INTRODUCTION

Making Chromosome Abnormalities Treatable Conditions

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Individuals affected by the classic chromosome deletion syndromes which were first identified at the beginning of the genetic age, are now positioned to benefit from genomic advances. This issue highlights five of these conditions (4p-, 5p-, 11q-, 18p-, and 18q-). It focuses on the increased in understanding of the molecular underpinnings and envisions how these can be transformed into effective treatments. While it is scientifically exciting to see the phenotypic manifestations of hemizygosity being increasingly understood at the molecular and cellular level, it is even more amazing to consider that we are now on the road to making chromosome abnormalities treatable conditions. © 2015 Wiley Periodicals, Inc.

KEY WORDS: chromosome abnormalities; gene dosage; haploinsufficiency; hemizygosity

How to cite this article: Cody JD,Hale DE. 2015. Making chromosome abnormalities treatable conditions. Am J Med Genet Part C Semin Med Genet 169C:209–215.

WHAT IS THE GOAL OF THIS ISSUE?

Emerging science on chromosomal abnormalities suggests that we now have the opportunity not only to expand our understanding of these multigene disorders, but also, for the first time, to develop specific targeted interventions. The goal of this series of articles on five of the earliest described chromosome deletions (4p-, 5p-, 11q-, 18p-, and 18q-) is to fundamentally shift the medical discussions and research directions about chromosomal abnormalities away from phenotypic description and toward genetically informed treatment. Although these conditions have been well described for almost 50 years, current treatment remains largely empiric, for example, hearing aids for those that are hearing impaired, rather than scientific, for example, therapeutic gene up-regulation. This journal issue will provide the most contemporary information on genotype/phenotype correlation, thus setting the stage for new and more integrative approaches for both chromosome abnormality research and treatment.

WHAT IS THE MAJOR CHALLENGE?

One of the major challenges confronting the study of chromosome abnormalities is that they suffer from a perception problem. This perception, set when they were first described decades ago, has not evolved, despite startling changes in many other medical disciplines. The problematic and pervasive attitude, recently voiced in the September 2014 issue of *Discover Magazine*, in an article about a child with 22q11.2 deletion is, "But there is no treatment for contiguous gene syndromes like 22q11.2 deletion; too many genes and complex biological systems are affected." The authors in this *Seminars in Medical Genetics* issue obviously disagree with the widely-held sentiment and will suggest possible pathways to effective treatments.

We also wished to address the perception problem in a more literal sense. The images shown in this issue are of people with the classic chromosome abnormalities who were all photographed by Rick Guidotti of Positive

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Drs. Cody and Hale have authored over 50 publications on the chromosome 18 conditions. In an effort to create a scientific community and ignite research interest on all chromosome abnormalities, they along with Dr. John Carey, hosted the World Congress on Chromosome Abnormalities in 2004 with over 500 families and scientists in attendance.

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DOI 10.1002/ajmg.c.31447

Article first published online 3 August 2015 in Wiley Online Library (wileyonlinelibrary.com).

Exposure. This program is dedicated to portraying the beauty of individuals regardless of their differences. And we, in like fashion, dedicate this issue to these individuals, many of whom we know as truly remarkable people from amazing families, who embrace their challenges with a smile and welcome us into their lives with open arms and hearts.

The critical breakthrough to transform simple phenotypic description into effective interventions was the mapping of the human genome. Hence, we now know exactly which genes are present in an abnormal number of copies in an individual. The next step is to determine the consequences, or lack of consequences, when a particular gene is present in an abnormal copy number, thus defining the dosage sensitive genes. This is our current challenge. Once those critical genes are identified, the potential pathways to treatment will become clear.

HOW DO CHROMOSOME ABNORMALITIES CAUSE DISEASE?

The molecular mechanisms leading to adverse outcomes in hemizygosity (i.e., haploinsufficiency) are fundamentally different from those of most single gene diseases. In recessive conditions, there are no functional copies of a gene and in most dominant conditions, mutations confer an adverse gain of function. In contrast, in hemizygous conditions, there is almost always one perfectly normal gene. This raises the question; "How hard can it be to upregulate a gene?"

