2012 William Allan Award: Adventures in Cytogenetics[1]

Uta Francke[2,3,*]

Ladies and gentlemen, members and guests, colleagues and friends,

I am deeply honored to be chosen for this award; thank you, Madam President, members of the Awards Committee, and Tayfun Özçelik for your kind introduction. I accept this award on behalf of a large group of scientists, numerous postdocs, graduate and undergraduate students, research associates, and technicians. I am grateful to all of them, some of whom are here, and to the many collaborators who contributed to our success.

You heard about the wide range of research activities of my laboratory, so for this address, I had to select what to cover. The microscope in the Allan Award medal inspired me to focus on my adventures in cytogenetics.

Let's start from the beginning. In the gymnasium, the German equivalent to high school plus junior college, I majored in mathematics and physics and studied Latin for the language requirement, so I couldn't speak much English when I came to this country. In medical school in Germany, I thought biochemistry was the most interesting subject. I remember that one day the professor walked in, all excited, and told us that mRNA had been discovered and that now we understood how the genetic information is transmitted from the nucleus to the cytoplasm.

Therefore, after my postgraduate medical training, which ended with a pediatrics residency at Children's Hospital Los Angeles in California, I wanted to subspecialize in endocrinology, a field that offered some understanding of the role of molecules and pathways in disease processes, and rational approaches to treatment. However, coming from a foreign medical graduate, my application for a pediatric endocrinology fellowship was not even considered.

Meanwhile, at University of California, Los Angeles (UCLA), a new fellowship program in pediatric genetics had been established under Stanley Wright, and they gladly accepted me. So, I got into genetics at the beginning of a new era, and I have not regretted it for a moment. This step allowed me to embark on an unbelievable journey and to participate in the development of our field for the past 40 years. It has been a constant learning experience.

Chromosome Banding and Identification and High-Resolution Ideograms

When I started my fellowship at UCLA, human chromosomes were uniformly stained and, with a few exceptions, could not be individually identified. Then one day, Stan Wright announced the big news that Caspersson in Sweden (1988 Allan Award recipient) had told him that with the fluorescent dye quinacrine they could reliably distinguish chromosomes 17 and 18. This was exciting, and I had to try it. I dug up a microscope with a movable mirror—it looked like the one in the Allan Award medal—and a fluorescent lamp and spent many hours in the dark room identifying each human chromosome,[1] as well as the chromosomes involved in translocations in cases that the Genetics Division had previously collected.[2]

When mouse geneticist Muriel Nesbitt joined the faculty, we collaborated on constructing the first quinacrine-stained mouse karyotype. The bands were fuzzy and needed to be documented further by densitometry profiles and interpreted in ideograms.[3] Identifying the mouse chromosomes was a momentous advance for mouse genetics because linkage groups had already been

[1]This article is based on the address given by the author at the meeting of the American Society of Human Genetics (ASHG) on November 9, 2012, in San Francisco, CA, USA. The audio of the original address can be found at the ASHG website.

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associated with abnormal yet unidentified chromosomes. By identifying the mouse chromosomes involved in translocations, we were able to assign entire linkage groups to their respective physical locations.[4–6] O.J. Miller and colleagues were also working on mouse chromosome identification, but we were unaware of it at the time.[7] So, I got an early taste for gene mapping and mouse genetics, areas which I continued to pursue.

As Giemsa (G)-banding was introduced and our chromosomes were banded at higher resolution, Muriel and I constructed banding ideograms for each mouse chromosome while taking into account the relative staining intensity of each band. We used calipers for band measurements, and I drew the chromosomes with shapes as I saw them in the microscope. We divided each mouse chromosome into major regions, designated by capital letters, and then subdivided the regions into numerals that could be further subdivided by decimals.[8] That numbering system was carried over to the current standard mouse chromosome nomenclature.[9]

Meanwhile, the International Committee for Human Cytogenetic Nomenclature (ISCN) had devised a system for human chromosome bands with ideograms that were based on impressions, not measurements.[10] Subsequently, cell-synchronization methods enabled the study of longer prometaphase chromosomes, resulting in high-resolution banding (HRB). In my lab, then at the University of California, San Diego (UCSD), we decided to do band measurements and design accurate chromosome representations that also included various intensities of staining, just as Muriel and I had done for the mouse chromosomes.[11]

In 1981, ISCN incorporated HRB information by subdividing their original bands into arbitrary subbands. The resulting HRB-ISCN ideograms had little resemblance to the looks of actual high-resolution G-banded chromosomes, so I decided to take our measurements and adapt them to the ISCN numbering system. The rules that white bands had to separate dark (or gray) bands necessitated compromises.[12] Our contribution was included in the official ISCN report.[13]

To continue the ideogram story, in the early 1990s the Human Genome Project (HGP) got underway, and people coming in with molecular biology and computer science backgrounds were talking about the genome as a one-dimensional string of four letters, an informational entity, whose function they would be able to unravel given enough computing power. I felt that they were missing the point because the single molecule of DNA that runs from one end of the chromatid to the other is packaged into metaphase chromosomes in distinctly uneven patterns, e.g., dark bands condense earlier than light bands in the continuous process of prophase and prometaphase. The location of the DNA sequences that were being assembled by the HGP with respect to chromosome bands must have functional implications. As Holger Hoehn had already pointed out in 1975, trisomies for chromosomes with relatively more dark-staining G-bands are compatible with development to term and live births, which contrasts with trisomies for equally sized chromosomes that contain more lightly staining chromatin and are only found in spontaneous abortions.[14]

To call attention to the contribution that chromosome-banding information could make to the HGP, I had our ideograms professionally redrawn and published them together with the underlying measurements in an article pointing out their features and potential uses (Figure 1).[15] The paper is not available online, and today's graduate students are not likely to venture into the stacks of the library, but the ideograms survive. You can find them in numerous illustrations, textbooks, databases,[16] and commercial products—and nobody remembers where they came from. Although this makes me proud, I also know their imperfections. No cytogeneticist with access to better-quality chromosomes has done what we did: caliper measurements of prometaphase chromosome bands at distinct stages of condensation. Would there be any reason to repeat the work now with higher-quality preparations?

