Trends in Genetics

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REVIEWS

Review

The Consequences of Abnormal Gene Dosage: Lessons from Chromosome 18

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Accurate interpretation of genomic copy number variation (CNV) remains a challenge and has important consequences for both congenital and late-onset disease. Hemizygosity dosage characterization of the genes on chromosome 18 reveals a spectrum of outcomes ranging from no clinical effect, to risk factors for disease, to both low- and high-penetrance disease. These data are important for accurate and predictive clinical management. Additionally, the potential mechanisms of reduced penetrance due to dosage compensation are discussed as a key to understanding avenues for potential treatment. This review describes the chromosome 18 findings, and discusses the molecular mechanisms that allow haploinsufficiency, reduced penetrance, and dosage compensation.

Highlights

Abnormalities of gene dosage result in a wide number of effects ranging from being causal for disease to acting as a risk factor or having no clinical effect.

Those affects may manifest congenitally or later in life.

Reduced penetrance for any of the effects is a common finding and is an essential element of the clinical interpretation of genomic copy number variation.

Understanding the mechanisms underlying reduced penetrance due to dosage compensation could provide important insight into potential treatment strategies for people with chromosome abnormalities.

How Does an Abnormal Number of Copies of Normally Functioning Genes Cause Disease?

Detecting genomic **copy number variation (CNV)** [1] (see Glossary) is now routine in clinical practice with utility throughout the lifespan. CNVs are important prenatally in the detection of life-limiting syndromes [2], postnatally as explanations of birth defects or developmental delay [3], and later in life as edifying or anticipatory for adult-onset conditions [4–7]. While detecting CNVs has become straight forward, predicting their impact is a more elusive and nuanced endeavor .

Understanding the effects of abnormal gene dosage, both gene duplication as well as **hemizygosity** are equally important. However, this discussion focuses on the effects of hemizygosity because there are significantly more data about the effects of individual gene deletions due to the widespread use of knockout mouse models. Additionally, the molecular simplicity of hemizygosity resulting in an absent and therefore nonfunctional allele make determinations of the effect of hemizygosity clearer. By contrast, an allele that is individually duplicated cannot be presumed to be as appropriately functional as the wild-type allele, unless all of the *cis* regulatory elements are duplicated as well and the nuclear topological domains remain unchanged. Until these elements of gene regulation and function are determined, the effects of individual gene duplication are beyond precise definition.

In particular, the examples provided here will largely involve the genes on chromosome 18, and the insights gained from those data because of the comprehensive chromosome-wide evaluation of their hemizygous effects. Chromosome 18 is unique because of the large number of nonrecurrent gene deletions. Nearly every unrelated individual with a chromosome 18q deletion and half of those with an 18p deletion have a unique region of hemizygosity, which in total span almost the entire chromosome [8–10]. Because the affected individuals have unique regions of hemizygosity, this facilitates genotype–phenotype correlations and the calculation of the penetrance of those effects [11]. The result is a chromosome-wide map of the hemizygous effect of almost every gene on the chromosome [11]. These data show that the

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effects of hemizygosity are not dichotomous, but rather span a spectrum of severity, from causing significant disability to no detectable effect and everything in between [11].

Chromosome 18 Genes as Exemplifications

Chromosome 18 contains 263 genes; of which, 238 have sufficient information on which to hypothesize a potential hemizygosity effect [11]. These data, combined with the phenotypic and genotypic data from a cohort of almost 700 individuals, with chromosome 18 CNVs, revealed a spectrum of effects linked to abnormal gene dosage for these individual genes [12]. This spectrum includes genes that when hemizygous cause phenotypes with high penetrance; phenotypes with low penetrance; conditional phenotypes resulting from the effects of a single additional factor; genes whose hemizygosity poses a risk factor for disease; as well as the majority of genes for whom hemizygosity has no clinically significant effect (Figure 1, Key Figure).[i]

High-Penetrance Genes

A small number of genes on chromosome 18 cause an abnormal phenotype in at least 50% of people with hemizygosity [11]. Of the 238 genes on chromosome 18, only one (*TCF4*) has a detrimental effect that is 100% penetrant and is the cause of Pitt–Hopkins syndrome [13,14]. In total, only 19 genes (7% of 263) have a clinically significant effect with >50% penetrance when hemizygous (Table 1 and Box 1) [11].

Low-Penetrance Genes

There are a smaller number of genes with rarer effects that cause an abnormal phenotype in 1% to 50% of individuals with hemizygosity for that gene [11]. Nine genes were identified in this category (3% of the chromosome 18 genes) associated with an abnormal phenotype and exhibit a low penetrance (Table 1 and Box 1) [11]. These represent a particularly interesting group of genes, whose hemizygosity phenotypes must have one or more buffering mechanisms, such as other genetic or environmental factors that are either protective or contributory, thereby changing susceptibility. Recognizing the potential effect of these low-penetrance genes is important for several reasons. First, with regard to clinical implications, physicians should be aware of the low probability effect of these genes for appropriate counseling of patients, as well as increasing surveillance for these conditions. Second, these genes can provide insight into the complex biological processes involved in maintaining homeostasis from development to physiology, as they play a necessary but not sufficient role in producing an abnormal phenotype.

