2018 Victor A. McKusick Leadership Award: Molecular Mechanisms for Genomic and Chromosomal Rearrangements[1]

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Thank you, Dave Valle, for that kind introduction and thanks to our Society for this terrific honor of the McKusick Leadership Award, which has very special meaning coming from the Society in which I grew up professionally. Experiential advice for the students, postdocs, and junior faculty that are present here today is that often some of your most important mentors are not at your institution, but rather sitting next to you here at the American Society of Human Genetics Annual Meeting; introduce yourself and get involved in your Society.

Genetic and Genomic Variation of the Human Genome

When we think of the human genome and how specific variation at a locus may result in different phenotypic outcomes, it is perhaps a good analogy to think of a diploid (i.e., two copies of each volume) 23-volume encyclopedia of the information required to sustain biological life (Figure 1). In this encyclopedia one has several "variant types:" a chromosome might be thought of as one volume of the encyclopedia. Single, simple, or small nucleotide variants (SNVs; Watson-Crick base pair changes), a type of variation that has also been referred to as a SNP (single-nucleotide polymorphism), could be analogized as a change in the alphabet that could change the meaning of a word. Likewise, indels, or small (<50 bp) insertion/deletions of Watson-Crick base pairs, could also result in a change in the meaning of the language of genetics by altering a word (codon), sentence (exon), or paragraph (gene) (Figure 1). However, there is another type or category of genetic and genomic variation, of a size between that which can be visualized microscopically by changes to a chromosome and that readily discerned molecularly by short read nucleotide sequencing—i.e., structural variants (SVs), including copy number variants (CNVs) (Figure 1). These CNVs can alter the gene copy-number and dosage at a genetic locus, causing deviations from the normal diploid state. Such deviations can have implications for haploinsufficiency and triplosensitivity disease traits; affect the interpretation of marker genotypes at a locus, sometimes distorting the results of linkage analyses;[4–6] potentially inflate the significance of association studies;[7] and if involving genes or even just single exons may contribute to disease gene discovery[8] and molecular diagnoses (CNV + SNV).[9,10]

In some respects, trisomy 21 (47,XY,+21), which is the variation of the human genome that causes one of the most commonly observed clinical phenotypes that we see as pediatricians and clinical geneticists, Down syndrome, is due to one large CNV. In trisomy 21, there are not really changes to specific genes, in terms of what you might observe as *de novo* DNA sequence changes specifying the sporadic trait, but instead three rather than the usual two copies of all genes on chromosome 21; i.e., at the chromosome 21 locus. From an alternative viewpoint, Down syndrome might be considered a multigenic trait with all the dosage-sensitive, i.e., triplosensitive, genes that map to chromosome 21 contributing to trait manifestation.

Structural Variants, Gene Dosage, and Genomic Disorders: The Evolution of a Human and Medical Genetics Concept

In genetics and genomics, it is of interest to trace the origins of the appreciation of CNVs, their contribution to gene dosage-dependent phenotypes or trait manifestations, and the evolution of our thinking regarding SV mutagenesis mechanisms (Figure 2). One of the first seminal papers that documented dosage, i.e., trisomy related to

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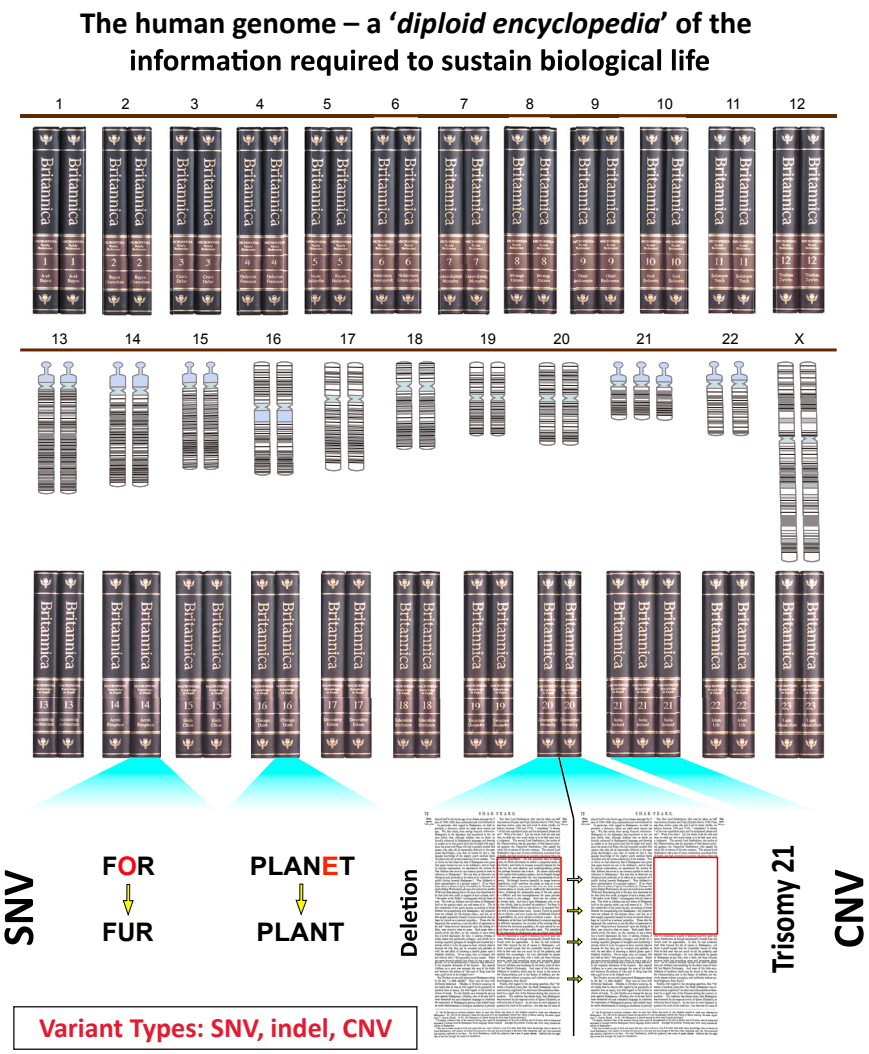


Figure 1. The Diploid Human Genome Can Be Analogized by a Duplicate Copy of a 23-Volume Encyclopedia Representing the Two-Copy "Diploid" Encyclopedia of Information Required to Sustain Human Biological Life