In 2009, a *Science* paper entitled "Comprehensive mapping of long-range interactions reveals folding principles of the human genome" reported on genome-wide chromatin-conformation analyses assessing the physical proximity of DNA segments.[17] Using a "Hi C" method, the authors probed the three-dimensional architecture of whole genomes by coupling proximity-based ligation with massively parallel DNA sequencing. Their principal-component analyses revealed that the genome is compartmentalized into closed and open compartments and that chromatin packaging is an integral element. The compartments correspond to gene density, specific histone-methylation patterns, and DNase I sensitivity profiles.[17] As soon as I looked at Figure 3G in this paper, I realized that I had seen the pattern before. It lined up with our ideogram, leading to the surprising insight that the chromatin organization of the genome in interphase appears to be carried through to metaphase and is reflected in the banding patterns (Figure 2). The closed and open chromatin regions correspond to dark and light prometaphase G-bands, respectively, and gray bands are an unresolved mixture of both types. I was thrilled, and told my undergraduate molecular genetics class the next morning. However, the lineup did not work perfectly for all chromosomes. Last year, I finally met Dr. Lieberman-Aiden and told him about my hypothesis, and he is now actively testing it.

Mapping Genes onto Chromosomal Regions

One reason for generating chromosome-banding maps is to have a template onto which genes can be placed. Fortunate circumstances enabled me to enter the field of physical mapping of mammalian chromosomes at its beginning. In 1971, after my year of genetics fellowship at UCLA, my first husband, Bertold Francke, and I moved to San Diego, and I started to work in Bill Nyhan's biochemical genetics laboratory at UCSD. There, I studied the X-inactivation mosaicism in women who were carriers for

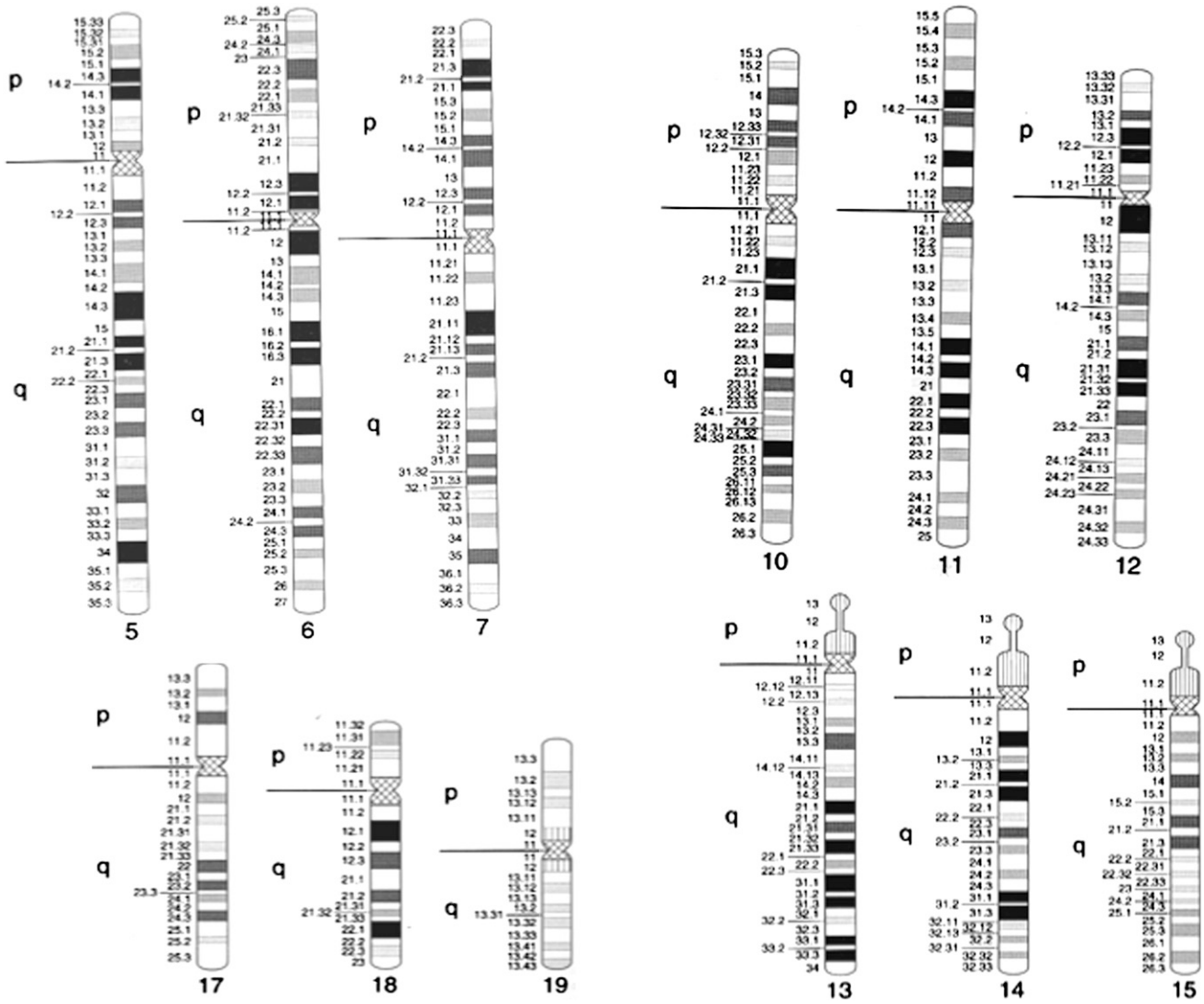

Figure 1. High-Resolution Human Chromosome Ideograms Based on Trypsin-G-Banded Prometaphase Lymphocyte Chromosomes
Band widths are based on measurements, and relative staining intensities are represented by black, white, and three shades of gray. The band numbering system is consistent with ISCN nomenclature.[13] This figure was modified from Figure 1 in Francke.[15]

