some 40 genes there. Are there particular forms of those genes that affect whether you do or you don't develop a heart defect or neuropsychiatric behavior differences, etc" (Interview with Beverly Emanuel, November 9, 2011). Emanuel is pointing here to the same issue that Hatchwell did previously: when an individual only has one copy of a particular gene because of a deletion, there is always the possibility that a mutation may be uncovered on the remaining chromosomal copy that will influence clinical outcomes. This also ties back in with the ongoing discussion over the extent to which DiGeorge/VCF syndrome is a 'contiguous gene syndrome': one in which a variety of impacted genes in one particular genomic region, 22q11, contribute to clinical expression.

While Tbx1 appears to play an important role in causing this disorder, it seems likely that other genes in this region are significant in at least some cases. Researchers generally agree that the size of the 22q11 deletion does not correlate with the severity of clinical outcomes (Carlson et al, 1990), but this does not discount the possibility that larger deletions at least increase the probability that other mutant gene effects could arise. Indeed, despite its association with the 22q11 deletion, DiGeorge/VCF syndrome continues to pose significant challenges when it comes to clinical, and in particular prenatal, diagnosis. In at least 10-15% of cases, children inherit the 22q11 deletion from one of their parents (in most cases the deletion occurs *de novo* during the reproductive process). Most of the parents passing down the mutation have had such mild effects that they were never diagnosed themselves, and did not need any clinical interventions. If they possess the deletion, a parent has a 50% chance of passing it on to each additional child they have. The severity of its clinical impact however, remains impossible to predict (Driscoll, 2001).

Indeed, there is no way to know another fetus found prenatally to have the 22q11 deletion will be more severely affected than their existing sibling, have an almost entirely normal life like their previously undiagnosed parent, or fall somewhere in between. This continued uncertainty greatly complicates the diagnostic process. As Emanuel put it to me,

> We have a lot to learn. The good news is that we understand a fair amount of the syndrome, i.e. we know it's due to the deletion. But the good news

> from the scientific perspective is that there are still many, many questions to ask in answer. And you can look at it from the reverse, the bad news is that we don't know the answer, but the good news is that someone is interested in finding the answers (Interview with Beverly Emanuel, November 9, 2011).

Indeed, while clinicians face many challenges when it comes to offering reliable and reproducible diagnoses, this uncertainty continues to offer researchers with interesting and potentially valuable questions and opportunities. Variations of the 22q11 deletion and genomic region found in patients diagnosed with DiGeorge/VCF syndrome, or not, offer additional opportunities to identify new genomic mutations and interactions that play a central role in the clinical expression of this disorder, as well as in human disease and biology more broadly.

**Conclusion**

In this chapter, I explore the clinical and laboratory history of two previously distinct disorders that have since come to be understood as one. As I demonstrate here, the 'look' of a particular clinical disease may be impacted by a variety of genetic, social, and institutional factors. Historical distinctions between DiGeorge and VCF syndrome seem to have resulted from the different medical specializations, and related patient populations, of those who first described the disorder. Angelo DiGeorge identified the syndrome, later named after him, based on his experience with severely impacted

newborns at a children's hospital, while Robert Shprintzen primarily saw school-aged patients who were brought to his clinic due to their speech and palate abnormalities. Indeed, before the two disorders were linked, the natural history of DiGeorge syndrome was widely assumed to end by age two, and that of VCF syndrome largely seemed to go unnoticed until age four or five.

Though the identification of the 22q11 deletion in both subsets of patients represented an important turning point in the history of DiGeorge/VCF syndrome, it was certainly not the first indication that these disorders were somehow linked. The openness to identifying a wide array of potential symptoms associated with VCF syndrome led the Shprintzen group to notice the impact of immune system abnormalities in their patients, and link this to existing accounts of DiGeorge patients. Based on this recognition, when a genomic deletion was found in most individuals diagnosed with DiGeorge syndrome in 1992, it seemed an obvious next step to also look for it in patients who showed signs of VCF syndrome, even if they were not previously diagnosed with this disorder. Ultimately, the 22q11 deletion was pointed to, by way of the parable of the blind men and the elephant, as proof that when it came to differentially diagnosing DiGeorge and VCF syndromes, there had always in fact been just one 'elephant' in the room.

The nosological status of the clinical disorder Angelo DiGeorge first identified – 'syndrome' or 'sequence' – has remained a point of contention. However, following the introduction of laboratory testing for the 22q11, it has been clearly established that individuals who have the 'look' described by DiGeorge, as well as this genomic aberration, are affected by a distinct syndrome. Indeed, while the clinical attributes of

DiGeorge/VCF syndrome in an individual, or their child, remains the primary indication for genetic testing, the genomic deletion itself has become so central to the diagnosis of this disease that its absence would almost certainly overturn a clinical diagnosis.