Volumes 1 through 12 of the encyclopedia; directly underneath are shown the chromosome karyograms and books for volumes numbered 13 through 23 (i.e., chromosome X in 46,XX females). The bottom of the page shows the different variant types and how they affect information flow for the words, sentences, paragraphs, pages, and volumes of the analogized human genome encyclopedia. Note, a typical SNV or Watson-Crick base pair changes that can alter the reading of the genetic code at that locus can completely distort the meaning of a word. In this analogy, trisomy 21 is shown to represent a large copy-number variation with three copies of volume 21 rather than the usual two in the diploid genome. Of note, the current version of the human genome is a haploid reference genome that does not account for copy-number variant information or SV haplotypes.[1–3]

phenotype, was a study published almost a century ago in 1922 by Albert Blakeslee at the Cold Spring Harbor Laboratories in New York.[12] He showed that the plant *Datura*, which has 12 chromosomes, could be found to have an extra chromosome of each one of the individual chromosomes (he coined the term simple trisomy, 2n+1), and there was an observed different plant phenotype for each of the different chromosomal trisomies (Figure 2). In 1936, Calvin Bridges used a relatively new genome technology, polytene chromosomes, to demonstrate that duplication of the *Bar* gene locus causes the bar eye phenotype. Bridges was part of the "fly group" at Columbia University but also performed some of these experiments at CSHL. Importantly, he showed that reversion of the duplication genotype results in a wild-type phenotype whereas triplication at the locus gave a more severe phenotype termed *ultraBar*[13] (Figure 2). It is interesting to note his discussion and interpretation of his data as the concept of "position effect" was perhaps prominent in the genetic thinking of the time: "The respective shares attributable in the total effect to the genic balance change (i.e., dosage) and to the position-effect change seems to be at present a matter of taste."[13]

In 1991, we documented that a 1.4 Mb duplication at the *CMT1A* locus resulted in Charcot-Marie-Tooth disease type 1A.[4] CMT1A (MIM: 118220) is a distal symmetric polyneuropathy (DSP)[14,15] usually of adult onset and observed as an autosomal-dominant (AD) trait with an age-dependent penetrance. This disease phenotype is caused by a gene dosage effect as shown by experimental evidence in a paper that appeared in the first issue of the new journal *Nature Genetics*.[16] Initial evidence that showed the specific dosage-sensitive gene was *PMP22* came from four groups simultaneously showing that this peripheral myelin gene maps within the CMT1A duplication genomic interval.[17–20] Other evidence included rare patients who did not have a duplication, but instead had point mutation, gain-of-function (GoF) missense alleles, of *PMP22*.[21]

Further mechanistic studies of the CMT1A duplication showed that *PMP22* was flanked by a unique human genome architecture of directly oriented low-copy repeats (LCRs), the 24 kb LCRs that were termed CMT1A-REP, and these could act as homologous recombination substrates and cause duplication by unequal crossing over and ectopic recombination,[22] a mechanism later termed by Pawel Stankiewicz as non-allelic homologous recombination (NAHR).[23] The mechanism predicted a reciprocal recombination product of deletion and this predicted

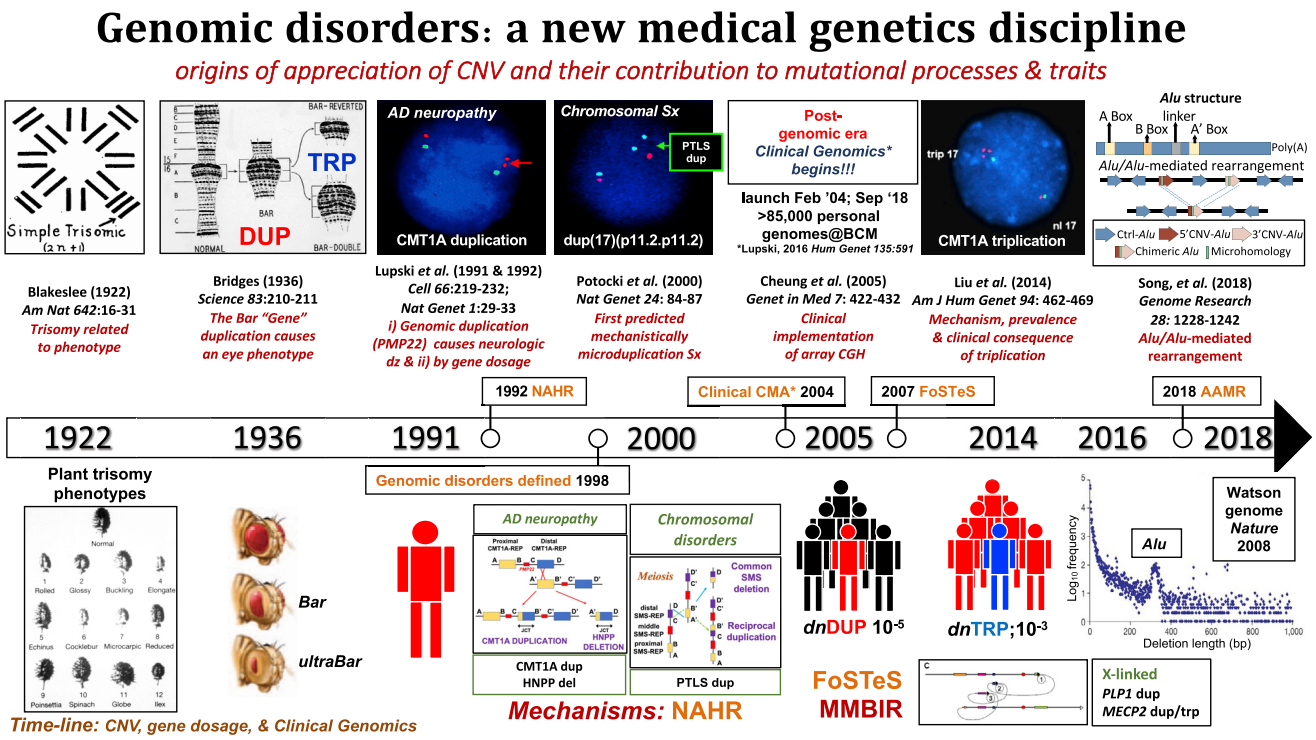


Figure 2. Timeline of Genetic Observations and Genetic and Genomic Variation Tied to Trait Manifestation Regarding Genomic Disorders and Medical Genetics/Genomics Practice

Above, key findings and papers. Below, phenotypes and traits. Also noted on the timeline, with the genome "phenotype" below, are the three major SV mutagenesis mechanisms: NAHR, FoSTeS/MMBIR, and AAMR and the *de novo* mutation frequencies for CMT1A duplication and triplication. The CMT1A duplication discovery opened a new field of genomic disorders "diseases caused by rearrangements of the genome incited by a genomic architecture that conveys instability." The conceptualization and mechanistic understanding (NAHR, FoSTeS/MMBIR, *AluAlu*-mediated rearrangements) of genomic disorders have prompted some medical geneticists to maintain that the clinical implementation of chromosomal microarray analysis (CMA) to detect genomic disorders, including their enormous role in causing developmental, cognitive, and behavioral disabilities, was the greatest clinical benefit to emanate from the human genome project—this was until 2013 when personal genome analyses by clinical ES became a reality. A timeline of the origins of appreciation of structural variation, copy-number variation, and dosage effects and their contribution to mutational processes and trait manifestations is shown. This timeline chronicles the development of the concept of genomic disorders and the implementation of such conceptual information into clinical genomics - an emerging new medical genetics discipline. (Figure modified, expanded, and updated from Lupski.[11])

deletion was shown in 1993 by Phil Chance's group to cause hereditary neuropathy with liability to pressure palsies (HNPP [MIM: 162500]).[24] Together our groups demonstrated experimentally that the CMT1A duplication and HNPP deletion were reciprocal NAHR products.[25] Consistent with a HNPP disease mechanism of *PMP22* haploinsufficiency was the finding of frameshift mutation, i.e., a loss-of-function (LoF) null allele, in association with rare non-deletion neuropathy in patients with HNPP.[26]