Lesch-Nyhan syndrome, an X-linked recessive disorder caused by mutations in the X-linked gene *HGPRT*, and developed a carrier-detection test by using electrophoresis of hair-root lysates.[18] When Jerry Schneider, a faculty member in the department, returned from a sabbatical in Ephrussi's lab in Paris and brought back the somatic cell hybridization (SCH) technology,[19] I had an idea for a doable project: I proposed to regionally map genes on chromosomes by fusing human cells carrying a balanced reciprocal translocation to a mouse or Chinese hamster cell line. Initially, human chromosomes are randomly lost from such interspecies hybrid cells, but by repeated subcloning, one can obtain cell lines with a stable subset of human chromosomes. Being able to identify human and rodent chromosomes by their banding patterns and to separate human and rodent isozymes by gel electrophoresis of hybrid cell extracts, I could assign species-specific cellular phenotypes to defined chromosome regions. To get support for this work, I applied to the National Institutes of Health for an R01 grant. The study section voted in favor and even recommended to increase the budget so I could buy the best microscope on the market. As funding was contingent upon UCSD's giving me a faculty position, I became an assistant professor in residence in pediatrics.

In my first somatic cell hybridization experiment, I fused mouse cells with a reciprocal X-autosome translocation to a Chinese hamster cell line. By studying the hybrid cell clones, we were able to map genes within the X chromosome and chromosome 16 in the mouse.[20] Subsequently, we produced many human translocation-derived hybrid cell lines and constructed hybrid panels that allowed us to map any human gene or cellular phenotype to a chromosome and, in many cases, to a chromosomal region. Physical mapping by SCH had been promoted mostly by Frank Ruddle (1983 Allan Award recipient) at Yale,[21] and he arranged for me to be invited to the second Human

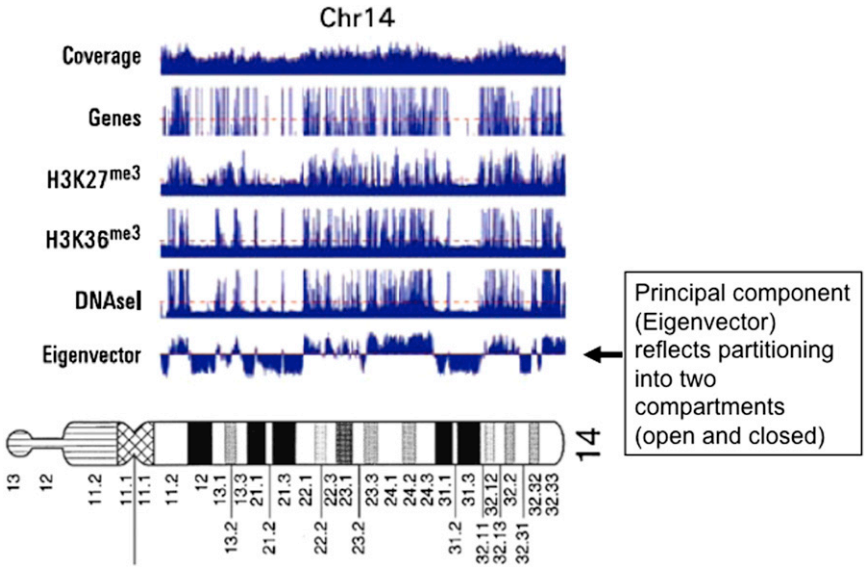

Figure 2. Interaction Map, Reflecting Spatial Proximity in the Interphase Nucleus, of Chromosome 14 at a Resolution of 100 kb

The principal component (eigenvector) correlates with the distribution of genes and with features of open chromatin. This interphase-chromatin organization is carried over to metaphase and is reflected in the chromosome-banding patterns. The closed and open regions correspond to dark and light prometaphase G-bands, respectively. This figure was modified with permission from Figure 3G of Lieberman-Aiden et al.[17]

Gene Mapping Conference in Rotterdam in 1974 to present our initial work.[22,23] At these biannual, or later annual, conferences supported by the March of Dimes Birth Defects Foundation, researchers from all over the world came together to share their latest physical and genetic-mapping data, which were then integrated and compiled by chromosome-specific committees. The updated mapping reports were published in *Cytogenetics and Cell Genetics*. In the mid 1990s, when data acquisition accelerated and the meetings became too large, they were split up into separate international chromosome-specific workshops. What many young people today do not realize is that when the HGP started with a focus on large-insert clone tiling paths and DNA sequencing technology, a rather dense physical map was already available to facilitate the assembly of the first human reference genome.

Initially, the phenotypes we could map in somatic cell hybrids were limited to expressed proteins for which the human-specific forms could be distinguished from the rodent forms, e.g., metabolic enzymes,[22–24] cell-surface antigens, such as human leukocyte antigen,[25] polypeptide spots on two-dimensional protein gels,[26] or yet-unidentified factors responsible for virus replication in cultured human cells.[27]

In the early 1980s, with the advent of molecular probes, we were able to map hybridizing restriction fragments on Southern blots made with DNA from interspecies somatic-cell-hybrid clones. The first human cDNAs that became available were for hemoglobins, and we mapped the β-globin gene cluster to the short arm of chromosome 11 in collaboration with Alec Jeffreys (1992 Allan Award recipient).[28] Restriction-fragment-length polymorphisms (RFLPs) were found to represent a powerful new set of genetic markers, and we collaborated with Ray White and David Botstein (1989 Allan Award recipients) to map the first highly polymorphic human RFLP locus (*D14S1*) to a region of chromosome 14[29] and to discover the first molecular-defined locus (*DXYS1*) present on both the X and Y chromosomes.[30]