Glossary

Conditional-effect genes: genes with a hemizygous effect only in the presence of a single additional factor; either genetic or environmental.
Copy number variation (CNV): genomic deletions or duplications of 50 base pairs of DNA or larger.
Hemizygosity: the state in which an autosomal gene is present in one copy instead of two.
Haploinsufficiency: the mechanism by which a gene in hemizygosity causes an abnormal phenotype.
Haplosufficient: the mechanism by which a gene in hemizygosity maintains a normal phenotype.
Ohnologs: gene duplicates resulting from whole genome duplication.
Topologically associated domains (TADs): a type of genomic organization within the cell nucleus in which the chromatin loops maintain a close configuration that impacts gene expression.

Key Figure

Schematic Depicting a Rough Estimate of the Proportion of Genes on Chromosome 18 for Each Classification That Contributes to a Hemizygosity Effect

Figure 1.

Table 1. Chromosome 18 Gene Dosage Classifications[a]

Gene symbol	Hemizygosity phenotypes	Refs
Genes with highly penetrant hemizygous phenotypes		
DLGAP1	Behavioral abnormalities	[57–59]
FAM210A	Decreased bone mineral density	[60]
GATA6	Congenital heart defects	[61,62]
LAMA3	Dental enamel pitting	[63]
OSBPL1A	Low HDL cholesterol	[64]
ZNF521	Behavioral abnormality	[65]
MAPRE2	Facial dysmorphology	[66]
ZNF24	CNS dysmyelination	[67]
SETBP1	Speech delay	[68]
SMAD4	Polyposis	[69]
TCF4	Pitt–Hopkins syndrome	[70]
TXNL1	Multiple congenital anomalies	[13]
MALT1	Decreased IgM	[71]
NETO1	Learning/memory	[72]
CYB5A	Cryptorchidism	[73]
TSHZ1	Aural atresia	[47]
MBP	Sensorineural hearing loss	[74,75]
Genes with low-penetrance hemizygous phenotypes		
AFG3L2	Spinocerebellar ataxia 28	[76]
PTPN2	Inflammatory bowel disease	[77]
GREB1L	Renal agenesis	[78]
CABLES1	Cushing's disease	[79]
NPC1	Motor or anxiety disorders	[80]
DSG1	Keratosis palmoplantaris	[81]
CELF4	Seizures	[82]
SLC14A2	Vesicoureteral reflux	[83]
DCC	Congenital mirror movements	[84]
Genes with conditional hemizygous phenotypes		
TYMS	Tetralogy of Fallot	[85]
SMCHD1	Facioscapulohumeral dystrophy type 2	[86]
LPIN2	Majeed syndrome	[87]
TGIF1	Holoprosencephaly	[21]
ZBTB14	Congenital heart disease	[88]
LAMA1	Poretti–Boltshauser syndrome	[89]
TWSG1	Holoprosencephaly	[90,91]
RALBP1	Oxidative stress	[92]
PIEZO2	Arthrogryposis	[93]
GNAL	Dystonia	[94,95]
PSMG2	CANDLE	[96]
SEH1L	Anonychia congenita	[97]
MC2R	Primary adrenal insufficiency	[98]

Table 1. (continued)

Gene symbol	Hemizygosity phenotypes	Refs
RBBP8	Jawad syndrome	[99]
TAF4B	Azoospermia	[100]
DSC3	Hypotrichosis	[101]
DSC2	Arrhythmia	[102]
DSG4	Hypotrichosis	[103]
DSG2	Cardiomyopathy	[104]
B4GALT6	Connective tissue disorder	[105]
ELP2	Intellectual disability	[89]
MOCOS	Xanthinuria type II	[18]
EPG5	Vici syndrome	[106]
PSTPIP2	Osteomyelitis	[107]
ATP5A1	Mitochondrial complex deficiency	[108]
LOXHD1	Hearing loss	[109]
IER3IP1	Microcephaly/encephalopathy	[110]
DYM	Smith–McCort dysplasia	[111]
MYO5B	Microvillus inclusion disease	[112]
CCDC11	Heterotaxy	[113]
RAB27B	Griscelli syndrome	[114]
WDR7	Amelogenesis imperfecta	[115]
FECH	Erythropoietic protoporphyria	[116]
ATP8B1	Intrahepatic cholestasis	[117]
RAX	Anophthalmia	[118]
LMAN1	Factor V/VIII deficiency	[119]
CCBE1	Lymphangiectasia–lymphedema	[120]
MC4R	Obesity	[11]
PIGN	Multiple congenital anomalies	[121]
TNFRSF11A	Paget disease	[122]
KDSR	Erythrokeratodermia variabilis	[123]
SERPINB7	Palmarplantar keratosis	[124]
SERPINB8	Exfoliative ichthyosis	[125]
TMX3	Ichthyosis	[126]
RTTN	Polymicrogyria, seizures, dwarfism	[127]
CNDP1	Carnosinemia	[128]
ZNF407	ID/Autism	[129]
CTDP1	Congenital cataracts, facial dysmorphism, and neuropathy	[55]
TXNL4A	Burn-McKeown syndrome	[130]
Genes that are risk factors for hemizygous phenotypes		
LRRC30	Autism	[55]
IMPA2	Eosinophilic esophagitis	[131]
ZNF519	Epilepsy	[132]
CHST9	Hematological malignancy	[133]
ZNF125	Sjögren syndrome	[134]