In 1997, we showed the same homologous recombination mechanism, NAHR, for the Smith-Magenis chromosome microdeletion syndrome (MIM: 182290) and suggested that reciprocal duplication could cause a chromosomal microduplication syndrome;[27] 3 years later, the predicted reciprocal recombination product was identified and it was shown to be responsible for the condition that bears the eponym Potocki-Lupski syndrome (PTLS [MIM: 610883]).[28,29] This paper that utilized chromosomal, molecular biological, and molecular cytogenetic studies of human subjects appeared in the first *Nature Genetics* issue of the beginning of the year 2000 and the new 21st century[28] (Figure 2), 100 years after Marcella and Theodor Boveri's work helped establish the chromosome theory of inheritance and the distinction of nuclear and cytoplasmic inheritance.[30] It established that SV mutagenesis mechanisms acting on genomes could generate reciprocal recombination rearrangement end products that explain chromosomal abnormalities.[28] The concept of genomic disorders was delineated in the early 1990s and formally summarized in 1998; it was predicated on two major premises: (1) disease phenotypes are due to DNA rearrangements, not DNA sequence changes, and (2) genome architecture incites genome instability.[31]

The first SV mutagenesis mechanism of NAHR causing genomic disorders was firmly established by experimentally finding the predicted reciprocal recombination products at several loci and defining the clinical conditions associated with them. Moreover, by establishing the NAHR mechanism as underlying chromosomal microdeletion/microduplication syndromes, it became clear that SV mutagenesis mechanisms were genomic DNA rearrangements underlying chromosomal abnormalities. Whereas

the mechanism for aneuploidies in humans were established as the cellular mechanism of meiotic nondisjunction, i.e., explained by perturbations in chromosome biology, little information was known regarding mechanisms for other types of chromosomal abnormalities. However, the evidence from studies of the SMS deletion[27] and reciprocal PTLS duplication[28] implicated perturbations in genome biology as underlying some types of chromosomal abnormalities.

During the next decade, much was learned about genomic disorders[32] and more was learned about downstream mechanisms for clinical manifestations in the decade that followed to the present day.[33] I have to admit I was quite excited to read Victor McKusick's comments published in 2007 in our Society's journal that stated: "Molecular cytogenetics and molecular genetics have narrowed the gap between Mendelian genetics and the classic cytogenetics of clinical disorders. This is illustrated by the conditions termed genomic disorders by Lupski, many of which show Mendelian patterns of inheritance."[34]

By 2004, there was a very good reference haploid genome enabling one to array different probes that mapped throughout the human genome and interrogate a personal genome for structural variation that might cause the disease trait ascertained in the clinic. The clinical genomics[35] assay that was initially implemented utilized bacterial artificial chromosomes (BAC clones) as probes for the array comparative genomic hybridization (aCGH) assay. Among the BACs arrayed were those that were used clinically in different fluorescence *in situ* hybridization (FISH) molecular cytogenetics assays to screen for microdeletion syndromes. Rapid expansion of that clinical genomics technology took place by having oligonucleotide arrays interrogating the human genome with more and more "pixels" allowing greater and greater resolution of the human genome and the detection in patients of more and more pathogenic rare variant SVs/CNVs in the human genome.[35]

In 2007 (Figure 2), after mapping the size, extent, and gene content of genomic CNVs, and extensive breakpoint junction analyses of the nonrecurrent rearrangements observed in patients with Pelizaeus-Merzbacher disease (PMD [MIM: 312080]), we proposed a new SV mutagenesis mechanism. This genomic rearrangement mechanism appeared to be a DNA replication mechanism for generating structural variation associated with genomic disorders.[36] The name proposed was fork stalling template switching (FoSTeS), emphasizing the interpretation of the data that showed evidence for long-distance template switching (TS) in a template-driven manner resulting in juxtaposition of genomic sequence from different human genome map positions and potential breakpoint complexity. Thus, rather than two pieces of DNA simply recombining or joining together, the breakpoint junction results from the bringing together or recombining of non-contiguous haploid reference genome sequence from two different map positions. At the breakpoint, one could often observe microhomology at the substrate human genome sequences that were joined, i.e., join-points. Thus, this was not a homologous recombination reaction, but rather the microhomology observed was interpreted as reflecting "primer binding" for the replicative recombination reaction. Moreover, this replicative recombination was "subtractive" for the microhomology versus "additive" for the microhomology,[37] the latter additive microhomology as evidenced by base pair duplications at the site of insertion for replicative transposition by the Shapiro model.[38]

Thus, from these data generated from studying nonrecurrent *PLP1* duplications associated with the genomic disorder PMD, experimental evidence showed that one was not dealing with simple end-joining. Instead, there was microhomology-mediated joining which was proposed to reflect the priming of DNA replication (that is, breakpoint can demarcate one or multiple join point(s) of discontinuous sequences). The evidence suggested long-distance TS facilitating template-driven juxtaposition of DNA sequences separated by large genomic distances in the reference haploid genome. Furthermore, breakpoint complexity could take place because of iterative TS. This idea of iterative template switching could explain breakpoint complexity but also could result in tremendous complexity to the genomic rearrangement or a rearrangement end product that revealed a complex genomic rearrangement (CGR).[37]

The FoSTeS concept of replicative rearrangements was further refined by combining observations and experimental data from bacteria and yeast to describe the mechanistic details of the DNA chemistry involved and this mechanism was termed microhomology-mediated break-induced replication, MMBIR.[39] The precise molecular mechanistic details that were delineated put forward the idea that a collapsed replication fork (likely resulting from replication proceeding through a nick in the DNA) generates a single-ended, double-stranded DNA, seDNA—this is distinct from a double-strand break which results in a two-ended, double-stranded DNA molecule. In the MMBIR mechanism, the 3′ end of one strand was exposed after extensive exonuclease degradation (5′ to 3′) that generates a long ssDNA with a 3′ end which can become a flexible primer for initiating DNA replication. This is followed by a new low processivity replisome that can disassociate repeatedly and reform with a different template, i.e., TS, and the replication then becomes a more well established and processive replisome that continues to copy the DNA. Such iterative TS can result in the breakpoint complexity.