The major challenge is the sheer number of genes involved in the relatively large chromosome abnormalities described in this issue. As many as 300 contiguous genes may be hemizygous in any affect individuals. However, this is not as big a challenge as it may appear on first consideration. As we proposed several years ago, [Cody and Hale, 2011] emerging data from three different lines of research continue to support our hypothesis that only 5–10% of genes are dosage sensitive. First, was the discovery that principle contributors to normal genetic variation (i.e., without overt adverse phenotypic effect) are gene copy number variants (CNVs). These CNVs, identified in large control populations, show that 91.24% of the genes in the OMIM Morbid Map and 91.18% of transcripts are overlapped by CNVs [MacDonald et al., 2013]. If 91% of genes and transcripts are found in CNVs in an apparently normal population, then these data imply that, at a minimum, 91% of gene products are not sufficiently sensitive to hemizygosity to be haplosufficient and produce an abnormal phenotype.

Second, the 1000 Genomes project demonstrates that each individual has roughly 80 heterozygous and 20 homozygous loss of function mutations [MacArthur et al., 2012]. A heterozygous loss-of-function mutation would be functionally equivalent to hemizygosity. Additionally, 26% of genes were determined to have heterozygous loss-of-function mutations in an Icelandic population [Sulem et al., 2015]. Together these suggest that many cellular processes are unaffected when there is a single functional copy of a gene.

Third, the converse of the dosage insensitive hypotheses is data on the percent of genes that are dosage sensitive. These data show that 5% of all genes have very low heterozygosity [Samocha et al., 2014]. This means that these genes are evolutionarily constrained with little functional coding variation demonstrating the evolutionary pressure for their being present in two functional copies.

Given these estimates, Table I shows the number of hemizygous genes for each of the conditions described in this issue and the estimated number of dosage sensitive genes supporting processes that are haploinsufficient. These numbers are shown in comparison to the actual numbers of dosage sensitive and conditionally dosage sensitive genes based on current data.

These data support the concept that the task of devising treatments for the classic chromosome abnormalities may not be as big a challenge as originally assumed because the number of genes to be upregulated is only a small percentage of the total.

HAPLOINSUFFICIENCY

Three basic mechanisms have been suggested for haploinsufficiency; subunit imbalance, metabolic rate limiting steps, and developmental regulation [Wilkie, 1994; Veitia and Birchler, 2010]. As we learn more about the molecular mechanism of specific examples these mechanisms are likely to be both expanded and refined.

One example of haploinsufficiency resulting from subunit imbalance is illustrated by protein components of cell adhesion complexes such as δ-catenin (cadherin-associated protein), delta 2 (*CTNND2*) on 5p15.2 at 11 Mb. δ-catenin is involved in neural cell adhesion and dendritic spine formation through its role with cadherin to create a cell adhesion complex [Yuan et al., 2013]. Reduced levels of δ-catenin could disrupt the integrity of the adhesion complex resulting in failed cell adhesion and signal transduction. Hemizygosity of this gene (in 5p-) leads to intellectual disability and impaired angiogenesis [DeBusk et al., 2010; Turner et al., 2015].

Haploinsufficiency caused by a metabolic rate limiting step is illustrated by *CYB5A* on 18q22.3 at 70 Mb. *CYB5A* enhances the activity of Cytochrome P450 17A1 (CYP17,20 lyase) which is in the biosynthetic pathway to dihydrotestosterone. Males who are hemizygous for this enzyme are at risk for cryptorchidism, hypospadias, and/or micropenis [Cody et al., 2014].

An example of developmental regulation haploinsufficiency can be found in individuals with hemizygosity for the *FLI-1* gene on 11q24.3 at 127 Mb. This winged helix-turn-helix transcription factor is involved in the regulation of megakaryopoiesis. Hemizygosity of the *FLI-1* gene can result in Paris–Trousseau thrombocytopenia [Hart et al., 2000].

OTHER MECHANISMS

In addition to haploinsufficiency, there are other potential mechanisms by

TABLE I. Hemizygous Versus Dosage Sensitive Genes

Condition	Number of genes in hemizygous region	Estimated number of dosage sensitive genes	Known number of dosage sensitive genes	Known number of conditional dosage sensitive genes	Initial Description
4p-	28	7	0	0	Cooper and Hirschhorn [1961]
5p-	314	16–28	5	6	Lejeune et al. [1963]
11q-	150	15	6	0	Jacobsen et al. [1973]
18p-	66	3–6	7	5	de Grouchy et al. [1966]
18q-(largest terminal deletion)	101	5–9	13	2	de Grouchy et al. [1964]
18q-reference group	37	2–3	5	2	