(continued on next page)

Table 1. (continued)

Gene symbol	Hemizygosity phenotypes	Refs
ZNF396	Autism	[135]
PIAS2	Autism	[136]
KATNAL2	Autism	[137]
SMAD2	Heterotaxy	[138]
ZBTB7C	Stroke	[139]
SMAD7	Arterial arch defects	[140]
MBD1	Autism	[141]
SERPINB4	Atopic dermatitis	[142]
CD226	Autoimmune disease	[143,144]
NFATC1	Lumbar scoliosis	[145,146]

[a]Abbreviations: CANDLE, chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; CNS, central nervous system; HDL, high-density lipoprotein; ID, intellectual disability.

Conditional-Effect Genes

Conditional-effect genes cause an abnormal phenotype only in the presence of a single additional factor either genetic or environmental. Therefore, conditional genes have a low probability of causing a hemizygosity effect and include 49 of the 263 chromosome 18 genes (19% of 263) (Table 1 and Box 2). The majority of these genes (42 of 49) cause disease by a recessive mechanism, thereby causing an abnormal phenotype only in the presence of a loss of function mutation in the remaining allele [11]. These genes actually function in a **haplosufficient** manner. An individual with hemizygosity for one of these genes is functionally equivalent to being a carrier of a disease causing allele with double the chance of manifesting the disease compared with that of a noncarrier. As such, hemizygosity for one of these genes has potential clinical relevance for hastening diagnosis, should symptoms of one of these conditions appear.

Additionally, a second type of conditional mechanism includes those few cases in which disease is caused by digenic loss of function variants (Box 2) [11]. This mechanism is not dependent on the remaining allele but rather another gene which could be closely linked or on another chromosome [15].

Although this is the largest group of genes to potentially cause disease associated with hemizygosity, the actual probability of disease in someone with hemizygosity of anyone of these genes is small. The probability of someone with hemizygosity for one of these genes to also have a loss of function mutation of the extant allele is quite low in the range of 0.5% [16].

Box 1. Examples of Hemizygous Gene Classifications Most Likely to Cause an Abnormal Phenotype

High-Penetrance Genes

Hemizygosity of the *TSHZ1* gene can cause aural atresia without microtia [47]. In our cohort, this phenotype is 78% penetrant in those individuals who are hemizygous for this gene [12].

Low-Penetrance Genes

Examples from this small group of genes include *PTPN2*, which can be a contributory factor in inflammatory bowel disease [48]. *AFG3L2* is a dominant cause of spinocerebellar ataxia type 28 (SCA28) [49]. One adolescent with SCA28 has been reported with hemizygosity for this gene [50]. However, individuals with hemizygosity for this gene have also been identified in control populations [51,52]. In our cohort of 94 individuals with hemizygosity of *AFG3L2*, no individuals have been diagnosed with SCA28, in spite of our active surveillance program (unpublished data).

Box 2. Examples of Hemizygous Gene Classifications Less Likely to Cause an Abnormal Phenotype

Conditional-Effect Genes

The gene *FECH*, which encodes the enzyme ferrochelatase, is associated with erythropoietic protoporphyria (EPP) but the inheritance pattern has been confusing because it appears to be inherited in a dominant pattern in some families and a recessive pattern in others. This is because the disease manifests when enzyme levels are 10–25% [53] due to one non-functional or absent allele and one hypomorphic allele [54]. In the case of EPP, the risk of the condition becoming manifest in someone who is hemizygous for the *FECH* gene is correlated with the population frequency of the hypomorphic allele.