More recent work from yeast has further supported some of the details of the MMBIR model including the TS mediated by microhomology of human *Alu* repetitive sequences placed in a yeast experimental system that allows induction of seDNA[40] and evidence for involvement of translesion polymerases.[41] As noted, seDNA can be generated at collapsed replication forks, but such DNA ends can also be generated at the end of a chromosome, i.e., telomeres,

as part of the end-replication problem or terminal deletions.[42,43] This mechanism might also generate an interrupted inverted duplication as can be observed with a breakage-fusion-bridge cycle accompanying telomere healing.[44]

For human genetics studies, the MMBIR model could readily explain nonrecurrent duplications and deletions, inversion, translocation, triplication, and higher order amplification—the latter from a proposed rolling circle replication. Furthermore, the MMBIR mutagenesis mechanism could begin to also explain a multitude of rearrangement end-products that were perceived as CGR, such as duplication-triplication-duplication (DUP-TRP-DUP) and DUP-NML-DUP array CGH patterns observed in humans with associated genomic disorders. Moreover, the MMBIR model made several predictions including: (1) increased SNV mutagenesis concomitant with CGR, (2) copy number neutral AOH when template switch occurs to the chromosome homolog versus the sister chromatid, and (3) higher order amplification, e.g., triplication, quadruplication, and beyond, might take place via a rolling circle type mechanism. All three of these predictions were borne out by experimental studies in human subjects.[45–48]

Perhaps one of the most interesting rearrangement end-product structures delineated was shown to result from just two iterative template switches (TSs): the initial breakpoint junction 1 (jct1) occurring by recombination between inverted repeats and junction 2 by a microhomology-mediated TS. This rearrangement end-product structure was termed DUP-TRP/INV-DUP.[45] Interestingly, by inverting a segment of DNA, new genes can be formed at the junction by now reading the reverse complement strand; and indeed the predicted transcripts were identified.[49] Thus, with only two TSs, complex structural variants could be derived and new genes generated. Moreover, studies on DUP-TRP/INV-DUP demonstrated that inverted repeats[45,50] and smaller-sized LCR (<1,000 bp) were important architectural features for human genome instability.

CGR can involve only a single exon, a single gene, or multiple genes.[51] They may include more than a single dosage-sensitive gene that can contribute to the disease phenotype due to triplosensitivity and as manifestations of a multigenic trait as shown for the Yuan-Harel-Lupski (YUHAL [MIM: 616652]) syndrome.[52] For deletions, multigenic haploinsufficiency traits[53] perhaps extend the Schmickel[54] contiguous gene deletion syndrome concept. With deletion CNV, the complexity of gene variants that may contribute to clinical phenotypic traits is sometimes greater than duplication CNV as the deletions cause a locus to be hemizygous and may unmask rare variant recessive alleles[55,56] or regulatory SNPs.[57] The multilocus complexity of a CGR can include an interstitial fragment of a chromosome or only its terminal end[43,58] or involve an entire chromosomal arm.[51] Multigenic contributions to the disease phenotype may also relate to gene interruptions at the breakpoint junctions and/or altered gene regulation due to position effects resulting from a rearranged genome or chromosomes.[59]

The MMBIR mechanism could explain insertional translocations,[60] small marker chromosomes (SMCs),[61] and some apparently balanced translocations.[62] It may also explain some cases of the somatic mutagenesis resulting in the phenomena of chromothripsis[63] originally described in cancer genomes and also such complexity observed as chromoanasynthesis[64] in association with developmental disorders. The extent of chromosomal/genomic structural changes that accompany chromothripsis/chromoanasynthesis/chromoanagenesis is staggering.[65] Finally, the perizygotic mutagenesis observed as a potential SV mutator phenotype appears to occur by MMBIR.[66]

By 2014, after almost a decade of extensive experimental analyses and implementation of clinical genetics testing for the CMT1A duplication in thousands of patients with neuropathy,[67] we determined the mechanism, prevalence, and clinical consequence of triplication in association with a more severe CMT1A peripheral neuropathy phenotype.[68] The NAHR mechanism driven triplication at the *PMP22* locus occurs as a 100-fold more frequent event from duplication to triplication than the frequency for *de novo* CMT1A duplication[69,70] (Figure 2). It was notable that after three quarters of a century we were given a chance in our Society's journal to illuminate some of the questions raised by the observations of Bridges and brought up in the discussion of his 1936 one-page paper,[13] although by genetics work from a completely different model organism, *Homo sapiens*.[68]

Consistent with the dosage hypothesis, triplication at the *PMP22* locus and the *MECP2* locus caused a more severe neurological disease phenotype than that which was observed with the duplication.[45,68,71] As anticipated by the gene-dosage hypothesis, CMT1A triplication gave a severe DSP and with similar severity to that observed with homozygous CMT1A duplication;[72] both instances have four copies of *PMP22*, the former with three copies in *cis* (i.e., heterozygous triplication) and the latter with two copies each in *trans* (i.e., homozygous duplication) having different implications for transmission genetics.[73]

The gene-dosage hypothesis for phenotypes, i.e., triplosensitivity and haploinsufficiency as mechanisms for disease traits, was strongly supported by data from the CMT1A duplication and HNPP deletion studies that solidified the concept of gene dosage. However, it is perhaps of heuristic interest that earlier human genetics data on gene dosage and disease were not appreciated and perhaps even misunderstood; the latter possibility might relate to understanding the difference between a disease population (i.e., a group of patients with the same diagnostic classification) and a disease trait. In 1987 French investigators demonstrated *APP* gene duplication in early-onset Alzheimer families and karyotypically normal (46,XY with likely submicroscopic duplication on chromosome 21) Down syndrome.[74] This was of interest

because of the known clinical association of early-onset Alzheimer dementia in patients with Down syndrome due to trisomy 21. Two reports then argued against APP duplication in Alzheimer disease.[75,76] Of note, these arguments were based on negative data studies in fewer than ten patients with Alzheimer dementia! It would take 20 years to overcome these negative data. Seven pages of negative data published in two papers, more than twice the three pages of positive data in the original paper. Twenty years later APP duplication was definitively shown to cause early-onset autosomal-dominant (i.e., AD) Alzheimer disease in five French kindreds.[77] I often wondered what would have happened if we had found the *PMP22* point mutation,[21,78] initially in 1 of about 100 CMT families studied, and not the CMT1A duplication?[4,79]

Finally, to bring the timeline (Figure 2) up to date from an SV mutagenesis mechanism perspective, this year we have provided evidence for perhaps a different mechanistic way to think about CNV of smaller sizes (e.g., <10 kb), particularly with respect to single exon drop out alleles. Evidence from the Watson genome, the first personal genome determined by massively parallel next generation sequencing technology,[80] documented an intriguing SV mutagenesis finding. Interestingly, allele frequency spectrum data for CNV deletion size showed that, for those deletion alleles <1,000 bp in size, there is a logarithmic increase in the frequency for which deletion CNVs are found in a personal genome. Remarkably, there is a large peak frequency observed at the ~300 base pair size that corresponds to the *Alu* repetitive sequence element (Figure 2). *Alu* is a repetitive sequence initially described by re-association kinetics, not genomic sequencing; it is distributed in ~1,000,000 copies as an interspersed repetitive element throughout the human haploid reference. Specifically, this ~300 bp peak allele frequency represents dimorphic *Alu* in the Watson personal diploid genome at a given map position whereby on one chromosome the *Alu* is present and on the other chromosome it is not present. It suggests that *Alu* transposition, or *Alu*-mediated rearrangement, might be responsible for a tremendous amount of evolution of the human genome.