which hemizygosity can lead to an abnormal phenotype. These include a revealed recessive mutation, a conditional or compound effect and a position effect. A mechanism for the infrequent phenotypes associated with a hemizygous region could be through revealed recessive mutations when the remaining single copy of a gene has an inactivating mutation. This is not expected to be a common mechanism because human genomes each typically have approximately 100 loss-of-function mutations [MacArthur et al., 2012]. If there are approximately 20,000 genes in the human genome then 100/20,000 genes or 0.5% of genes would have a loss-of-function mutation. Therefore, phenotypes occurring in 0.5% of the cohort with a particular deletion might be due to a revealed recessive mutation. The study cited above also determined that each genome harbors approximately 20 homozygous loss-of-function mutations indicating that not all revealed recessive mutations will have a deleterious effect. Phenotypic characteristics, or more extreme versions of common phenotypes occurring with a low frequency, may be the result of a revealed recessive mutation. Conversely, revealed recessive mutations are unlikely to be the mechanism of the common phenotypes associated with a hemizygous deletion.

Secondly, abnormal gene dosage may be a risk factor or conditional factor contributing to, but not solely causing, an abnormal phenotype. For example, hemizygosity of the *TWSG1* gene on 18p is associated with holoprosencephaly, but only in the presence of a second mutation in the *SHH* gene on chromosome 7 [Billington et al., 2014]. Less direct connections to disease may include gene deletions identified more commonly in people with conditions such as autism than in controls [Krumm et al., 2013]. Hemizygosity could be one of many factors that change the risk for of a condition without being a direct cause. This means that copy number variations identified in self-declared normal individuals, may still be risk factors for disease or variants that are conditional and requiring an additional genetic variant to be present in order to cause disease. This is distinct from genotypes with low penetrance because in conditional genotypes a secondary variant is always required in order to produce an abnormal phenotype.

Thirdly, an abnormal phenotype could result from functional hemizygosity when the position effect of a gene(s) near the deletion breakpoint renders it inactive. There are two ways in which this could happen. First, the existence of regulatory elements in a hemizygous region for genes that are nearby and not themselves hemizygous may render the gene functionally hemizygous. Second, the formation of a neo-telomere at a breakpoint could mask a gene within heterochromatin. This position effect mechanism would not necessarily apply to all copy number changes but would be dependent on the breakpoint and the morphology of individual genes and their regulatory elements near the breakpoint [Ibn-Salem et al., 2014].

WHAT IS THE ROAD TO MAKING THESE TREATABLE CONDITIONS? GENOTYPE/PHENOTYPE MAPPING

The well-established approach for identifying dosage sensitive genes in cohorts of patients with hemizygosity is genotype/phenotype mapping or more
appropriately termed; phenotype mapping. The goal is to link a specific a specific phenotype with a specific gene within a hemizygous region. This is an iterative process that hones in on smaller and smaller-shared

hemizygous regions to identify critical regions within which the causative gene lies, until a single gene is identified. There are three factors that are essential for this approach to be successful. The first is high resolution genotyping to determine exactly which genes are hemizygous. This is now standard practice in today's molecular genetic laboratories. The second is a cohort of a large number of affected individuals with a particular chromosome abnormalities with a variety of deletion sizes and locations; the most informative being those participants with interstitial deletions. The more variety in the size and position of hemizygous regions, the more potential there is to define small regions. The third, and most important factor today, and assuredly the most complex, is precise phenotyping.

By precise phenotyping, we mean several things. Firstly, the phenotype should reflect as closely as possible its physiological basis. Second, the phenotype is objectively observed and described. Third, the phenotype is unique in that it is not associated with numerous known genetic causes. The widely used phenotypes of "intellectual disability," "hypotonia," and "failure to thrive" are so generic that they can be applied to almost all chromosome abnormality. The use of such imprecise phenotypes led to the concept that chromosome abnormalities affected virtually all biological systems and therefore, the individual hemizygous genes involved were not relevant [Shapiro, 1989]. However, there are numerous causes of failure to thrive, ranging from complex congenital heart disease, to inherited metabolic conditions, to hormonal deficits. Each of these involves different biological systems and unique genes. New tools, such as MRI, and mass spectrometry, and new understanding, such as executive function versus simple IQ, provide new insight and allow increasing precision in phenotyping.