As Shprintzen has argued, if the 22q11 deletion is not present in a patient, then the clinical diagnosis of VCF syndrome was almost certainly incorrect (Shprintzen, 1994, 1998). And similarly, as McDonald-McGinn has told me, "It's the 22q, the only thing that everyone has in common is that deletion. Even if it's a smaller deletion it's the same thing" (Interview with Donna McDonald-McGinn, November 10, 2011). The move towards a more universal use of '22q11 deletion syndrome' instead of DiGeorge and VCF, only further re-enforces the clinical boundaries of this category. At least in terms of diagnosis, this genomic finding, of a 22q11 deletion, now seems to be privileged in the clinic over bodily expression.

This said, while clinicians and researchers have agreed for 15 years now that DiGeorge and VCF syndrome are the same clinical disorder, a universally agreed about upon name for this disease has not been reached. In this chapter, I have noted the various institutional and professional reasons why a common name is so difficult to establish. Organizations have been created, research dollars spent, and careers built around the historical names of these clinical disorders. The identification of a common genomic abnormality may have quickly impacted how individuals are diagnosed with this disorder, but it has failed to so rapidly alter how the clinicians, researchers, patients, and families interested in and affected by DiGeorge/VCF syndrome identify themselves and promote their causes. Indeed, in merely attempting to refer to this syndrome in my own

scholarly analysis of it, I cannot help but make myself subject to the politics that swirl around it.

The outcome of the instance of genomic overlap discussed in this chapter may appear on its surface to have been more easily and simply resolved than in the case of Prader-Willi and Angelman syndromes. My goal in this chapter however, has been to demonstrate the various complications that remain when two disorders become one. Beyond just institutional and professional debates, the association of DiGeorge and VCF syndromes with the 22q11 deletion has also done little to explain or predict the great clinical variability of this disorder. While it is easy to diagnose DiGeorge/VCF syndrome prenatally, or during early childhood, it is impossible to predict how the disorder will ultimately be expressed.

During the 1980s, many hoped that the association of DiGeorge syndrome with aberrations at the genomic address 22q11 would lead to the identification of a single mutant gene for this disorder. However, as recognition of the clinical variability of this syndrome increased, particularly following its genomic link with VCF syndrome, many researchers began to assume that one gene alone could not explain the complicated 'look' of this disorder in the clinic. A number of theories since have been applied to explaining the variable expression of DiGeorge/VCF syndrome, including the idea that it is a 'contiguous gene syndrome', and the related concept that the 22q11 deletion unmasks mutant genes in some patients.

While clinical diagnosis would certainly benefit from the resolution of some of these uncertainties, their genomic implications do continue to drive valuable research in

human and medical genetics. The concept that this disorder could be explained by one-to-one correlations between gene mutations and clinical outcomes has now been largely overshadowed by the recognition of multiple-dimensional genomic functionality in this and other disorders. However, we should not discount the continued importance of the chromosomally defined genomic region 22q11 over the past 30 years, and in the present day understanding of DiGeorge/VCF syndrome. While the mechanism of this disease is complex and unclear, the 22q11 deletion remains an important visual genomic, and anatomical, marker of this disorder, which has undoubtedly improved the diagnosis, understanding, and significance of it in the laboratory and the clinic.

Indeed, the 'look' of this disorder is very much rooted in the types of visual evidence that clinicians and researchers choose to highlight and give epistemic priority. These standards are established and reinforced by the collective ways in which these biomedical professionals learn and agree to see their objects of study. These research objects have taken various forms in postwar biomedicine, including the clinically presented body, and microscopically visible human chromosome set. As this chapter described, the established 'look' of a disease may vary among different institutions, medical specializations, and over time as new evidence is introduced and re-conceived as being increasingly important and reliable.

# CHAPTER 5
## The Lasting Impacts of Chromosome Level Thinking and Analysis

In 2011, during the early days of my dissertation research, I interviewed Beverly Emanuel, a medical genetics researcher at the University of Pennsylvania and Children's Hospital of Pennsylvania. As our conversation was drawing to a close (and after I had already turned off my tape recorder), Emanuel was reflecting on her career, which has largely been defined by her research focus on a particular sub-region on the long arm of chromosome 22. As Emanuel mused, she held her thumb and 1st finger about a centimeter apart and spoke of how amazing it was that something so small could be the focus of an entire (quite productive and rewarding) career. Now, she knew as well as I did that the genomic region she works on is much smaller than the space between her fingers; in fact, it is almost imperceptibly small. That bit of space between her fingers however, was real to her in terms of how she understood chromosome 22 in her head. Visualized as an ideogram, the chromosome was a tangible entity, maybe a few inches in length. Small portions of this chromosome have been the focus of many a life's work.