Extensive analyses in patients suggest that for some loci a predominant mutational mechanism is exon deletion driving alleles within the disease gene and that these exonic deletions can be the result of *Alu-Alu*-mediated rearrangement.[50,81,82] Thus, in a recently reported paper we provided computational and experimental evidence that *Alu-Alu*-mediated rearrangement occurs by MMBIR.[83] In this *Alu-Alu*-mediated rearrangement (AAMR; Figure 2) mechanism, a TS occurs by using the *Alu* microhomology as a substrate sequence to form Watson-Crick base pairing of the primer and after TS initiate the replicative repair.[40,83] Of note, some of the first intragenic exonic deletion and duplication CNV alleles reported were found at the *LDLR* locus causing familial hypercholesterolemia with susceptibility to AD familial hypercholesterolemia (MIM: 143890) and atherosclerosis with early myocardial infarction as a complex trait.[84,85]

SV Mutagenesis Mechanisms, Mirror Traits, and Gene and Genome Evolution

In general, from experimental studies of personal genomes from individuals with genomic disorders, two types of simple deletion and duplication rearrangements have been delineated (Figure 3). One is recurrent rearrangements, sometimes also referred to as the "commonly found" rearrangement at a given locus, and the second is nonrecurrent rearrangements (Figure 3). What we have learned from the study of recurrent rearrangements is that the flanking LCRs represent a genome architecture, or intrinsic property of the human genome, rendering genomic instability. Moreover, these flanking LCRs can be used as homologous recombination (HR) substrates to mediate the rearrangement via NAHR. We have also learned that reciprocal recombination results in either deletion or duplication at the same locus.[88,89] The mechanism has further been shown to represent an end product of ectopic synapses and it is these ectopic synapses that then mediate an apparent ectopic recombination or an NAHR.[90]

Of note, from a human and medical genetics standpoint, for reciprocal recombination products the phenotypic features conveyed can sometimes be observed to represent mirror traits. The term mirror traits refers to opposite extremes of phenotype observed in association with copy-number loss versus gain at the locus (e.g., reciprocal duplication/deletion) with a copy number variant locus contributing to a quantitative trait.[91,92] Examples of mirror traits include obesity (metabolic syndrome) versus underweight (lean), microcephaly versus macrocephaly,[93,94] and short stature versus tall stature. Complex neurobehavioral traits, such as licking behavior in the chromosome engineered mouse models for PTLS and SMS,[95] can even represent mirror trait phenotypes. Some neurobehavioral and neurocognitive traits in human have been proposed to represent mirror phenotype extremes in a population distribution[96] —macrocephaly and microcephaly with autism and schizophrenia CNV, respectively, in association with reciprocal deletion or duplication at 1q21.1 and 16p11.2 provide further evidence for mirror traits.[97–101]

A number of mirror traits were described at the SMS/PTLS locus[86] including a mirror trait for weight/BMI (Figure 3B) also shown to contribute to metabolic syndrome in chromosome engineered mice.[87] Moreover, the mirror trait of "leanness" associated with duplication of this locus is resistant to environmental influences, such as a high fat diet (Figure 3C), on weight (gene by environment, G × E,[87] interactions).

NAHR can be the mechanism underlying some chromosome inversions, isodicentric isochromosome[102] formation [isodicentric17q, isoXq, and isoYq] (by nonsister chromatid recombination of inverted LCR on the short arm), or recurrent translocations.[103] Based on this mechanistic thinking of NAHR-driven reciprocal recombination, the

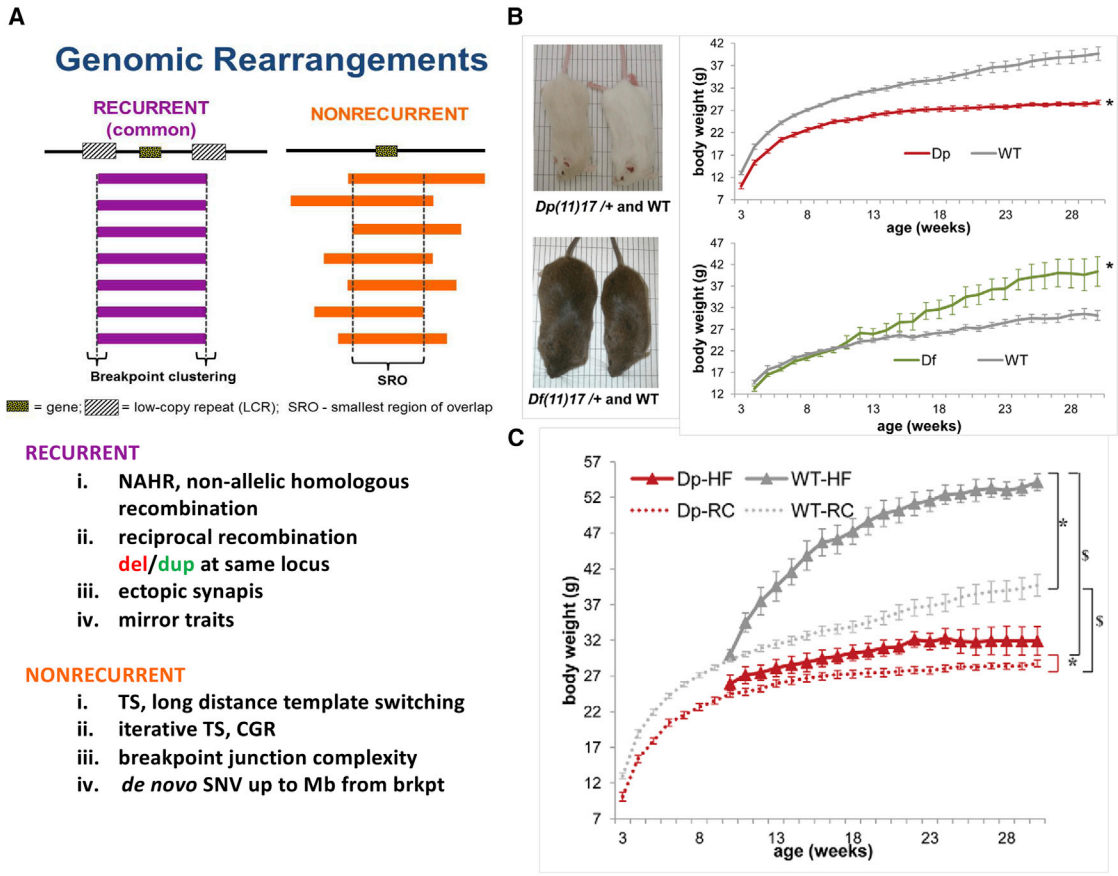


Figure 3. Two Major Categories of Simple Deletion and Duplication Rearrangement End Products: Recurrent and Nonrecurrent, and Lesson Learned from Studies of These Rearrangement End Products

(A) Definition of recurrent or common rearrangements where breakpoint clustering occurs in flanking low-copy repeat (LCR) sequences and a dosage-sensitive gene maps within the rearranged interval of the human genome. For nonrecurrent rearrangements, the breakpoints can be different in different patients, but the smallest region of overlap (SRO) identifies a potential dosage-sensitive gene(s).