As an example of a relatively precise phenotype, rare malformations are the prototype. This is illustrated by congenital aural atresia in the absence of microtia. This phenotype is not found in conjunction with numerous syndromes, but is a common phenotype in people with terminal deletions of 18q. In addition, the tools for assessing this condition are well standardized. Thus this is an excellent and relatively precise phenotype for phenotype mapping and consequently the critical region and then the gene were successfully identified on 18q- [Feenstra et al., 2011].

There will undoubtedly be individuals with hemizygosity for a critical region who do not have a specific phenotype and who are therefore non-penetrant. Such calculations have clinical relevance with regard to the genetic counseling of future patients with hemizygosity for the region. The potential for non-penetrance is also the reason that the absence of a phenotype in someone with hemizygosity cannot be used to narrow a critical region.

It is also important to point out that not all phenotypes are evident in the early years of life and even those that are evident may evolve over time. Therefore, the approach with the highest likelihood of yielding the optimal information on any phenotype is to to maintain ongoing relationships with a large cohort of individuals with a shared region of hemizygosity. When a new phenotype, or evolution of an established phenotype, is identified in one individual, the entire cohort can be assessed for that phenotype using a standardized protocol. Likewise, as the physiological or biological nature of a clinical phenotype is appreciated, additional testing of the cohort may be necessary. Additionally, as new data emerges on the function of genes within the hemizygous region those genes with data supporting a haploinsufficiency mechanism could prompt assessment or reassessment of participants. An example of this involves the *SMCHD1* gene on 18p described in this issue. Therefore, long-term relationships between scientist, participants, and lay advocacy groups are essential to the success of phenotype mapping and ultimately gene dosage annotation.

ANNOTATING THE GENOME—GENE DOSAGE MAP

One goal of the work that is described in the five articles in this series is to establish a paradigm for determining the consequence of having only a single copy of a gene. These consequences may range from having no detectable deleterious effect to lethality. Great progress has been made in identifying specific genes over the past five decades; however, there are still many new genes and novel gene functions and mechanisms to be explored. This is especially true for genes that are present in atypical numbers. As a means of tracking the emerging science of each gene on a particular chromosome, we have developed "custom tracks" on the University of California Santa Cruz Genome Browser. These custom tracks allow for a visual representation of the location of critical regions and genes. These tracks can also be labeled or color coded to indicate different meaning. In addition, they provide a mechanism to create linked pages containing details about data from other studies such as knockout mice or molecular genetics that specifically pertain to the effects of abnormal gene dosage. As an example, we have created a Gene Dosage Map for chromosome 18 [Cody et al., 2009]. Genes are categorized as haplosufficient, haploinsufficient, conditional haploinsufficient, or haplolethal and are revised continually as new information becomes known. In this way, the coordinates of a region of hemizygosity from a diagnostic molecular genetics laboratory report can be entered into the UCSC Genome Browser within the Gene Dosage Map custom tracks. The genes in the region in question are labeled by dosage category. At this point in time, 80% of the genes on chromosome 18 can be given a dosage designation.

The data used to create the Gene Dosage Map for chromosome 18 are primarily informed by data on the heterozygous knockout mice, the

existence of CNVs that encompass the gene of interest and data about the gene with regard to molecular mechanisms of disease. Additionally, we have extensive phenotype/genotype correlation data on our cohort of over 400 people with chromosome 18 copy number changes. For example, genes involved in dominant loss-of-function conditions are categorized as haploinsufficient. Conversely, genes involved in recessive conditions are by definition haplosufficient as well as genes that are hemizygous and in CNVs in multiple people in control populations. Data from all these sources are considered in order to determine a gene dosage category. Genes can be reassigned to a different category as new information is discovered.

One category which we anticipate will grow is the "conditional dosage sensitive" category. Genes in this category do not cause an abnormal phenotype by simply being present in an abnormal copy number. Instead they required a second genetic or environmental event before their effect is unmasked. As we study, more individuals with higher resolution phenotyping and genotyping involving the entire genome instead of just the hemizygous region, we will likely find that the majority of genes have an impact somewhere along a continuum from slightly shifting the risk of an abnormal phenotype to being a necessary but not sufficient conditional component in causing an abnormal phenotype. These more subtle effects will be more difficult to define.

MAKING ONE GENE DO THE WORK OF 2; HOW HARD CAN IT BE?