Most of the genomic entities and processes that Emanuel and thousands of other postwar medical geneticists dedicated their lives to studying were too small to ever be directly seen. However, as this dissertation has sought to demonstrate, the work objects of these researchers were far from invisible to them. In line with generations of biomedical researchers before then, these individuals relied on painstaking observation, and standardized ways of seeing and communicating, in practicing their trade. Nicholas Rose (2007, p. 12) and others (Clarke et al, 2010) have suggested that a new "style of

thought" (Fleck, 1979), the "molecular gaze" has "supplemented, if not supplanted" the longstanding centrality of the 'clinical gaze' in biomedicine. In this dissertation however, I demonstrate that an increasing focus on the 'genomic basis' of disease has not undercut the centrality of observational approaches in genetics in biomedicine. Rather, I suggest that the postwar period is perhaps best defined by the rise of a 'genomic gaze', which integrates molecular understandings of disease with visible and tangible genomic markers and conventions.

What is the point of retaining chromosome level conceptions and depictions of the human genome in an era when clinicians and researchers have a complete DNA reference sequence at their fingertips? Why has an 'antique' nomenclature developed in the 1970s for the visual description of chromosomes remained a prominent set of landmarks in the post-Human Genome Era? The answers to these questions are particularly perplexing when one considers that chromosomal banding nomenclature and genomic sequence data are incommensurable languages: banding boundaries can, at best, be located within a 100,000 DNA base pair range (Interview with David Haussler, February 29, 2012). In this chapter, I examine the forces and considerations that have helped to maintain the importance of chromosome level thinking and nomenclature, and reflect on why older languages of description based on chromosomal analysis remain intact in the face of newer, more exacting options. Following this, I offer a series of broader conclusions that tie together the various case studies examined in this disseration.

**From Sequencing to Browsing**

The official announcement of the completion of a rough draft of the human genome took place on June 26th 2000 at the White House. The draft DNA sequence itself however, was not made publically available until over a week later on July 7th, when the genome was for the first time posted on the Internet by the University of Santa Cruz Genome Bioinformatics Group. As David Haussler put it to me, "that was the day that the world got the first glimpse of the human genome". On that day however, the draft was little more than 2.7 billion letters, "It was nothing more than a waterfall of As, Ts, Cs, and Gs. So you had people counting how many times GATTACA appeared in it, or looking for secret biblical messages . . . it was something you could use for wallpaper" (Interview with David Haussler, February 29, 2012).

In order to make the human genome sequence more accessible to the thousands of clinicians and geneticists who expected to be begin using it for diagnosis and research, multiple genome 'browsers' were built during the second half of 2000. This included the Map Viewer, created by the National Center for Biotechnology Information (NCBI) at the NIH campus, the Ensembl Genome Browser, sponsored by the Wellcome Trust Sanger Institute and the European Molecular Biology Laboratory (EMBL), and the University of California, Santa Cruz (UCSC) Genome Browser (Wolfsberg et al, 2002). These browsers served as portals to the raw human genome sequence data, and provided the online software and annotations necessary to make the information useful to those who accessed it. As Haussler put it, in reference to the online release of the initial human

genome draft in July 2000, “In terms of usefulness it wasn’t until this browser was built that people could actually use it” ((Interview with David Haussler, February 29, 2012).

The UCSC Genome Browser and others, made analyzing the human genome a lot like browsing a bookshelf. These data portals offered a top down view of the genome, with the human chromosome set as the primary unit of analysis. One can jump straight to a particular gene or genome region, or begin with a specific chromosome and zoom-in from there. When I began my interview with Haussler at UCSC, he immediately asked me for my favorite gene, so that he could search for it in the Genome Browser. I picked SNRPN, a gene associated with Prader-Willi syndrome. Haussler typed this into the search mechanism, and the browser immediately brought us to a region near the centromere on the long arm of chromosome 15.

The UCSC genome browser has a horizontal orientation, with a series of customizable data tracks appearing on the screen. In its default mode, one is shown the nucleotide number of the region in question (the DNA nucleotides on each chromosome are numbered from 1 into the hundreds of millions, beginning at the farthest point from the centromere on the short arm). Below this, the expanse of the gene and its coding regions are shown, and continuing downward a number of additional data tacks are shown, including the sequence homologies with a number of other organisms. Above all of this information, featured prominently at the top of the page is a banded ideogram of the chromosome being explored: in the case of SNRPN this was chromosome 15. A red box (or line depending on how zoomed in the tracks are) shows the location and extent of

chromosome 15 being viewed. SNRPN falls into the chromosomal band 15q11.2, and appears to be quite close to the boundary with the next visible band, 15q12 (Figure 10).