In addition to mapping human genes, we also studied SCH between Chinese hamster and mouse cells that had retained reduced sets of mouse chromosomes and mapped the murine orthologs to mouse chromosomes, initially in collaboration with Peter Lalley and John Minna.[31–33] As human cDNA probes cross-hybridized to rodent restriction fragments and vice versa, we used mouse-hamster hybrid panels, in addition to the human-rodent panels, to map any newly cloned gene simultaneously in mice and humans and thus contributed to the recognition and delineation of conserved syntenic chromosomal regions.[34]

With molecular probes in hand, we also employed in situ hybridization of labeled probes directly to banded chromosomes. This independent approach enabled us to confirm and further refine the regional map positions. Initially, small cDNA probes were tritium labeled, autoradiographic grains were counted, and the counts were statistically analyzed.[34] In the late 1980s, radioactively labeled cDNA probes were replaced with fluorescently labeled cosmid probes for which hybridization signals were detected in a fluorescent microscope. This technology—known as fluorescence in situ hybridization—was pioneered by Peter Lichter in David Ward's lab at Yale while I was in the same department,[35] and we adapted it to our mapping experiments.[36]

With our reliable gene-mapping tools, we had the great fortune to collaborate with the premier gene-cloning labs and mapped *HRAS* with Robert Weinberg;[37] *NGFB*, *IGF1*, *IGF2*, *INSR*, *EGFR*, *TGFB*, *PDFRA*, and other genes with Axel Ullrich, Rick Derynck, and other scientists at Genentech;[38–42] genes for the low-density-lipoprotein receptor with Mike Brown and Joe Goldstein (1985 Allan Award recipients);[43] genes for adrenergic receptors with Brian Kobilka and Robert Lefkowitz;[44] the gene encoding synaptophysin with Thomas Südhof;[45] and many more.

Finding Disease-Associated Genes by Mapping

While actively contributing to the human and mouse genetic maps, our underlying motivation in gene mapping

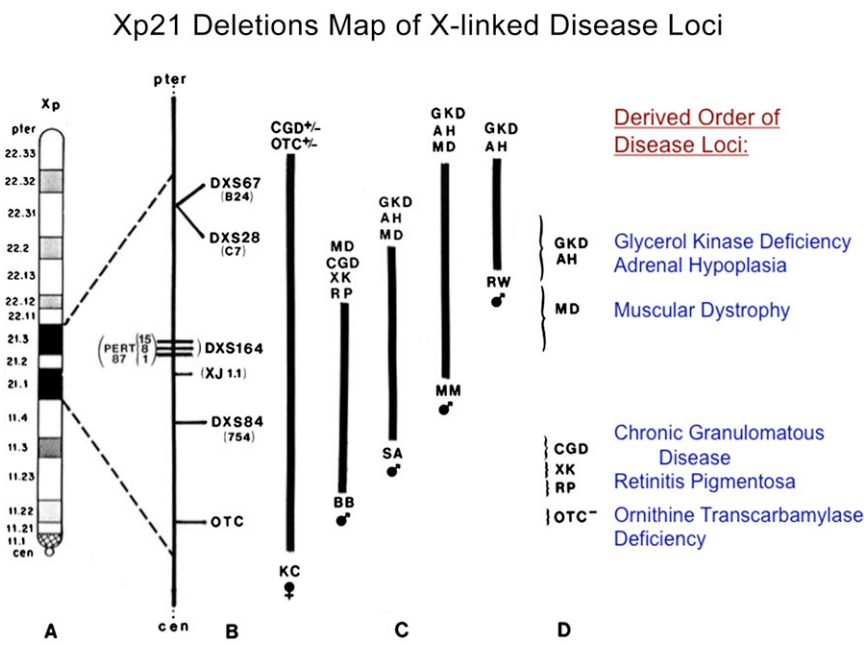

Figure 3. Xp21 Map of Molecularly Defined Intraband Deletions and Derived Localization of Disease Loci
(A) G-band map of Xp.
(B) Location of some DNA probes.
(C) Extent of deletions based on DNA segments missing from individual cases.
(D) Brackets indicate derived localizations of disease loci and of *XK*, mutations in which cause McLeod syndrome.
This figure is the precursor to Figure 3 in Francke et al.[57]

was to find genes associated with human disease. I never liked the metaphor "gene hunting" because the genes that when mutated cause human disease are not running away—they just sit there in hiding while waiting to be discovered. If we could map a cloned gene to a location that coincided with a previously mapped disease locus or mouse mutant phenotype, we might be able to directly identify the disease-causing gene. Over the years, we tested many positional candidate genes in DNA samples from affected individuals or mutant mouse strains and usually had negative results. However, we did get lucky one time: when the gene-encoding peripheral myelin protein 22 (PMP-22) was cloned in Eric Shooter's lab at Stanford, we mapped it near the gene for the Trembler phenotype in mice, and a *Pmp22* mutation was detected in these mice.[46] Trembler had already been considered as a mouse model of Charcot-Marie-Tooth disease, and we mapped *PMP22* to the small chromosomal duplication known to be associated with Charcot-Marie-Tooth disease type 1A.[47]

Locating Disease-Associated Genes by Cytogenetic Analysis

Direct cytogenetic analysis can also lead to mapping and discovery of disease-associated genes. In this approach, genes can be localized, or even identified, if the disease phenotype is associated with a chromosomal aberration that is visible in the microscope, e.g., a tiny deletion might remove the relevant locus, or a translocation might disrupt it.