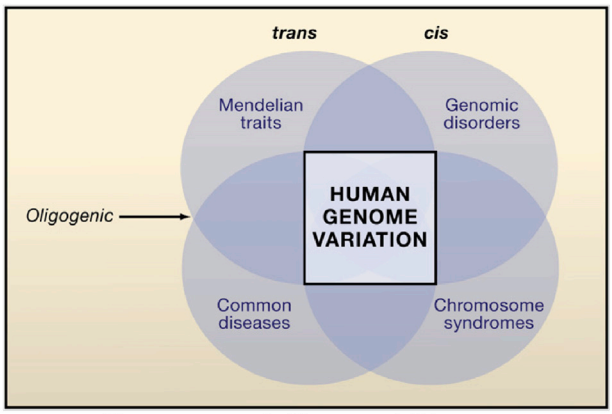
(B) Lessons learned from recurrent rearrangements include the concept of mirror traits whereby copy-number gain of a specific interval results in one phenotypic extreme or copy-number loss results in the mirror image trait phenotypic extreme as illustrated here for weight and the animal models for PTLS [*Dp(11)17*] and SMS [*Df(11)17*].[86]

(C) Documents that a mirror trait is truly a genetic trait at the PTLS locus in that there is protection from weight gain, obesity, and the metabolic syndrome, when the environment changes to that of a high fat diet fed to the animal model. Note, the wild-type littermates gain a tremendous amount of weight due to this environmental change and the G × E interaction, but heterozygous duplication animals are protected from weight gain and metabolic syndrome. Parts of figure modified from Lacaria et al.[87]

reciprocal recombination products, and genomic architecture of flanking LCR driving rearrangements associated with several genomic disorders, the specific loci underlying neurocognitive phenotypes/syndromes due to reciprocal recombination were found at many human genome map positions.[104,105] Evidence for new genomic disorders and CNV loci affecting neurocognition provide an opportunity to better describe and further characterize neurodevelopmental traits,[106,107] enabling potential biological insights allowing potential avenues for variant tailored therapeutic interventions and educational programs.

The second major class or type of observed rearrangement end product is nonrecurrent rearrangements (Figure 3A). The major mechanism involved here seems to be long-distance TS. Iterative TS can result in breakpoint junction complexity and can also result in CGR.[37] Because the replicative repair mechanism may use a polymerase that has reduced processivity, one may see breakpoint complexity as the fork collapses and undergoes another TS before the replisome is stabilized and a processive replisome is established. This processive replisome may replicate large genomic distances. Moreover, in this replicative repair mechanism, evidence suggest that the polymerase utilized has less fidelity than the intergenerational DNA polymerases and can generate SNV during replicative repair. Thus, one can create SNV hypermutation in proximity to the breakpoint junctions because of *de novo* SNV up to a megabase from the breakpoint junctions or join points formed during the TS in the MMBIR mechanism.[108]

This FoSTeS/MMBIR replicative repair mechanism appears also to play a critical role in the evolutionary processes as it may contribute to a host of observations regarding the evolution of genes and genomes, including: (1) gene duplication and divergence, (2) gene amplification (e.g., methotrexate resistance in cancer, via rolling



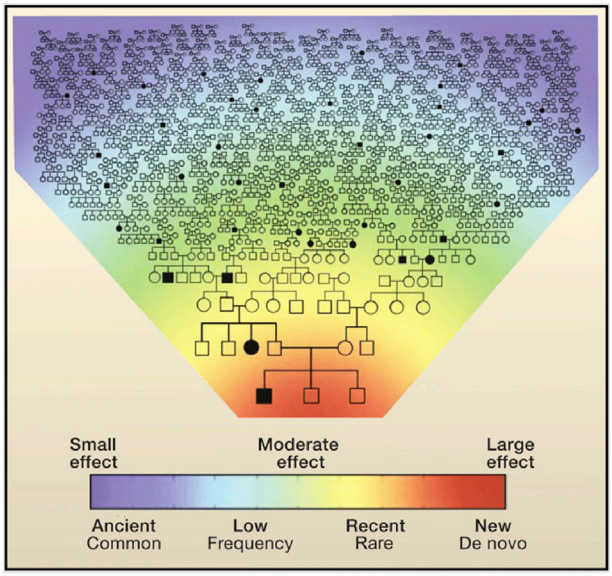


Figure 4. Clan Genomics, Rare Variants, and Medical Actionability
Above shows the types of conditions that can be influenced by genetic variation and human genome variation. These include chromosomal syndromes as analogized in Figure 1 and genomic disorders delineated and defined in Figure 2. Mendelian disease traits are due to single locus variation while some of complex diseases and multi-factorial traits may result from digenic and oligogenic inheritance or multi-locus pathogenic variation. Below analogizes the concept of a clan genomics whereby rare variants that have arisen recently in the patient, family, or clan are more likely to be the medically actionable variants and provide insight into the genetic influences on disease phenotypes as this variation is tied to disease biology and disease trait manifestation. Figure modified from Lupski et al.[109]

circle replication), (3) exon shuffling, (4) new gene creation by inversion, reverse complementary strand read at SV breakpoint junctions, (5) locus-specific mutation rates for CNV versus SNV at the locus = between 10^{-4} and 10^{-6} versus 10^{-8}, respectively, (6) one can regulate mutation rates via the FoSTeS/MMBIR mechanism. Thus, regarding the latter two points (5 and 6), structural variant mutagenesis mechanisms may contribute to the "mutational rheostat" essential to evolution—NAHR (meiotic) will require one or two generations to respond to environmental change whereas for FoSTeS/MMBIR this can happen during early embryogenesis and it may perhaps be that this mechanism is particularly prone to occurring during a perizygotic mutagenesis period with rapid replication/cell division during early embryogenesis.[66]

Rare Variants, Clan Genomics, and Disease Traits

SV mutagenesis and the genomic disorders caused by such rare variant CNVs have taught us more about human genome variation in the context of clearly observed clinical phenotypes. In general, four major types of disorders are observed in the clinic for which there are genetic influences. These include chromosomal syndromes, genomic disorders, Mendelian traits, and common diseases that may be complex traits. The latter complex traits are those disease traits for which there are clear genetic influences, but the phenotype does not show a Mendelian segregation pattern consistent with a single locus effect. Such traits may be multi-factorial traits with both genetic and environmental influences (Figure 4A). As DNA sequencing technologies were applied to personal genomes from many individuals, and one could begin to study variation of individual loci and personal genomes at scale, it has become apparent that the genome of any one individual has a tremendous amount of rare variation for which one did not begin to see its existence until the personal genomes from many different individuals and populations were studied.

This rare variation for SNV has similar observations to that which was discerned for CNV in clinical populations. The variant causing pathology might either be *de novo* or recently inherited within the family or clan structure. But perhaps more important and pragmatic for the individual and for clinical medicine was the conceptual realization of what rare variation and medical actionability actually might mean. It is the conceptualization of this realization that lead to the clan genomics hypothesis,[109] a term coined by Richard Gibbs. Simply stated, the clan genomics hypothesis argues that genome variation with respect to human disease and medical actionability has to do with rare variants (SNVs and/or CNVs) that have large effect and which have arose recently in the population history. Therefore, new mutation in you and your recent ancestors, and novel combinations of mutations you inherit from your parents at a locus through transmission genetics, account for many medically actionable variants.

The clan genomics model provides a powerful framework to contrast the importance of rare variants versus common variants in disease. It is distinct from the rare variant/rare disease (RVRD) hypothesis. Thus, clan genomics provides a theoretical framework for aligning human disease biology with evolutionary biology and genetics, the latter of which deals with per locus variation and trait manifestation. Thus, if the phenotype that is observed clinically is treated as a disease trait, the medically actionable variants will be the rare variants that represent

potentially pathogenic variation contributing to disease trait manifestations. Moreover, one can utilize a series of rare variant filtering strategies to discern this potentially pathogenic rare variation from the tremendous amount of variation found in each personal genome. Using such rare-variant, family-based genomic studies, one can narrow to a potentially small group of rare variants those which might contribute to the disease phenotype; this has ramifications for disease gene discoveries and for molecular diagnosis.