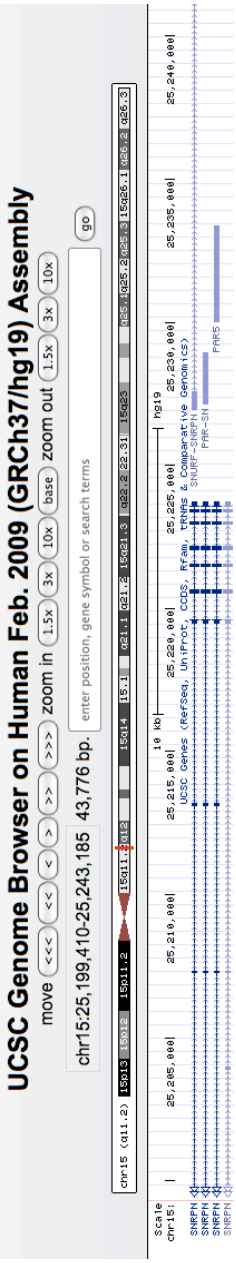


**Figure 10** Output impage from the UCSC Genome Browser, showing the prominient position of a chromosome 15 ideogram above all other genetic tracks. "The UCSC Genome Browser, http://genome.ucsc.edu", Human genome assembly Feb. 2009.

Chromosomal ideograms have a similarly prominent position in the other major genome browsers. In Ensembl, one begins with a vertically oriented view of all 24 human chromosomes, the "whole genome", and from here can click on a particular band, or drag the cursor and select a larger region of one chromosome. From here, Ensembl offers a similar experience as the UCSC Browser, with a horizontal ideogram of the chromosome being examined above and a series of data tracks below. The NCBI Map Viewer also has a number of common experiential features. For instance, one begins by choosing a chromosome from a vertical depiction of 24 unbanded chromosomes. The interface is noticeably different at the chromosome level however, because it is vertically oriented, with a chromosomal ideogram on the far left, and a series of data tracks to the right. The NCBI depiction of chromosomes is more in line with early genomic representations, which depict chromosomes vertically with their short arm on top. In the era of widescreen computers however, horizontal depictions of ideograms may become more standard.

Initially, UCSC and NCBI provided their own, slightly different assemblies of the human genome. However, it was quickly decided that for clarity, there should be one reference genome sequence shared by all browsers, for which the NCBI assembly was chosen (Wolfsberg et al, 2002). Each browser continues to have its own annotational and organizational strengths. The Ensembl browser specializes in highlighting protein structure and function, while the UCSC Browser is more focused on the genetic code itself. Human geneticists therefore often go to the USCS browser first, and then jump into the Ensembl browser for protein analysis (Interview with David Haussler, February

29, 2012). The NCBI Map Viewer is particularly closely integrated with OMIM, and tends to highlight genes and their chromosomal positions.

The official publication of the draft genome sequence came in issues of *Science* and *Nature* in February 2001. By agreement, papers concerning the results from the publically funded Human Genome Project appeared in *Nature*, and those from Craig Venter's competing private venture, which also finished its draft in June 2000, were published in *Science*. The primary collaborative paper from the public project in *Nature* highlighted the newly constructed UCSC and Ensembl genome browsers (Lander et al, 2001). Published along side a series of related papers on different aspects of the genome project and its preliminary results, was a foldout map depicting the genome at the level of chromosomes. A vertical, microscopic image of each chromosome was placed next to a series of horizontal data tracks. Similar to the genome browsers, these tracks broke down each chromosome into cytogenetic banding units and nucleotide base pair distances. Known genes were also listed along each chromosome.

When I walked into his office at UCSC, Haussler had the *Nature* foldout displayed prominently on his wall. I asked why the contributors to the Human Genome Project's primary publication decided to plot the large mass of information that they had sequenced and compiled in this very visibly oriented format. Haussler responded,

> People needed to have something tangible, they wanted a fold out. It was a monumental achievement, so you wanted something you could physically touch and look at to get an idea of the scope of the work.

> Previously you would publish a paper in *Nature* based on five years work of locating one of those genes, and Figure 1 would be the chromosomal ideogram and your location of your gene. And that was an incredible achievement, and I think this made a statement of, wow, look at the scale, all at once, all those genes (Interview with David Haussler, February 29, 2012).

Having spent a decade sequencing and assembling the entire human genome, it remained the case that the best way to capture and make apparent the broad implications of this great accomplishment was to visually depict it a much lower level of resolution. Even on this poster sized, foldout figure, each linear inch represented 10 million DNA base pairs. In this sense, the figure certainly conveyed the immensity of the data set that had been obtained.