For single gene disorders, there are several examples in which modest increases in gene expression can alleviate a medical condition One example is, erythropoetic protoporphyria, caused by loss of function mutations in both copies of the *FECH* gene can be rescued if only 1 allele is able to sustain the expression necessary for 30% enzyme activity [Gouya et al., 2006]. Therefore, increasing expression to 100% normal activity may not be necessary in order to effect a treatment. Thus, it is reasonable to assume that were modest changes in gene expression feasible, these changes might modify certain consequences of the hemizygous state. While there are not yet examples of this in the five papers in this series, there are a variety of medications already in use that increase gene expression and ameliorate clinical conditions.

Therefore on a theoretical basis, treatment should be straight forward. It may not even be necessary to double the expression of a hemizygous gene. It may only be necessary to increase the expression by a much lesser extent in order to attain normal functionality.

Even for those conditions that are not recognized until after birth in most children, there is still a rationale for understanding the causative genes, since the same gene that plays a role in differentiation early in embryonic life, may play a different role in the child. Additionally, knowledge of a gene's role in development can impact the treatment plan. For example, the *TSHZ1* gene is responsible for causing congenital aural atresia in 18q-. The treatment for this malformation is canaloplastic surgery. However, in our natural history database, we have observed that a large number of these surgeries are unsuccessful in the long-term because the ear canals collapse and additional surgery is required. If we better understood the growth factors involved in the failure of the ear canal to form properly in the first place, we might be able to effect those factors and improve the long-term surgical outcome.

The ultimate goal of the investigators and families is to make chromosome abnormalities treatable in order that those affected should have healthy and automatous lives. Reaching this goal will require investigation on several simultaneous fronts as shown in Figure 1. The figure is organized to indicate the earliest steps at the top moving more toward treatment knowledge at the bottom. This work will involve investigators with expertise in clinical trials, clinical evaluation, cell biology, genetics, and mouse models of disease. Conceptually, there are three types of treatments. Standard treatments include the use of medications that are already efficacious in typical children, for example, treating growth hormone deficiency with growth hormone replacement therapy. Another potential source of treatment might be "repurposed drugs." These are drugs that have been approved for human use, but were ineffective for the intended therapy. The timeline for the use of such therapies conceivable could be much shorter because preliminary human studies have already been completed. Lastly, there is the development of novel compounds specifically developed for treatment of a specific genetic defect. These will obviously take the longest to develop.

The rate limiting steps in this plan are the ability to understand the pathophysiology of specific human phenotypes and the capacity to develop biomarkers for treatment endpoints that are relevant and meaningful for cognitive and social impairments. These gaps in knowledge, while expensive and time consuming to address, are critical in moving treatment forward.

CONCLUSION

When these five conditions were first described, it was thought these were the rare variants with catastrophic effects. We now know that genome copy number changes are not necessarily rare and don't necessarily have catastrophic consequences. Indeed, 1 of every 60 babies born has a de novo genomic copy number change large enough to include at least 1 gene [Itsara et al., 2010] and that these deletions can involve any chromosome. This suggests that gene copy number changes are potentially the single most common cause of congenital disability. Except that we also know that genomic copy number variation is a common genetic variant without phenotypic effect. Resolving which changes have phenotypic consequences and which do not is of considerable importance.