This approach started with a little girl who we found to have sporadic bilateral retinoblastoma and an interstitial deletion of 13q.[48] Comparing this case with scattered literature reports allowed me to predict the location of *RB* in band 13q14,[49] and this information assisted in the cloning of the gene.[50] In a similar fashion, aniridia and Wilms tumor were both known to segregate as autosomal-dominant traits. In collaboration with Vincent Riccardi, our discovery of a 11p13 microdeletion in individuals affected by both conditions placed both loci into this region[51,52] and facilitated the cloning of the responsible genes.[53]

In 1984, we received a blood sample and lymphoblastoid cell line from an adopted boy (BB) who was apparently affected by four different X-linked diseases and who had been studied by several specialists in Seattle but for whom no genetic abnormality had been identified. On high-resolution chromosome banding, we discovered a tiny interstitial deletion of subband Xp21.2, but we needed a molecular probe to confirm that. We obtained Xp probes from collaborators, and there were few available at the time, but none were missing in BB. If the missing Xp21.2 material had been inserted somewhere else, we would not be able to see that microscopically. Therefore, we made somatic cell hybrids with a Chinese hamster cell line and isolated BB's X chromosome away from all his other chromosomes. Ultimately, one probe, called 754, from Peter Pearson's lab in the Netherlands, was deleted from his X chromosome and from his total DNA, proving that he indeed had a deletion.[54] We interpreted this finding as indicating that genes in the deletion might contribute to the expression of Duchenne muscular dystrophy (DMD), chronic granulomatous disease (CGD), retinitis pigmentosa, and McLeod syndrome, all manifested by BB.

Louis Kunkel (2004 Allan Award recipient) used the cells from BB to isolate DNA fragments from the deletion,[55] and Brigitte Royer-Pokora and colleagues subsequently used them to clone the gene for X-linked CGD.[56] This was the first human disease-associated gene cloned on the basis of its location with no prior knowledge of its function ("positional cloning"). Subsequently, we identified additional cases with overlapping Xp21 deletions and various phenotypes that allowed the mapping and ordering of disease genes in this chromosome band (Figure 3).[57–59]

To directly identify the DMD gene, we studied a female diagnosed with DMD and a reciprocal X-autosome

translocation. X-inactivation studies revealed the preferential inactivation of the normal X chromosome, and we separated the translocation chromosomes in SCH. Hypothesizing that the translocation had disrupted the DMD gene, we figured that cloning the translocation breakpoint might lead us directly to the gene. During a sabbatical at the European Molecular Biology Laboratory in Heidelberg with Hans Lehrach, I made a cosmid library from this individual. Joe Giacalone, a graduate student in my lab at Yale, isolated breakpoint-bridging cosmids and sequenced across the translocation breakpoints.[60] However, by that time, others had already succeeded in finding the DMD gene.

Finding Disease-Associated Genes by Positional Cloning

Moving to Stanford in 1989 as a Howard Hughes Medical Institute investigator in the new Beckman Center for Molecular and Genetic Medicine allowed me to expand work on a number of disease-specific projects. In the early 1990s, as the human linkage map became enriched by highly polymorphic RFLPs and microsatellite markers, more genes for Mendelian diseases were mapped and became candidates for positional cloning. One early success story involves the isolation, in my lab, of the gene associated with the X-linked immunodeficiency Wiskott-Aldrich syndrome (WAS). Jonathan Derry, a postdoctoral fellow, constructed a yeast artificial chromosome (YAC) and cosmid contig covering the region of Xp11.22–p11.23, to which the *WAS* locus had been assigned. Hans D. Ochs, a pediatric immunologist (who had also referred case BB) in Seattle provided us with lymphoblastoid cells from several of his WAS cases. Jonathan isolated cDNA fragments complementary to the YACs and tested them on blots of cases' RNA. Lack of expression in two of them led to the gene,[61] and mutations confirmed it.[61,62] The gene was expressed exclusively in hematopoietic tissues and encoded a previously unknown protein that we called "Wiskott-Aldrich syndrome protein" (WASP). Its function in modulating the actin cytoskeleton only became clear after collaboration with Arie Abo's lab.[63]

I had long been fascinated by Roberts syndrome, a rare autosomal-recessive condition characterized by limb and other malformations and associated with distinct chromosomal features: premature separation of centromeres and repulsion of heterochromatin regions.[64] The unique challenge here was to find the gene associated with a Mendelian disorder that can be diagnosed by cytogenetic analysis, and we approached it by looking for genes that could complement the cellular and/or chromosomal phenotype. First, while at Yale University, we showed that the characteristic chromosomal features are corrected in interspecies somatic cell hybrids.[65] David van den Berg, a graduate student in my lab at Stanford, then focused on cellular abnormalities in order to devise a selective system for complementation.[66] However, this extensive effort failed to lead to the discovery of the gene, which was later identified by Vega and colleagues via homozygosity mapping in inbred families as *ESCO2*, one of two human orthologs of a yeast gene required for sister chromatid cohesion.[67] Birgitt Schüle, a postdoc in the lab, documented *ESCO2* mutations not only in Roberts syndrome but also in individuals diagnosed with SC phocomelia, thus confirming that the two clinically defined conditions are allelic.[68]

Since the mid 1980s, I had been intrigued by Rett syndrome, a sporadic neurological disorder limited to females and characterized by postnatal onset and developmental regression, loss of hand use, truncal ataxia, apraxia, seizures, and acquired microcephaly.[69] Among many possible genetic hypotheses, de novo mutations in an X chromosome gene seemed to be the most plausible. We collected samples from families with girls affected by Rett syndrome, established lymphoblastoid cell lines, and tested their DNA for mutations in neuronal genes that we and others had mapped to the X chromosome, e.g., *SYN1*, *SYP*, *GLUD2*, *GDI1*, and *GRPR*, but we had no success. To narrow down the X chromosome region that could contain a gene associated with Rett synrome, we turned to exclusion mapping by studying rare familial cases.[70,71] In collaboration with Huda Zoghbi, we continued candidate-gene testing in the unexcluded Xq28 region, which led to the discovery of mutations in *MECP2* in Huda's lab.[72,73] The gene encodes MeCP2, a methyl-CpG-binding protein that binds to chromatin-modification factors and that was thought to function as a global transcriptional repressor.[74,75] I had considered this gene an unlikely candidate for Rett syndrome because chimeric mice harboring *Mecp2*-knockout cells had been reported to die in utero,[76] whereas Rett-syndrome-affected girls who are mutation heterozygotes and X-inactivation mosaics survive and are born normal.