This foldout published along with the initial Human Genome Project publications in early-2001 is representative of continuity of conceptions about the genome before and after the completion of a draft sequence. Clinician and research understandings of the genome as a visible, tangible entity did not immediately fade away or become irrelevant. Rather, they remained central to the thinking and practices of post-HGP era genetics and biomedicine. In the next section, I trace this continuity more extensively through my interviews with geneticists.

**Cytogenetic Thinking and Analysis in the Era of Whole Genome Sequencing**

One of the publications found in *Nature* along side the initial paper on the completed human genome draft sequence, reported on a large project to integrate cytogenetic landmarks into the genomic DNA sequence (Cheung et al, 2001). 7600 probes had been utilized in an attempt to correlate the chromosomal banding nomenclature with the draft sequence of the human genome. Each probe was fluorescently tagged, and under the microscope, which chromosome and band it annealed with noted, along with the relative ordering of each probe. The probes were associated with specific genomic fragments, and so their location in the draft sequence was also known. The goal of all this was to correlate two existing physical maps of the genome: one based on observational analysis of the human chromosome set going back to the 1970s, and the newly completed draft DNA sequence.

To improve the accuracy of the genome browsers, researchers wanted to know where boundary lines should be drawn between each consecutive chromosomal band within the genome sequence. Realistically, these data sets are incommensurable. Asked how closely the banding boundaries could be associated with the reference sequence, Haussler told me that the estimations were within, "100,000 bases at best, and that's assuming fairly dense mapping, in the optimum conditions. In sparse places, it is a million bases" (Interview with David Haussler, February 29, 2012). Indeed, where one chromosomally visible band ends and another begins primarily has to do with the physical compaction of chromosomes, a process that is only indirectly related to the DNA codes itself (areas with fewer genes tend to be less compacted and appear as lighter G-bands).

Knowing the approximate correlation between chromosomally visible bands and the genomic code is of value in part for research purposes. As Cheung et al (2001, p. 954) put it in their 2001 report, "To proceed from cytogenetic observation to gene discovery and mechanistic explanation, scientists will need access to a resource of experimental reagents that effectively integrates the cytogenetic and sequence maps of the human genome". Often times, as is described throughout this dissertation, the first indicator of the genomic location of a disease etiology comes from the identification of a chromosomal abnormality in a number of similarly affected individuals. A better correlation between chromosomal band and genomic sequence location can help to target the search for genes in that region, potentially involved in the disorder. In a similar study reported in 2003, and based on 9000 probes, UCSC postdoctoral researcher Terrence Furey made a similar argument about the importance of linking the chromosomal banding and genomic sequence maps, "The integration of the cytogenetic map with this draft sequence provides cytogeneticists with the necessary link to this molecular-based resource. Given a chromosomal abnormality in a diseased cell where the affected region has been cytogenetically mapped, the corresponding area in the draft sequence can be easily determined, and then investigated for potential disease genes" (Furey and Haussler, 2003, p. 1037).

In practice however, correlating the cytogenetic banding map to the draft human genome sequence was about more than helping to target the molecular search for new disease genes. From a conceptual perspective, the chromosomal banding map offer clinicians and researchers a way to find themselves in the genome, and to communicate

genomic locations to their patients and colleagues. As medical geneticist David Ledbetter has put it to me, "It's hard to talk about a gene using genome sequence coordinates, because how do you visualize that, how do you wrap your brain around genome sequence coordinates?" (Interview with David Ledbetter, March 21, 2012). Here Ledbetter is referring to the system by which each nucleotide in the human genome reference sequence is numbered on each chromosome from 1 into the hundreds of millions. So, for instance, one can refer the gene SNRPN as being located on chromosome 15 between the nucleotide coordinates 25,217,650 and 25,224,945, or as being with the visible band labelled 15q11.2. The first method is more exacting quantitatively, but it does not give one a sense of where they are in the genome or on chromosome 15.

Many other clinicians and geneticists have offered similar accounts of how their conceptions of the genome remain grounded in the human chromosome set. As medical geneticist Beverly Emanuel put it,

> If you go to any of the sequence websites, like the [UCSC] Genome Browser, and you focus in, there is an ideogram that is still there . . . because for so long we have used that information in that way, and it does help to put that in a perspective, as opposed to a long string of numbers. A long string of numbers, from 17 to 20 million, doesn't necessarily put you into a visual of where in the genome it is (Interview with Beverly Emanuel, November 9, 2011).