An example of a digenetic condition is facioscapulohumeral dystrophy type 2 (FSHD2). The manifestation of FSHD2 results from aberrant expression of a permissive *DUX4* gene. This can occur through a specific configuration of and around the *DUX4* gene (4q35.2) in combination with hemizygosity of the *SMCHD1* gene (18p11.32). This is because the function of the *SMCHD1* gene product is to maintain the methylation and hence repression of the *DUX4* gene. One functional copy of the *SMCHD1* gene provides insufficient methylation and thereby insufficient repression of *DUX4* leading to disease [15]. *SMCHD1* is therefore an example of a gene whose dosage sensitivity is conditional because the specific *DUX4* configuration is also required in order for disease to be manifest.

Another example is the combination of *TWSG1* and *TGIF* hemizygosity that can cause holoprosencephaly. What makes this interesting is that these two genes are just 5.9 Mb apart on chromosome 18p. Hemizygosity or loss-of-function mutations in both genes results in holoprosencephaly but only in about 10% of cases [21]. Because they are tightly linked they are more likely to both be hemizygous in an individual than they would be had they been on different chromosomes thereby greatly increasing the probability of an abnormal phenotype.

Risk-Factor Genes

Additionally, some genes are risk factors for certain polygenic disorders such as autoimmune disorders, epilepsy, or autism (Box 3). Although the clinical relevance to someone with hemizygosity of one of the risk-factor genes is negligible, this designation of risk factor is relevant for two reasons. First, with regard to clinical relevance, knowledge of existing risk factors may shorten the time to diagnosis should first symptoms begin to appear. Second, with regard to furthering research, someone with hemizygosity of one of these risk factors genes who has one of the associated polygenic phenotypes means that there are fewer additional genes to identify in order to reveal the underlying genetic basis of these conditions. This makes the risk factor designation a useful research tool and people with a polygenetic condition and hemizygosity important research participants.

Dosage-Insensitive Genes

At the opposite end of this continuum of effect from 100% probability of an abnormal phenotype are those genes with no discernable hemizygous effect (Box 3). Over 1000 genes in the human genome have been identified as dispensable genes because they were homozygously deleted in healthy individuals [17,18]. Additionally, there are an even greater number of genes identified as loss-of-function variants, in which one copy is predicted to be nonfunctional in apparently healthy individuals [19].

On chromosome 18, 146 of 263 genes (56%) have no evidence for a clinically significant effect when hemizygous [11]. This group includes genes whose hemizygous effect is not clinically significant, although it may be measurable (Box 3) [20].

Box 3. Examples of Hemizygous Gene Classifications Likely to Cause an Abnormal Phenotype

Risk Factors

Several genes on chromosome 18 have been found to be hemizygous more often in people with autism than in people without autism [55] (see Table 1 in main text).

Haplosufficient Genes

These are illustrated by data from the mouse with a deletion of the *SLC14A1* gene. Although these mice have a urea transport deficiency, they do not manifest any clinical syndrome, suggesting the existence of compensatory mechanisms [20]. Another measurable but not clinically significant example not involving a chromosome 18 gene is hemizygosity of the *OCA2* gene associated with red hair [56].

This discussion on the role of individual genes in hemizygosity should not imply that all the phenotypes associated with a hemizygous deletion of chromosome 18 have the specific causative genes identified and that conversely every gene's role is defined. There are in fact 47 phenotypes linked to critical regions of chromosome 18 that have yet to be narrowed down to the causative genes [9]. Most of these phenotypes are low incidence or potentially polygenic, making it more difficult to identify the role of individual genes. Adding to this genetic complexity are the potential for conditions caused by tightly linked conditional-effect genes within the same CNV [21]. There is clearly more work to be done to completely define the role of each chromosome 18 gene, especially with regard to its hemizygosity.

The data show that the effects of hemizygosity are not a pathogenic or benign dichotomy but rather a continuum that includes a low probability of causing disease, to contributory to disease, to risk factor for disease. Using what we have learned about the hemizygosity effects of the chromosome 18 genes can help to inform the fundamental questions about the effects of abnormal gene dosage. There are three main questions to address. First, what molecular mechanisms allow **haploinsufficiency**? Second, what molecular mechanisms create variable penetrance? Third, what are the mechanisms of gene dosage compensation? Together these should highlight areas for future research and potential strategies for remediation and treatment.

What Molecular Mechanisms Allow Haploinsufficiency?

Are there characteristics of the genes themselves that make them dosage sensitive? One hypothesis is that genes with an evolutionarily conserved sequence, with few functional coding variants, are more likely to be dosage sensitive [22]. The idea being that genes that evolutionarily cannot tolerate sequence variation are components of systems or pathways that can also not tolerate variation in protein quantity. However, not all sequence variation results in a change in the protein quantity and could just as likely be synonymous mutations that do not change the amino acid content, or missense mutations that change the amino acid but do not alter the protein function. Therefore, sequence variation is not a guarantee of protein quantity variation.