From one perspective, one might state that this concept of clan genomics is nothing new. We already knew that common variants have a small effect and rare variants have a large effect, and that is why Mendelian diseases perhaps are rare and that these were highly penetrant allelic variants in association with Mendelian disease. Nevertheless, the clan genomics hypothesis back in 2011 had profound implications for family-based genomics and rare variant filtering strategies to examine per locus variation in the context of the patient, family, or clan. Clan genomics is about patient, family, and clan—disease traits not disease populations.

This concept of clan genomics is further illustrated by case reports including, for example, one documenting cousins with Charcot-Marie-Tooth disease, in whom testing revealed one cousin had recessive Charcot-Marie-Tooth disease due to *MTMR2* homozygous mutation while the other cousin, one of fraternal twins, had a *de novo* *PMP22* duplication, the CMT1A duplication, causing the disease.[110] Of note, case/family study reports of molecular cytogenetic testing[111] and gene panel testing applied clinically[112] also resulted in some of the first clinical observations of multi-locus pathogenic variants in a patient. Using a family-based genomics approach and a trio analysis (proband with sporadic disease trait and unaffected parents) one can readily observe the new CMT1A duplication mutation associated with the sporadic trait in the nuclear family. Also, a quad approach in the cousin's nuclear family will find the homozygous mutation or biallelic variation associated with the recessive CMT trait in the cousin; homozygosity due to an identity-by-descent (IBD) of a rare variant that might be private to the clan. Thus, every time a search for a molecular diagnosis by clinical exome is performed and the rare-variant family-based analysis undertaken, one is testing the clan genomics hypothesis. No evidence has been obtained to reject the hypothesis, whereas a substantial amount of evidence in tens of thousands of exomes applied in research studies[113,114] and clinical studies[9] support the clan genomics hypothesis.

Family-based genomics can also be approached by determining whether there is evidence for a potential IBD due to consanguineous relationship between the parents. This can be performed on the exome data by determining a B allele frequency and using BafCalulator to find genomic intervals with absence of heterozygosity (AOH).[115] Whereas genetics is the study of single locus variation in the context of a family history, genomics takes into account both the family history and *de novo* mutation as well as the aggregation of pathogenic alleles at a locus by transmission genetics. Moreover, genomics allows direct assay of potential multilocus pathogenic variation to be assessed. Clan genomics studies the individual, the family, and the clan, which is a different emphasis from what is investigated by a genome-wide association study (GWAS) approach, which concentrates on a disease population. Thus, for medical actionability the most important thing for individuals regarding clinical implementation of their personal genome is what their nearest relatives (e.g., parents through transmission genetics) gave them and *de novo* events at that locus. The population from which the subject comes is not that relevant.

The clan genomics hypothesis has been tested in a broad study of the Centers for Mendelian Genomics (CMG) and collaborators. To date, about 60,000 samples have been collected from more than 22,000 families and the CMG and collaborators work to date includes almost 4,000 collaborators with subject samples coming from 80 countries worldwide. The CMG have begun to analyze more than 2,200 different phenotypes. Thus far, about 47,000 exomes and more than 1,000 whole-genome sequencing studies have been performed. The work of the CMG and collaborators continues to be disseminated through different means of making the information public. This includes more than 500 publications with 5,832 contributing co-authors from 1,540 organizations in 77 countries.[114] About 1,700 candidate disease genes have been identified, with the level of evidence in support of these disease candidates being quite extensive for about 600 of them. The remaining candidates will need verification in other families or with other variants being tied to the same or similar phenotype before being clearly established as a "disease gene." About 400 genes were known disease genes, but the phenotype observed expands beyond what the known phenotype had been associated with that gene to date as detailed in the clinical synopsis of the disease trait in the OMIM entry. This could be due to different allelic variants and an allelic series establishing the extent and variability of phenotypes that one may see with pathogenic variation at that locus. Alternatively, observed phenotypic expansion in an individual could be because there is a second locus for pathogenic variation and this makes it appear that there is phenotypic expansion.[115] Currently, the CMG and collaborators identify a novel candidate disease gene in about 1 in every 30 exomes that are performed on different subject samples.[114]

The clan genomics hypothesis has also been tested in the clinic where indeed a high rate of identifying patients with sporadic disease traits due to new mutations has been observed.[9] Current molecular diagnostic yield of clinical exomes is approximately 25%–30% of cases. Notably, research strategies for optimizing family-based analyses of exomes could be employed for molecular diagnosis.[116] Re-analyses of exome data[116] once or twice a year increases the molecular diagnostic rate. In a study following up the