Chromosome bands offer a better sense of location and nucleotide numbers do. In addition, banding nomenclature locations also help to historicize a disorder and its genomic association. Medical geneticist Kurt Hirschhorn described the important of chromosomal context to me in this way, once again referencing the UCSC Genome Browser, "Some of this is historical, a number of difficulties have been described by virtue of a chromosome and a position in a chromosome. So, if you want to understand what the background of the whole thing is, you really need to see the chromosome. And I think they have done a very good job of that at Santa Cruz" (Interview with Kurt Hirschhorn, January 26, 2012).

Chromosomal locations are also more useful than genomic coordinates when communicating with patients. As medical geneticist Uta Francke noted to me, "It's hard to visualize just DNA . . . if you talk to parents, or people who are affected with a chromosomal imbalance, it helps a lot to show them a picture of a chromosome, and say look, this is the piece that is now translocated. It gives them some coordinate numbers" (Interview with Uta Francke, February 27, 2012). Indeed, it is much easier to offer patients a visual representation of a genomic abnormality than a sequence level, quantitative account. Deborah Driscoll offered a similar narrative of how she uses chromosome level explanations to counsel patients,

> We have come a long way [with molecular genetics], and I think it has really changed the way we can counsel families, but visually you still

> think of a chromosome. It is kind of where the DNA lives, the genes live, so that's how I think of it. When I talk to patients, I talk about chromosomes, and then what I try to do is explain to them what is a gene (Interview with Deborah Driscoll, November 29, 2011).

Along similar lines, many of the clinicians and geneticists I interviewed told me that those who work with the human genome have a communal sense of its geography based on the chromosomal banding nomenclature initially developed in the 1970s. Indeed, one cannot know the entire genome at the level of resolution that the DNA reference sequence provides. But geneticists are quite used to visualizing the genome under the microscope as a karyotype. Referring to Victor McKusick's 'morbid anatomy' of the human genome, Reed Pyertiz suggested to me, "I think if you had psychoanalyzed people back then and tried to get them to express what image flashed in their mind when they thought of the genome that [McKusick's chromosome level gene and disease maps] would be it . . . I still have tucked away the notion of the karyotype . . . but now I think of a cloud, it's just a mass of data" (Interview with Reed Pyeritz, April 18, 2012). Indeed, even in the era of whole genome sequencing, it is difficult for geneticists to conceive of the human genome without referring back to the chromosomes.

While the human genome reference sequence is an impossibly large and repetitive data set, chromosomal nomenclature offers researchers and clinicians with a satisfying sense of place. Medical geneticist Dorothy Warburton has suggested to me that she thinks of the genome as a familiar neighborhood, full of landmarks that make navigating

it easy and intuitive. To remove these landmarks, and replace them only with consecutive numbering she told me, is to take away the native elements that make a neighborhood recognizable Interview with Dorothy Warburton, May 11, 2011). Ledbetter expressed a similar feeling that chromosomal locations offer him a sense of place and context,

> If I am in a seminar or talking with somebody and they start talking about a gene, the first question I ask is, what chromosome is it on, where does it live? And, it is sort of like saying, where are you from? Just the geography of where somebody lives or comes from just helps you . . . If a gene is on chromosome 18 or it is on chromosome 16, I'm not really asking because I want to know what the individual gene neighbors are. I just can't imagine a gene without thinking where it is in the genome (Interview with David Ledbetter, March 21, 2012).

As Ledbetter went on to explain, there really is not an explicit functional purpose for knowing on which chromosome a gene is located. Indeed, genomic proximity does not suggest that there is a functional relationship or interaction among gene functions or products.

Knowing where a gene is located in the human genome however, may offer context more generally about what other genetic entities or regulatory elements are in the area, which could be relevant in cases involving chromosomal aberration or genomic

imprinting (as in the instance of Prader-Willi and Angelman syndrome). In this sense, chromosomal banding offers a set of signposts that may help to guide one through the genome. As Robert Nicholls put it in our interview, "for me the chromosome correlation is a guidepost as to the underlying genes or regulatory sequences" (Phone Interview with Robert Nicholls, April 5, 2012). Driscoll similarly referred to cytogenetic banding nomenclature as "the signposts along the genome" (Interview with Deborah Driscoll, November 29, 2011). In fact, the genome offers many useful signposts (expressed sequence tags, ESTs, and restriction enzyme sites have been used in this way). However, chromosomal ideograms offer a broadly shared, visual language for describing the human genome, which is known to geneticists worldwide, thereby making it highly useful for positional communication.