Another observation is that monoallelically expressed genes are under-represented in pathogenic CNVs compared with normal-variant CNVs, which led a study to hypothesize that genes that are monollelically expressed are less likely to be dosage sensitive [23]. For the 10–30% of genes that are monoallelically expressed, a single gene level of expression is sufficient for normal function [23]. These authors point out that monoallelically expressed genes have more variable expression than biallelically expressed genes, yet do not fully compensate for the lack of expression from one of its alleles.

In addition to sequence-determined and expression-determined hypotheses, another is based on the spatial context in which the gene lies within the nucleus. The nuclear localization hypothesis suggests that haploinsufficiency is a combination of a potentially dosage-sensitive gene located within a region of hemizygosity that then alters the 3D structure and position of the chromatin within the interphase nucleus [24]. Changes in these **topologically associated domains (TADs)** can change the expression of the genes within those domains, by changing the proximity of a gene to *cis* regulatory element such as promotors or enhancers by changing the epigenetic modifications that then impact gene expression [24]. While this is not a feature of the specific hemizygous gene or its product, this could be a mechanism impacting the manifestation of the phenotype.

Another hypothesis is based on evolutionary retention and that dosage sensitive genes are more likely to be **ohnologs** [25]. The hypothesis is that evolutionarily, after whole genome duplication, some genes are retained, while others are lost. The genes that are retained are ohnologs and are

included within pathogenic CNVs, more often than in nonpathogenic CNVs [25]. Because ohnologs are more likely to be developmental genes and genes whose products are members of protein complexes, they are postulated to be more likely to be dosage sensitive. The functionality of multimeric protein complexes depends on the exact stoichiometry of the component proteins in these complexes [26]. For example, a protein that is part of a heterotetrameric complex, composed of two different proteins requires a 1:1 ratio of subunits. When one subunit is present in half the normal amount only a third as many of the complexes have the correct ratio of components and therefore are functional [26].

A similar hypothesis is that genes that are dosage sensitive are more likely to be hubs within biological networks [27]. This hypothesis is similar to the multiprotein complex hypothesis because haploinsufficiency is not a feature of the gene *per se*, but rather determined by the processes in which the gene product operates. The network hub hypothesis is based in the notion that in humans the more interactions a gene product has with other proteins or processes, the higher the likelihood that one of those processes with be adversely affected by a reduction in the amount of protein caused by hemizygosity [27].

What is not known is the correlation between evolutionarily sequence-constrained genes, monoallelically expressed genes, ohnologs, genes that code for network hubs, and the gene position within TADs. Additionally, do any of those data correlate with dosage-sensitive genes? More importantly, do any of these characteristics predict dosage sensitivity? Alternatively, it is possible that each hypothesis has some validity, and dosage-sensitive genes are those that possess all of these characteristics: they are sequence-restricted ohnologs that are components of multiprotein complexes that are also network hubs. The individually curated chromosome 18 gene-dosage classifications could be used to test these hypotheses.

What Molecular Mechanisms Create Variable Penetrance?

One aspect of haploinsufficiency that is often overlooked is the issue of reduced penetrance [28]. Complete penetrance is rare. Conceptually, the rarity of complete penetrance could be explained by two mechanisms potentially working in concert. First, abnormal phenotypes caused by hemizygosity and resulting in haploinsufficiency are the result of a chain of events beginning with one gene, instead of two generating an insufficient amount of mRNA, thereby producing insufficient protein resulting in tissue then organ dysfunction (Figure 2) [29]. Gene-dosage compensation could happen at any one of these steps. Conversely, all the steps in this process from gene to clinical phenotype must be haploinsufficient in order for an abnormal phenotype to become apparent [29]. Second, haploinsufficiency is caused by half the normal amount of gene product. However, even in recessively inherited conditions in which both genes have loss-of-function mutations, there are examples of individuals who are nonpenetrant for the phenotype [28,30,31]. It is therefore not surprising that hemizygosity creates an equivocal state that can be swayed toward normalcy or disease, by potentially numerous or even small fluctuations in network or pathway activity.

For the phenotypes resulting from conditional effects, the combination of a hemizygous and a loss-of-function allele is likely a rare event. For comparison, one of the most common recessive conditions is sickle-cell disease occurring in one in 12 African Americans or 8% of that population [32]. This prevalence of 8% for a common recessively inherited condition is lower than most of the low-penetrance hemizygosity conditions that occurs in the range of 20–30% of those people with hemizygosity for the causative gene [11]. This suggests that complete loss-of-function variants in the remaining allele are not the cause of an abnormal phenotype in a low-penetrance phenotype.