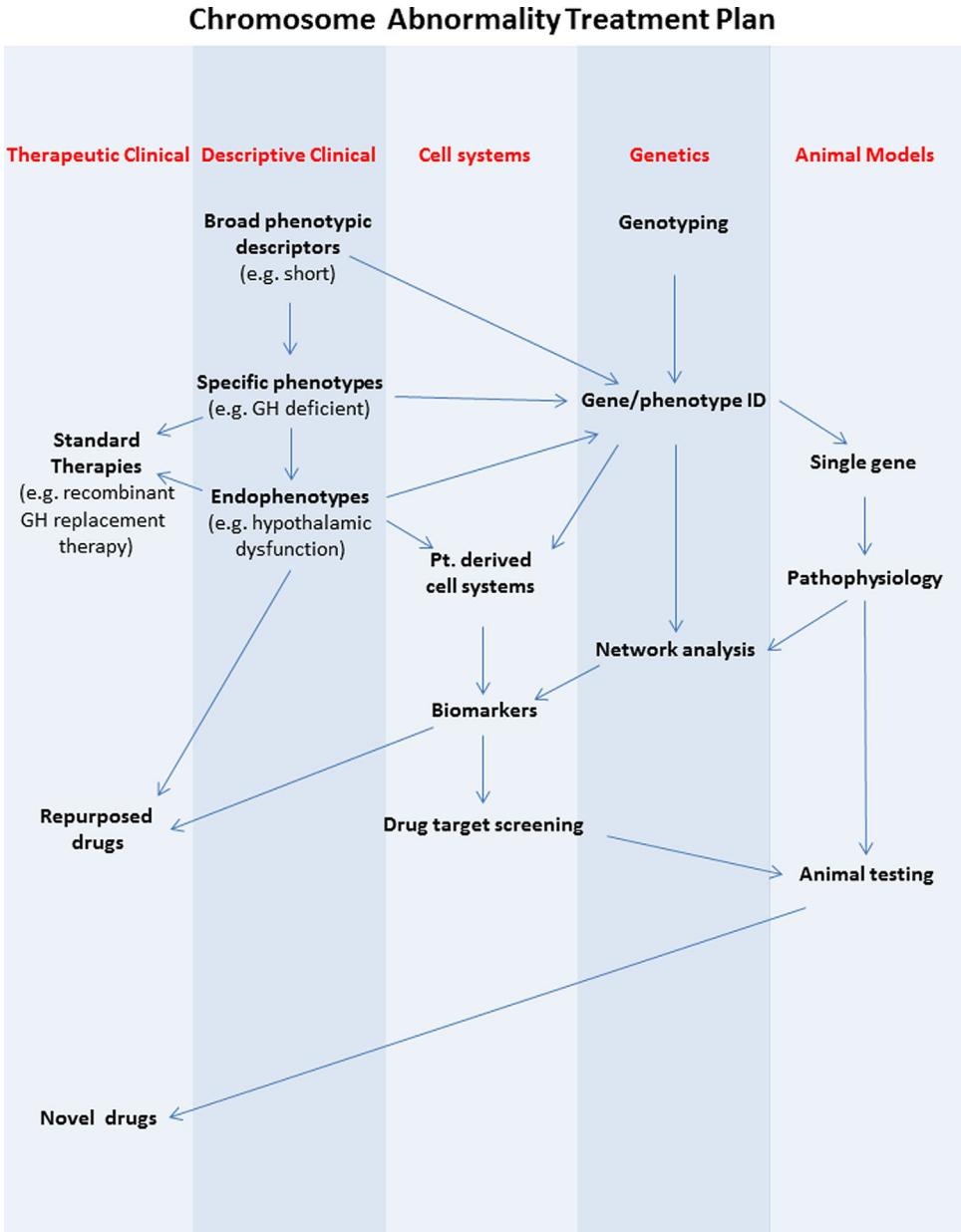

Figure 1. There are five broad categories of research that will be involved in the development of treatments for chromosome abnormalities. These are labeled across the top of each column in red. The remainder of the figure is arranged as a general timeline from top to bottom indicating the types of studies that need to take place. The relationship of these studies with regard to gaining information from other areas of research and contributing information to other areas is indicated by the arrows. One example is shown in parentheses. GH, growth hormone.

Second, few of these chromosome deletions involve recurrent copy number changes; meaning that the majority of people with chromosome deletions have unique copy number changes.

This means that the tactic of binning by common genotype to define a "syndrome" is not a feasible approach to clarity. Rather, a gene by gene annotation to understanding the consequences of a copy number change is a more useful approach. In this way, any hemizygous region can be understood by identifying the genes involved because the consequences of abnormal gene dosage for those genes is known.

The manuscripts in this issue of *Seminars in Medical Genetics* review the current state of the science for five of the classic chromosome deletion syndromes; each of which was identified at the beginning of the genetic age. While these were some of the earliest identified, they are by no means the only, or archetypal, chromosome deletion conditions. However, they do have the longest history and consequently a larger and older cohort of individuals than many other such conditions. As such, these five

conditions can serve a point of reference for other conditions involving hemizygosity.

Lastly, but most importantly, the conditions highlighted in this issue illustrate the challenges and opportunities of the field. The challenges are numerous but for the first time the steps necessary to make chromosome abnormalities treatable can be defined, planned, and executed. These defined steps present a grand scientific opportunity to address a whole category of conditions previously overlooked by much of the scientific community.

ACKNOWLEDGMENTS

The authors wish to thank Dr. John Carey for his many years of support, encouragement, and friendship in our common goal of making a better life for people with chromosome abnormalities. The authors have no conflicts of interest to declare.

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