Indeed, while the human genome may in many ways be an expansive cloud of data, as Pyeritz described it to me, in the post-HGP era, clinicians and geneticists continue to rely on familiar landmarks and low-resolution chromosomal representations of the human genome as they communicate about and interact with it. Chromosomal ideograms offer a tangible landscape within which clinicians and geneticists situate and contextualize their research, and offer a useful visual referent as they counsel patients on the genomic basis of a particular disorder. While genetic analysis now regularly takes place at the level of DNA sequence analysis, chromosomal level thinking and representation continue to be an important starting point for conversation.

**Concluding Remarks**

This dissertation is a contribution to the ongoing observational turn in the history of postwar genetics and biomedicine. In recent years, scholars have made significant contributions to our understandings of the visual practices of human and medical geneticists since the 1950s (Martin, 2004; Lindee, 2005; de Chadarevian, 2010; Santesmases, 2010). As part of this, historians of science have sought to better understand how the human genetic complement and chromosome set have been seen and standardized as a scientific object by genetics researchers. To this body of literature, I add a historical analysis of evolving conceptions and depictions of the human genome since the 1960s, an entity that has become increasingly central to the thinking, practices, and promotion of biomedical research over the past 30 years.

Each of the case studies presented in this dissertation seek to capture the development of a new nosolgical and diagnostic system in postwar biomedicine, in which clinical disorders have come to be understood as having a genomic basis. As I describe, the visible chromosomal markers associated with each of these disorders played a central role during the 1970s, 80s, and 90s in shaping their clinical delineation, diagnosis, understanding, and treatment. These visible genomic markers, specifically the fragile X site, 15q11-13, and 22q11 deletions, have served as an influential basis for delineating and naming a new disorder, identifying a relationship between two diseases that were otherwise clinically distinct, and ontologically (though not institutionally) unifying two previously separate syndromes into one.

As these case studies are meant to demonstrate, the ideal of one-to-one correlations between genetic mutations and diseases was often complicated during the postwar period by the unanticipated and confusing behavior of visible chromosomal markers. While a source of frustration for many clinicians and patients, such ambiguous findings attracted the attention of many more basic genetics researchers. Indeed, chromosomal analysis proved to be a very productive experimental system for human geneticists throughout the postwar period. As I describe, sustained observational analysis of chromosomes in patients impacted by various genetic disorders led to new, more complex, understandings of genomic functionality in the decades before the completion of the Human Genome Project. Far from a one-dimensional dataset, chromosomal analysis offered a window into the multi-level functionality of the genome.

Many of the disorders examined in this dissertation represented important (and sometimes short-lived) exemplars of particular forms of genomic disease in the postwar period. For example, Fragile X and Prader-Willi syndrome have long been, and remain, important teaching cases in human genetics and biomedicine. The deletion of the chromosomal region 15q11-13 was pointed to throughout the early-1980s as representative of how the loss of specific genomic information could cause a discrete clinical outcome, and since 1990 with the demonstration of genomic imprinting, has been used as a means for demonstrating the multi-dimensional functionality of the human genome. Likewise, the fragile X site similarly represented in the early-1980s a visible chromosomal feature that could be used to delineate a particular form of intellectual disability, which it in the 1990s was presented to clinicians and geneticists as the basis of

a new genomic mechanism, trinucleotide repeat expansions, which turned out to explain the unusual inheritance pattern of multiple genetic disorders.

As Angela Creager has suggested, exemplars are much more than fixed textbook renderings of established scientific theory. In fact, existing exemplars are constantly being renegotiated within productive experimental models (Creager, 2002). The case studies presented in this dissertation demonstrate that throughout the 1980s, chromosomal analysis remained an important and productive experimental system for human and medical geneticists, which brought about new exemplary understandings of the structure and function of the human genome and its role in disease. While during the 1980s, clinicians and geneticists had high expectations for the value of new molecular approaches to doing genetics, and for the potential of DNA sequence level mapping of the human genome, this did not undercut their willingness to take advantage of existing cytogenetic tools.

Particularly in the clinic where the treatment of individual patients could not be put on hold pending new techniques or understandings, researchers developed new approaches for better understanding genetic diseases through a process of bricolage. Using whatever tools were currently had available they built what was at once a technological and experimental system. As I have described throughout this dissertation, the questions and findings of the clinic often shaped more basic genetics research, and vice versa. The trading zone of problems, interests, and information between the laboratory and clinic, I argue, was greatly facilitated during the 1970s and 1980s by adoption of common conventions for describing the genomic basis of disease among

basic and clinical geneticists. These conventions were perhaps best embodied by the standardized chromosomal ideograms discussed throughout this dissertation.