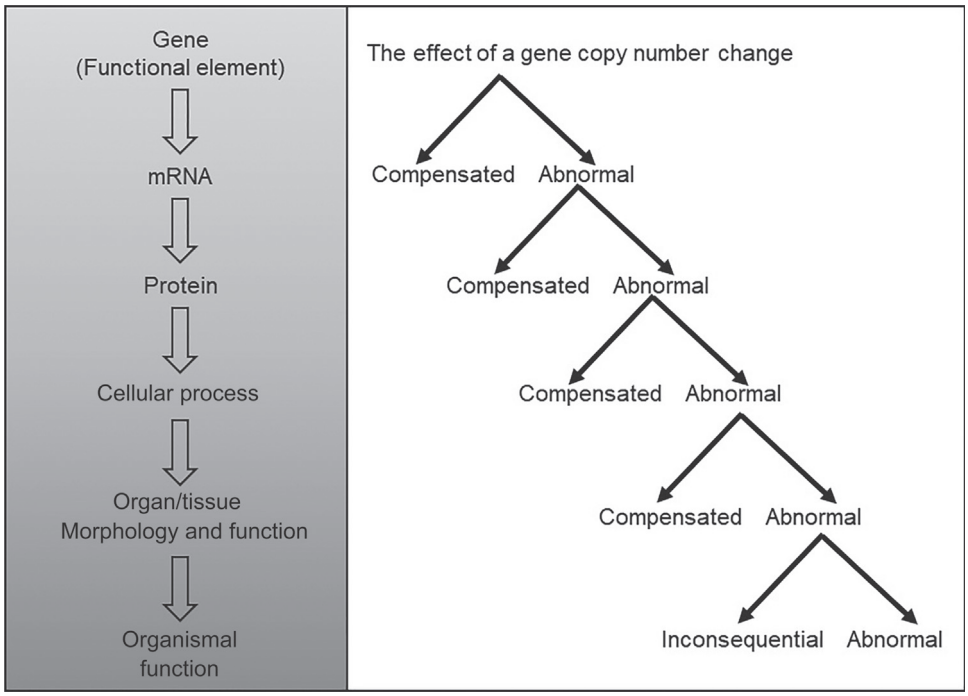

Figure 2. The Pathway from Abnormal Gene Copy Number to Abnormal Phenotype and All the Potential Points of Compensation in Between. The general pathway from gene to clinically apparent phenotype is shown down the left side of the figure. The right side illustrates the numerous stages at which compensation can happen between an abnormal gene copy number and an abnormal phenotype. Adapted, with permission, from [29].

However, the remaining allele may affect the penetrance by being hypomorphic or hypermorphic. Such variance in gene activity could be due to sequence variation or the result of epigenetic phenomenon. An interesting example of epigenetic variation involves the conditional-effect gene *FECH* (Box 2), requiring the remaining allele to be hypomorphic in order for hemizygosity to cause disease. In this case, the methylation status can affect the expression of the remaining allele and cause it to be hypomorphic [33] making the combination of hemizygosity and a silenced remaining allele result in the disease.

Disease penetrance can also be impacted by environmental factors. In the case of 22q11 deletions, the *TBX1* gene within that region of hemizygosity is a cause of cardiac and pharyngeal defects with a prevalence of 74% and 69%, respectively, and have a highly variable penetrance within affected families [34]. One study [35] has implied that mice hemizygous for the *Tbx1* gene when exposed prenatally to excess retinoic acid have markedly increased prevalence of cardiac and pharyngeal defects. A gene whose hemizygous effect exhibits low penetrance could be one in which an environmental factor is necessary for expression of the trait.

What Are the Mechanisms of Gene Dosage Compensation?

Understanding the mechanisms of reduced penetrance and gene-dosage compensation can provide insight into potential treatment strategies because they are two aspects of the same phenomenon normalizing the phenotypic outcome. Phenotypic modifiers in the context of hemizygosity could include multiple factors. (i) As previously mentioned, sequence variation in *cis* or *trans* affects regulatory factor effectiveness and can increase or decrease the severity of the phenotype from hemizygosity. Genetic modifiers of rare diseases as a topic has been recently

reviewed [36]. (ii) Epigenetic modification differences are becoming appreciated as potentially having a major effect on gene expression. Investigating the epigenetic differences between penetrant and nonpenetrant individuals could shed light on gene regulation [37]. (iii) Other proteins in the affected pathways or networks may provide compensation through upregulation [38]. (iv) Extrinsic factors such as environmental or dietary effects, could impact any of the above factors.

The probability of disease manifestation, that is, the penetrance, may largely depend on the activity of the remaining allele, which can be affected by sequence variation or epigenetic variation. Studies that compare the DNA sequence and epigenetic modifications between the affected and unaffected individuals may provide insight into the parameters necessary for an abnormal phenotype to become apparent. Data pertaining to each of the factors mentioned above could provide insight into the dysregulated pathway, create additional points of potential remediation, and shed light on the inflection points most amenable for dosage compensation. Examples of both transcriptional and post-transcriptional genetic compensation were recently reviewed [39].