Discovery of *MECP2* as the gene mutated in Rett syndrome generated a wave of scientific work in many laboratories with the goal of unraveling the reasons for the frequent recurrent de novo mutations and the pathophysiological mechanism of the postnatal neurological damage. Under the hypothesis of transcriptional dysregulation, we attempted to identify downstream targets by transcriptome-expression-array studies of single-cell-derived fibroblast clones from Rett-syndrome-affected girls with identified *MECP2* mutations[77] and from dissected brain regions of mouse mutants.[78]

To understand how *MECP2* itself is regulated and to define the "MECP2 functional expression module," Jinglan Liu, a postdoc in the lab, identified *cis*-acting control elements and employed chromosome conformation capture to generate an interaction map of enhancers and repressors with the *MECP2* promoter.[79] Despite the enormous amount of work in many neuroscience laboratories around the world, the complete pathophysiology of Rett syndrome is not yet understood beyond the recognition that the defect resides at the synapse.

In the interest of focusing on adventures in cytogenetics, I will skip over projects on genotype-phenotype

characterization of Marfan syndrome and related connective-tissue disorders; these were a collaboration with the Stanford Center for Marfan Syndrome and the experimental pathology lab of my late husband, Heinz Furthmayr.

Dissecting Microdeletion Syndromes

The next challenge in cytogenetics was the dissection of clinically and cytogenetically defined microdeletion syndromes. First, we needed to find all genes in the deletion and then determine which of the many deleted genes are responsible for which of the phenotypes by considering issues of haploinsufficiency and penetrance, gene-gene interactions, genomic imprinting, and potential effects of structural chromosome rearrangements on the expression of neighboring genes. The deletion syndromes we focused on, Williams-Beuren syndrome (WBS) and Prader-Willi syndrome (PWS), are caused by nonallelic homologous recombination (NAHR) between flanking repeats, and therefore the same genes are deleted in most cases.

WBS with multisystem manifestations and unique neurobehavioral features is caused by a barely visible 7q11.23 deletion that contains elastin (*ELN*).[80] Before the first draft of the human genome sequence became available, my lab developed a contiguous cosmid map across the 1.5 Mb WBS deletion and flanking regions by screening cosmid libraries and sequencing the ends.[81] Along the way, we discovered several protein-coding genes within the deletion.[82–88] To assess the functional consequences of gene loss, we knocked out one of them, *Fzd9* (encoding a Wnt receptor), in mice, but heterozygous-knockout mice did not show distinct phenotypes.[89]

Because cases with partial deletions of the WBS region are very rare, we decided to create *Wbs* partial-deletion mouse models by chromosome engineering. Mouse models would be useful for dissecting the molecular mechanisms underlying the unique features of WBS for physical and behavioral studies, for providing access to brain tissue, and for enabling control of the genetic background. The human 7q11.23 region is conserved in mouse chromosome band 5G2. Orthologous genes are in the same order, but the region is inverted with respect to the centromere, and there are no flanking repeats in the mouse. Rather than removing the entire orthologous *Wbs* deletion region, we generated mice with two complementary half deletions (PD and DD) (Figure 4). Interbreeding half-deletion heterozygotes produces litters comprising four different genotypes that can be compared in studies of phenotypes. Double heterozygotes (D/P) represent a model for the human WBS. The successful completion of this project required a huge effort by many skilled and devoted people over many years. The mice were studied extensively and found to reproduce some human WBS features that could be assigned to one or the other half of the deletion.[90] Increased sociability and acoustic startle response are associated with PD, and cognitive defects are associated with DD. Skulls are shortened and brains are smaller in DD mice, whereas in PD mice, the lateral ventricle volumes are reduced and neuronal cell density is increased in the somatosensory cortex. Motor skills are most impaired in D/P. Gene-transcript levels in the brain are generally consistent with gene dosage. Together, these partial-deletion mice replicate crucial aspects of the human disorder and serve for the identification of genes and gene networks contributing to the neural substrates of complex behaviors. The regions can now be dissected further by genetic complementation studies.[90]

In the case of PWS, the deletion involves an imprinted region, i.e., deleted genes are on the paternally inherited chromosome 15, and the maternal copies are silent. The PWS project started with our assignment of the first protein-coding gene, *SNRPN* (small nuclear ribonucleoprotein polypeptide N), to the deletion region in 1992.[91] We then systematically searched for expressed sequences in the region and found various expressed noncoding DNA segments[92] and a cluster of small C/D box nucleolar RNAs (snoRNAs) located in introns; we called these *PWCR1*.[93] Whereas the ~100 bp sequences of this snoRNA cluster were moderately conserved between humans and mice, the exons of the noncoding-RNA host genes were not conserved at all, suggesting a functional role for these intronic sequences.