Chromosomal ideograms offered idealized representations of what chromosomes looked like under the microscope, and at the same time tangible entities within which known cartographic and anatomical features of the human genome could be systematically represented. In this sense, following the distinction first made by Charles Pierce (1982), these ideograms were both iconic and indexical representations. Iconic images are those that capture the likeness of an object, such as a photograph of someone, whereas indexical representations point to something unseen within them, such as dark clouds suggesting an impending rainstorm.[37] Along these lines, ideograms roughly approximated what chromosomes looked like under the microscope, and at the same time have been used to represent the basic landscape and anatomy of the unseen DNA sequence of the human genome, which is compacted within them.

As I describe throughout this dissertation, the combined iconic and indexical status of chromosomal ideograms in postwar genetics and biomedicine was central to evolving notions of the human genome as a standardized object of research and analysis in the laboratory and the clinic. As iconic representations, ideograms helped clinicians and geneticists to distinguish and communicate about chromosomes and their visible anomalies. During the 1970s and 1980s, these ideograms also increasingly took on an indexical role as the framework upon which the genetic and disease related components

[37] For me on this distinction: http://www.cs.indiana.edu/~port/teach/103/sign.symbol.short.html. See also, Lukas Rieppel's (2012) recent paper on the museum exhibition of fossilized dinosaurs.

of the human genome were mapped. Over this period, these ideograms evolved both visually and conceptually, coming at once to represent the human chromosome set iconically and the human genome indexically.

Chromosomal characteristics that could be seen under the microscope and were represented on ideograms, such as dark and light bands, fragile sites, and deletions, were used as a basis for understanding unseen genomic structures and functionality. Light bands on ideograms suggested areas of the human genome with a much higher density of genes, while bands that were absent in patients diagnosed with particular disorders were assumed to contain the etiological basis for certain clinical outcomes. In this way, ideograms were used to represent knowledge about both the normal and pathological human genome.

Keating and Cambrosio (2003) have argued that central to postwar biomedicine has been a material and institutional realignment of the normal and the pathological, in the form of what they call biomedical platforms. In this dissertation, I suggest that McKusick's 'morbid anatomy' of the human genome captures a similar conceptual realignment, through its iconic use of normal chromosomal ideograms to point, indexically, to the genomic basis of hundreds of genetic disorders. Indeed, as the case studies presented here demonstrate, when diseases come to be understood as having a genomic basis, they may draw the attention of more basic genetics researchers, who are not interested in a particular disorder, so much as what it might reveal more broadly about the structure and function of the human genome. This continuity of in the questions and interests of basic biological and applied clinical researchers, I argue, has

been facilitated in part by shared conventions for mapping the human genome using the standardized banding patterns provided by chromosomal ideograms.

Chromosome level depictions of the human genome embody particular modes of thinking and sets of approaches that have been central to the development of contemporary biomedicine. Indeed, understandings of the human genome as visual, tangible, and anatomical have helped to facilitate a broad communication, exchange of interests, and sense of common relevance among the diverse array of individuals who are involved in biomedical research. Each day, in meetings, clinics, and laboratories, biomedical professionals look to chromosomal depictions of the human genome, and see in them iconic and indexical representations of what has been accomplished so far, and what territory remains to be explored. Mindful of this, as historians of science and medicine continue to probe the material and conceptual underpinnings of postwar biomedicine, I hope that they will keep an eye open to its visual cultures, which have been integral to the postwar success of genetic medicine – scientifically, clincally, and socially.

## LIST OF INTERVIEWS

Deborah Driscoll, Philadelphia, PA, November 29, 2011

Beverly Emanuel, Philadelphia, PA, November 9, 2011

Uta Francke, Palo Alto, CA, February 27, 2012, follow-up via email, March 28, 2012

Randi Hagerman, Sacramento, CA, March 2, 2012

David Haussler, February 29, 2012, Santa Cruz, CA

Kurt Hirschhorn, New York City, January 26, 2012

Edmund Jenkins, New York City, May 26, 2011

David Ledbetter, Danville, PA, March 21, 2012

Donna McDonald-McGinn, Philadelphia, PA, November 10, 2011

Loris McGavran, Denver, CO, August 20, 2012

Robert Nicholls, April 5, 2012 (Phone)

Reed Pyeritz, Philadelphia, PA, April 18, 2012

Charles Scriver, May 30, 2012 (Phone)

Dorothy Warburton, New York City, May 11, 2011

Charles A. Williams, March 16, 2012 (Phone)

Elaine Zackai, Philadelphia, PA, November 10, 2011

## LIST OF ARCHIVAL RESOURCES

International Standing Committee on Human Cytogenetic Nomenclature (ISCN) Papers, Office of Dr. Uta Francke, Stanford University School of Medicine, Palo Alto, CA

March of Dimes Archives, White Plains, NY

Victor McKusick Papers, Alan Mason Chesney Medical Archives of The Johns Hopkins Medical Institutions, Baltimore, MD

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