Nonpenetrance data can also yield important information about the parameters within which normal function can occur. For example, do the nonpenetrant individuals have normal levels of transcription? If not, these data can help define the range of transcription that is sufficient for normal function. This same type of comparison could be used to compare phenotype-relevant physiology or biochemistry between those who do and do not exhibit the phenotype. thereby more precisely defining the range of normal function?

Reduced penetrance and variable expressivity are not the products of stochastic events. There is an underlying biological cause. That cause stems from a genetic variation or an environmental trigger that enhances or suppresses the effect of the abnormal gene dosage. To quote Albert Einstein, 'God does not play dice with the universe.' There is much to learn about the myriad ways the human body copes, compensates, and counteracts in order to maintain homeostasis. This information can then inform studies aimed at treatment.

Concluding Remarks

Careful curation of the hemizygosity outcomes data for the genes on chromosome 18 revealed a spectrum of potential effects. Individual gene function data were then applied to the actual phenotype data from almost 700 individuals with chromosome 18 CNVs, showing that dosage insensitivity and dosage compensation are the norm. Multiple authors had previously created models and made predictions attempting to identify dosage sensitive genes [27,40–46] using small training sets to model genome wide. However, the potential of reduced penetrance and polygenic disease was not appreciated. This review touches on the potential mechanisms leading to haploinsufficiency and those by which dosage compensation can occur. Future work to define the gene composition and activity of individuals who exhibit a hemizygosity phenotype with those who do not could lead to insights relevant to treatment options. Many challenges remain; the greatest of which is the precise phenotyping of a large cohort of people with hemizygosity (see Outstanding Questions). Studies of normal CNV typically enroll subjects who self-declare their normalcy. While studies of abnormal CNV typically enroll subjects whose phenotype is described as global developmental delay or dysmorphic features. In both cases, this level of phenotyping is grossly inadequate. Considering the ubiquitous nature of genomic CNVs, an effort to catalog the precise phenotypic ramifications would have wide-reaching implications for both health and disease.

Outstanding Questions

In the continuum from annotating the genome to developing treatments for chromosome abnormalities there are numerous outstanding questions:

What are the phenotypes of the rarely published heterozygous knockout mice? In many cases these data already exist and if published could add to the knowledge base for the individual genes, as well as identify potential animal models for use in drug development.

On an individual cell level, how does hemizygosity affect the expression of monoalleleically expressed genes? Is there a randomness to which alleles are expressed so that half of the cells have no expression and the other half have single gene expression. Or, is there some sort of regulation so that in the absence of one allele the other allele is active?

What are the microforms and endophenotypes of the heterozygous parents of individuals with recessively inherited loss of function conditions and nonpenetrant homozygotes? This could identify both the more subtle effects of hemizygosity and identify the factors leading to nonpenetrance.

Is the current system of gene-dosage annotation both conceptually and logistically in need of re-evaluation?

What is that natural history of adults with chromosome abnormalities? Little attention has be given to the long-term effects of gene CNV.

What are the physiological and biochemical correlates of behavioral and intellectual disability that could be tied to single-gene hemizygosity? This concept is particularly relevant for the behavioral phenotypes known to be polygenic.

What are the potential modes of therapeutic gene regulation?

What features of a gene or protein network that correlate with haploinsufficiency?