To delineate the minimal deletion region responsible for the PWS phenotype, we studied reciprocal translocation cases by precise molecular mapping of breakpoints.[94] Taking our data together with others in the literature led us to propose that a small region between *SNRPN* and *UBE3A* and containing the *PWCR1* snoRNA cluster—discovered in our lab[93] and independently by Cavaillé et al. (who named it HBII-85)[95] and now called *SNORD116*—was responsible for the PWS phenotype.[96]

To test this hypothesis, Feng Ding in the lab took on the arduous task of making a mouse model in which the *Snord116* cluster was deleted by chromosome engineering in embryonic stem cells (Figure 5). In contrast to previous PWS mouse models that have a high rate of neonatal mortality,[98] the *Snord116*-deletion mice were viable. Extensive metabolic, dietary, and behavioral studies of mice carrying the deletion on the paternally derived chromosome revealed some PWS-like phenotypes, such as growth delay and hyperphagia, but lacked others, e.g., hypotonia and obesity.[97] Then, surprisingly, Art Beaudet's lab and others found that human cases with deletions of these snoRNAs met criteria for a PWS diagnosis, as discovered by array comparative genomic hybridization.[99,100] How the lack of SNORD116 snoRNAs produces the phenotype is a fascinating question. The answer involves discovering the normal function of SNORD116 snoRNAs. We know that they do not modify rRNA as most known C/D box snoRNAs do, but are they involved in other aspects of RNA processing such as mRNA turnover, alternative splicing, or RNA editing? Transcriptome expression arrays of dissected hypothalamic tissue at postnatal days 5 and 13 revealed similar expression profiles in mice with deletion and normal genotypes at both developmental

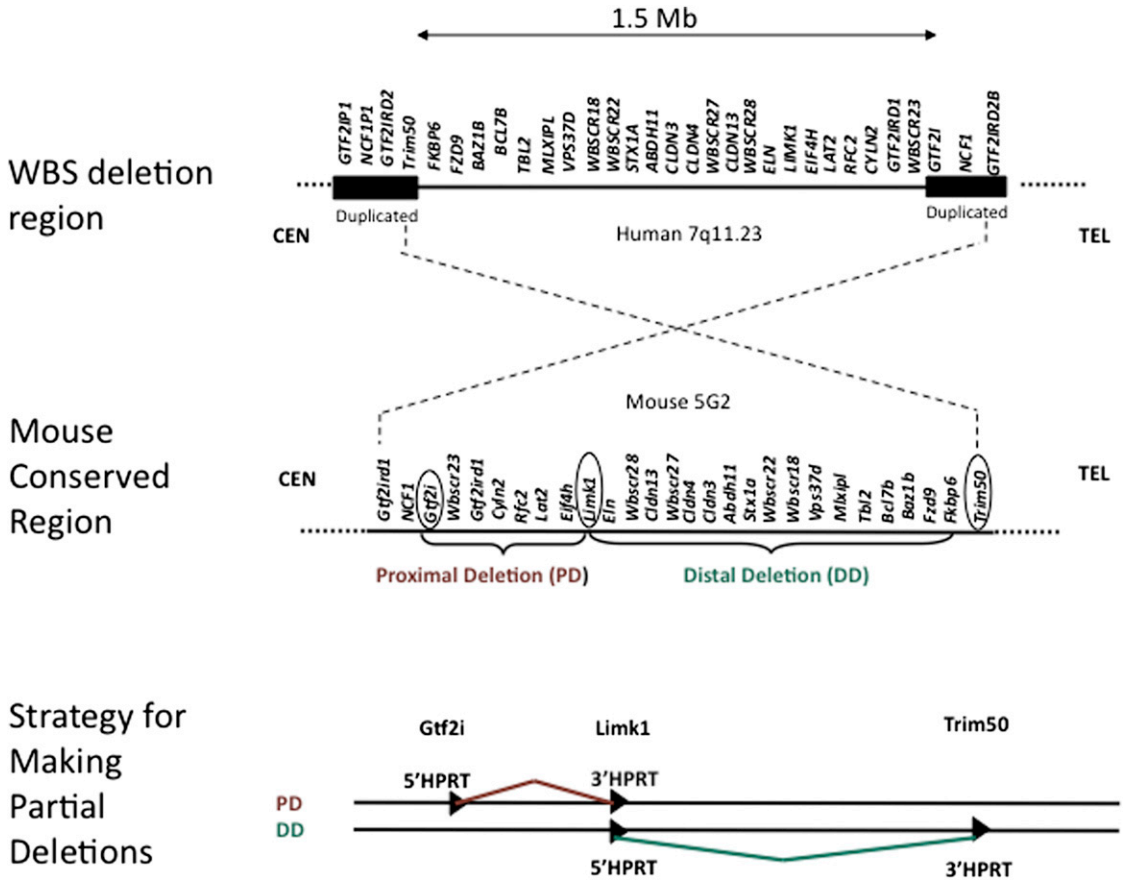

Figure 4. Generating Mouse Models for the WBS Deletion
The gene order across the WBS deletion region on 7q11.23 is conserved in mouse chromosome band 5G2 except for an inversion with respect to the centromere (CEN) and telomere (TEL). For the generation of partial-deletion mice, targeting constructs containing loxP sites (arrowheads) and partial *Hprt* sequences were inserted by homologous recombination in embryonic stem cells. *Cre*-mediated recombination between the loxP sites at *Gtf2i* and *Limk1* and between the loxP sites at *Limk1* and *Trim50* generated the proximal deletion (PD) and the distal deletion (DD), respectively. This figure was modified from Figures 1A and 1B in Li et al.[90]

stages.[101] When I closed my lab in 2008, Feng Ding took a faculty position in China and continues to study these mice. We also deposited them at The Jackson Laboratory to be available for any researcher who wants to tackle these questions.

Predicting the Future and Personal Genomics

In 1999, when ASHG last met in San Francisco, I served as president. In my presidential address, entitled "Human Genetics in the Information Age," I strongly advocated for open-access scientific publishing online. I did not submit my presidential address for publication in *The American Journal of Human Genetics* because the editor and board of directors at the time had not agreed to accommodate an open-access model for *The Journal* (the president has little power!), but a copy of my speech is available on the ASHG website.

Meanwhile, open-access publishing has been growing at an accelerating pace. Last year, about 17% of all scientific articles were published in open access-journals (biomedicine was the leading field), and the "online only with a fee" category is rising the fastest.[102] I believe that the next year will see further acceleration in the move to this publishing model hand in hand with the rapid expansion of massive open online courses.