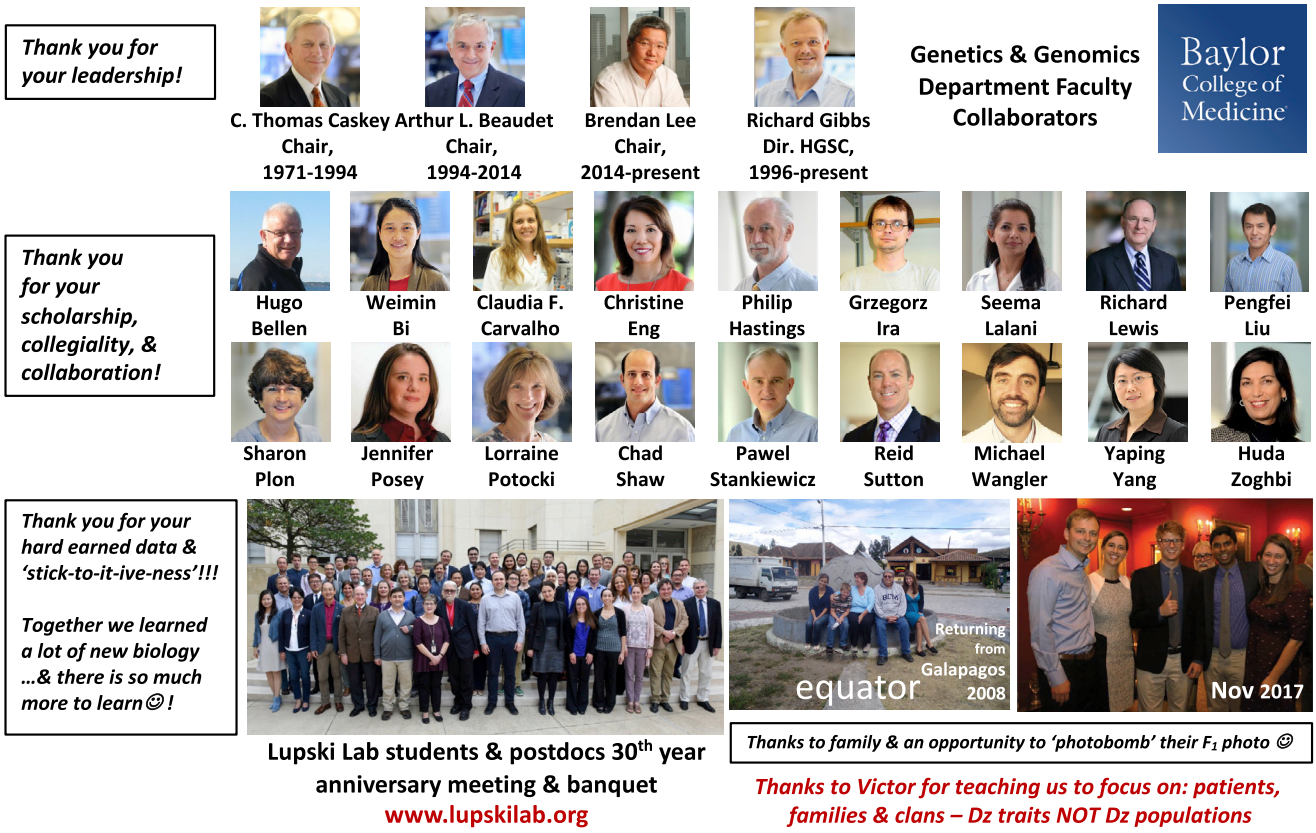


Figure 5. Many People to Thank and Apologize for Those Missing from the Collage

original publication of a pilot clinical genomics study using exome sequencing (ES),[117] it was shown that after a 5-year period, the molecular diagnostic rate had increased from 24.8% to 46.8% (unpublished data). Likewise, a follow up of the original 2,000 studied subjects who underwent clinical ES[9] documents that 3–4 years later, the diagnostic rate increased from 25.2% to 36.7%. Notably, in both of these re-analysis cohorts, the biggest contributor to new molecular diagnoses was new disease gene discovery. Other contributors were clinical updated information and utilization of additional family members for genomic studies (e.g., unaffected parents for trio analysis, affected siblings for analysis of shared rare variants). In the follow up of the *JAMA* study[117] a significant contributor besides variant re-classification was actual CNV detection.

An interesting finding to come from extensive analyses of thousands of clinical exomes is that the molecular diagnostic rate is approximately 30%. Of note, about 5% of the molecularly diagnosed cases actually have pathogenic variation at more than one locus.[118] Thus, about 1 in 20 families with a "solveable" clinical exome, i.e., productive for a molecular diagnosis, have a disease phenotype that results from genomic multi-locus pathogenic variation. Each variant at each individual locus may cause a dominant, recessive, or X-linked disease trait for the trait associated with each individual locus. For the loci including a monoallelic variant causing an autosomal-dominant (AD) trait, 68% were found to be due to *de novo* variants whereas of those causing an X-linked (XL) trait, 52% were found to be *de novo*. Remarkably, both variants were *de novo* in 44.7% (17/38) of cases diagnosed by mono-allelic variants (i.e., causing an AD+AD or AD+XL trait) at both loci.[118]

Patients having multiple molecular diagnoses can be challenging to clinicians because the patient can manifest a blended phenotype. These blended phenotypes can be further dissected into those whereby each locus causes *distinct* parts of the blended phenotype versus the blended phenotypes in which the clinical phenotype caused by each locus can have some *overlap* and it may just look like a more severe, for example, neurological disease if both of the gene loci affect the nervous system and cause cognitive phenotypes.[119] Dual diagnosis with *overlapping* phenotypes may represent a mutational burden within an interactome or pathway, biological structure/unit (e.g., cilia, neuron), or organ system.[118]

Recently, we documented that apparent phenotypic expansion can sometimes illuminate multi-locus pathogenic variation. Studies of intra-familial genotypic and phenotypic variability are able to show that a more severely affected individual in a family had multi-locus pathogenic variation while the less severely affected sibling inherited pathogenic variation at only one locus.[115] We have also recently obtained preliminary evidence that suggest the clan genomics hypothesis may extend to multi-locus pathogenic variation. In studies of arthrogryposis, a complex trait, we show that within the Turkish

population there is a high rate of multi-locus variation for which two or more loci both encode an AR disease trait and that the biallelic variants have come together because of IBD (unpublished data). Whereas in North American populations clinical diagnostic exomes have multi-locus pathogenic variation in about 1:20 solved cases, in the Turkish population of patients with arthrogryposis about 11% (11/101) of subjects had two or more loci with pathogenic variation. Remarkably, 88.9% (16/18) were AR+AR whereas the frequency of such cases in the North American cohort was 22% ($p < 0.0001$) (unpublished observation).

Studies using family-based genomics analyses from ES and whole-genome sequencing (WGS) have begun to elucidate genetic models that may apply to common disease and complex traits. These models include multi-locus pathogenic variation,[118] mutational burden,[120] and the compound inheritance gene dosage model.[121–123] The latter compound inheritance gene dosage model ties common variant alleles to disease biology and may be important at expression quantitative trait loci, eQTL. Compound inheritance may have particular relevance to birth defects as complex traits that result from perturbations in developmental pathways. Extensive studies in humans and mice on a spinal disease, congenital scoliosis, has elucidated this genetic model at the *TBX6*/*Tbx6* locus.[121–123] Recent work supports a role for compound inheritance in another birth defect—congenital anomalies of the kidney and urinary tract (CAKUT)[124] involving TBX6—and in lethal developmental lung disorders due to disruption of the TBX4-FGF10 pathway.[125]

In summary, clan genomics provides the theoretical framework for a rare-variant, family-based genomics approach in research and the clinic. Clan genomics also ties disease biology to evolutionary theory. SV mutagenesis[11] contributes to chromosomal syndromes, genomic disorders, and the allelic architecture (exon dropout alleles; CNV + dnSNV) of Mendelian disease traits. Of the molecularly diagnosed cases from a clinical referral population, about 1 in 20 individuals have two or more pathogenic variant alleles. Multi-locus pathogenic variation provides opportunities for exploring genetic models of complex traits. The compound inheritance gene dosage model[121–123] ties common variant alleles to disease traits and disease biology and may have particular relevance to birth defects where homozygosity for severe LoF alleles, or mutational burden in a developmental pathway, may result in embryonic lethality—a lot more of this to come during the next few years of study.

In closing, I would like to thank the students and postdocs who have passed through the Lupski laboratory for their hard-earned data and all that they have taught me, many thanks also to my terrific faculty colleagues and outstanding leadership of our Department and the Baylor College of Medicine Human Genome Sequencing Center (Figure 5). A special thank you to my family for taking this journey through life in science with me, and finally Victor McKusick for teaching us to focus on patients, families, and clans—disease traits not disease populations.

Declaration of Interests

J.R.L. has stock ownership in 23andMe and Lasergen, Inc., is a paid consultant for Regeneron Pharmaceuticals and the Regeneron Genetics Center, and is a coinventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting. The Department of Molecular and Human Genetics at Baylor College of Medicine derives revenue from chromosomal microarray analysis (CMA) and clinical exome sequencing (ES) offered in the Baylor Genetics (BG) Laboratory. J.R.L. is a member of the Scientific Advisory Board (SAB) of BG.

Web Resources

Baylor Genetics (BG) Laboratory, http://baylorgenetics.com
Centers for Mendelian Genomics (CMG), http://mendelian.org/
OMIM, https://www.omim.org/

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