In my 1999 presidential address, I also predicted that medical genetics services would be provided online: "There is a huge need to provide online genetic information tailored to consumers that is accurate, up-to-date, and accessible. There should be career opportunities for site developers and database curators," and "I have no doubt that interactive web-based systems will be developed that can provide accurate, timely, and individualized genetic information."

In 2006, when Anne Wojcicki and Linda Avers started the personal genomics company 23andMe and invited me to serve as consultant for the editorial team, I accepted because I knew that this was the direction I wanted to go in;[103] in 2010, I joined the company as a part-time employee. Working with the company's research team opened new opportunities for different kinds of research. With a rapidly growing database of more than 180,000 people genotyped (approximately one million SNPs) and a huge collection of self-reported phenotypes, 23andMe

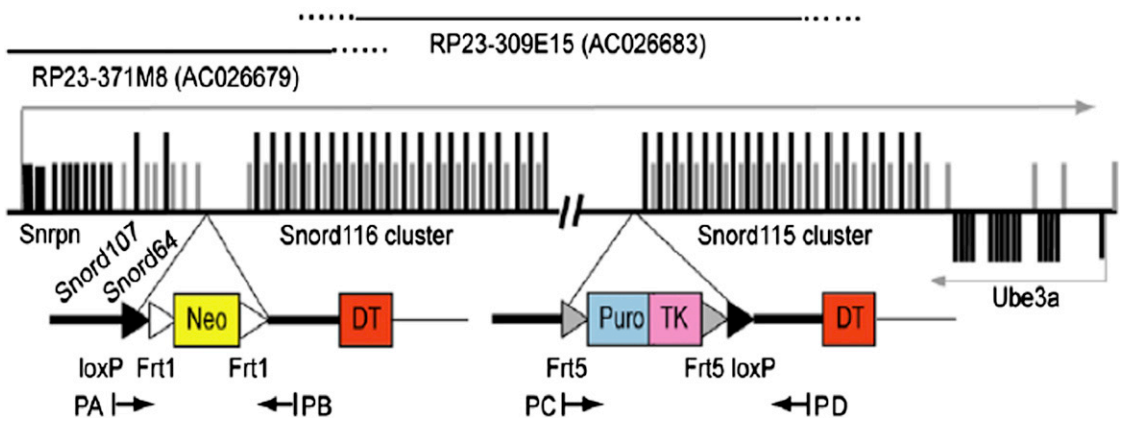

Figure 5. Generating a PWS Mouse Model in which the *Snord116* Cluster of Intronic C/D Box snoRNAs Is Deleted
A map of the PWS critical region that is conserved in mouse chromosome band 7C illustrates the location of the bacterial artificial chromosomes used for making targeting constructs. The large transcript originating from the *Snrpn* promoter (gray line with arrowhead) covers exons and introns. Short black vertical bars indicate coding exons for *Snrpn* on the left and for *Ube3a* on the right; short gray bars represent noncoding exons. High black bars represent intronic snoRNAs (these do not represent accurate numbers of elements in clusters; there are at least 41 copies of *Snord116*). Schemata of targeting constructs are at the bottom. Thick lines represent mouse genomic sequence, and thin lines represent vector sequence. Black arrowheads depict loxP sites, white arrowheads depict Frt1 sites, and gray arrowheads depict Frt 5 sites. Neo, Puro, TK, and DT boxes indicate the location of selection markers for PGK-Neo^r, PGK-Puro^r, HSV-TK, and diphtheria toxin, respectively. Positions and directions of the genotyping primers PA, PB, PC, and PD are indicated. This figure is from Figure 1A in Ding et al.[97]

researchers are able to quickly replicate published genome-wide association studies and detect novel associations[104,105]—as evidenced by the 19 abstracts the team submitted to this meeting.

In response to the controversy about direct-to-consumer genetic testing and the discussion about the sharing of incidental findings discovered by genome sequencing, we decided to implement an interview-based study on consumer reactions to BRCA test results. Our study provides data addressing one of the most urgent questions in the field of genetics and genomics: the impact of receiving unexpected highly penetrant genetic-risk information. The 23andMe Personal Genome Service only tests for *BRCA1* mutations c.185delAG and c.5382insC and *BRCA2* mutation c.6174delT, which are common in Ashkenazi Jews and convey a high risk of breast and ovarian cancer, and customers can choose whether or not they want to see their results. We invited all 136 BRCA-mutation-positive individuals who had chosen to view their BRCA reports to participate in this study and completed semistructured phone interviews with 32 mutation carriers (cases), of whom 16 were women and 16 were men, and 31 noncarriers (controls). None of the 11 women and 14 men who had received the unexpected result that they carried a BRCA mutation reported extreme anxiety, four reported moderate anxiety, and four women and six men described their emotional response as neutral. Female carriers sought medical advice and confirmatory testing, and many underwent risk-reducing surgery. Male carriers realized the risk for female relatives, and some felt burdened by this information. Sharing mutation information with family led to cascade screening of many relatives and the identification of additional BRCA-mutation carriers. Noncarriers did not report inappropriate actions, such as foregoing cancer screening. All but one mutation-positive participant appreciated learning about their BRCA status. Remarkably, six cases and six controls did not report Ashkenazi Jewish ancestry. Although the participants in this study might not be representative of the general population, their responses can inform the planning of future population screening programs.[106]

On the basis of what I learned during my personal journey in human genetics, what advice would I give to young people just starting out in the field?

- Acquire skills and knowledge in several areas; look for intriguing and important open questions that can be answered by the combination of various skills and approaches.
- Learn how to extract information from the rapidly growing -omics and medical databases, hone your informatics and computer skills, and don't be afraid of big data.
- Choose your collaborators wisely—to complement your own skills and knowledge—and treat them with respect; we can learn so much from each other.
- If you are a physician scientist, learn from your patients; the road between the clinic and lab is a two-way street, and with an anchor in both, you can be most productive.

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Web Resources

The URL for data presented herein is as follows:

American Society of Human Genetics, www.ashg.org

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