Resources

[i] https://wp.uthscsa.edu/chrome-18/research/

References

1. Freeman, J. *et al.* (2006) Copy number variation: new insights in genome diversity. *Genome Res.* 16, 949–961
2. Levy, B. and Wapner, R. (2018) Prenatal diagnosis by chromosomal microarray analysis. *Fertil. Steril.* 109, 201–212
3. Battaglia, A. *et al.* (2013) Confirmation of chromosomal microarray as a first-tier clinical diagnostic test for individuals with developmental delay, intellectual disability, autism spectrum disorders and dysmorphic features. *Eur. J. Paediatr. Neurol.* 17, 589–599
4. Kendall, K.M. *et al.* (2019) Association of rare copy number variants with risk of depression. *JAMA Psychiatry* 76, 818–825
5. Sriretnakumar, V. (2019) Copy number variant syndromes are frequent in schizophrenia: Progressing towards a CNV-schizophrenia model. *Schizophr. Res.* 209, 171–178
6. Fortier, N. *et al.* (2018) Detection of CNVs in NGS data using VS-CNV. *Methods Mol. Biol.* 1833, 115–127
7. Gentile, G. *et al.* (2020) The contribution of CNVs to the most common aging-related neurodegenerative diseases. *Aging Clin. Exp. Res.* Published online February 6, 2020. https://doi.org/10.1007/s40520-020-01485-4
8. Heard, P.L. *et al.* (2009) High resolution genomic analysis of 18q– using oligo-microarray comparative genomic hybridization (aCGH). *Am. J. Med. Genet.* 149A, 1431–1437
9. Cody, J.D. *et al.* (2015) Consequences of chromosome 18q deletions. *Am. J. Med. Genet. C (Semin. Med. Genet.)* 169C, 265–280
10. Hasi-Zogaj, M. *et al.* (2015) A review of 18p deletions. *Am. J. Med. Genet. C (Semin. Med. Genet.)* 169C, 251–264
11. Cody, J.D. *et al.* (2018) Chromosome 18 gene dosage map 2.0. Hum. *Genet.* 137, 961–970
12. Cody, J.D. *et al.* (2018) The Chromosome 18 Clinical Resource Center. *Mol. Genet. Genomic Med.* 6, 416–421
13. Zweier, C. *et al.* (2007) Haploinsufficiency of TCF4 causes mental retardation with intermittent hyperventilation (Pitt-Hopkins Syndrome). *Am. J. Hum. Genet.* 80, 994–1001
14. Hasi, M. *et al.* (2011) The role of the TCF4 gene in the phenotype of individuals with 18q segmental deletions. *Hum. Genet.* 130, 777–787
15. Daxinger, L. *et al.* (2015) Genetic and epigenetic contributors to FSHD. *Curr. Opin. Genet. Dev.* 33, 56–61
16. MacArthur, D.G. and Tyler-Smith, C. (2010) Loss-of-function variants in the genomes of healthy humans. *Hum. Mol. Genet.* 19, R125–R130
17. Sudmant, P.H. *et al.* (2015) An integrated map of structural variation in 2,504 human genomes. *Nature* 526, 75–81
18. Saleheen, D. *et al.* (2017) Human knockouts and phenotypic analysis in a cohort with a high rate of consanguinity. *Nature* 544, 235–239
19. MacDonald, J.R. *et al.* (2014) The Database of Genomic Variants: a curated collection of structural variation in the human genome. *Nucleic Acids Res.* D986–D992 (Database issue)
20. Jiang, T. *et al.* (2017) Generation and phenotypic analysis of mice lacking all urea transporters. *Kidney Int.* 91, 338–351
21. Rosenfeld, J.A. *et al.* (2010) Clinical characterization if individuals with deletions of genes in holoprosencephaly pathways by aCGH refines the phenotypic spectrum of HPE. *Hum. Genet.* 127, 421–440
22. Samocha, K.E. *et al.* (2014) A framework for the interpretation of de novo mutation in human disease. *Nat. Genet.* 46, 944–950
23. Savova, V. *et al.* (2017) Risk alleles of genes with monoallelic expression are enriched in gain-of-function variants and depleted in loss-of-function variants for neurodevelopmental disorders. *Mol. Psychiatry* 22, 1785–1794
24. de Wit, E. (2019) Gene regulation knows its boundaries. *Trends Genet.* 35, 883–885
25. Rice, A.M. and McLysaght, A. (2017) Dosage sensitive genes in evolution and disease. *B.M.C. Biol.* 15, 78
26. Bergendahl, L.T. *et al.* (2019) The role of protein complexes in human genetic disease. *Protein Sci.* 28, 1400–1411
27. Khurana, E. *et al.* (2013) Interpretation of genomic variants using a unified biological network approach. *PLoS Comput. Biol.* 9, e1002886
28. Cooper, D.N. *et al.* (2013) Where genotype is not predictive of phenotype: towards an understanding of the molecular basis of reduced penetrance in human inherited disease. *Hum. Genet.* 132, 1077–1130
29. Cody, J.D. and Hale, D.E. (2011) Linking chromosome abnormality and copy number variation. *Am. J. Med. Genet. A* 155, 469–475
30. Chen, R. *et al.* (2016) Analysis of 589,306 genomes identifies individuals resilient to severe Mendelian childhood diseases. *Nat. Biotechnol.* 34, 531–538
31. Tarailo-Graovac, M. *et al.* (2017) Assessment of the ExAC data set for the presence of individuals with pathogenic genotypes implicated in severe Mendelian pediatric disorders. *Genet. Med.* 19, 1300–1308
32. Hassell, K.L. (2010) Population estimates of sickle cell disease in the U.S. *Am. J. Prev. Med.* 38, S512–S521
33. Chiara, M. *et al.* (2019) Targeted resequencing of *FECH* locus reveals that a novel deep intronic pathogenic variant and eQTLs may cause erythropoietic protoporphyria (EPP) through a methylation-dependent mechanism. *Genet. Med.* 22, 35–43
34. McDonald-McGinn, D.M. *et al.* (2005) 22q11.2 Deletion Syndrome. In *GeneReviews* (Pagon, R.A. *et al.*, eds), University of Washington
35. Voss, A.K. *et al.* (2012) MOZ regulates the *Tbx1* locus, and Moz mutation partially phenocopies DiGeorge syndrome. *Dev. Cell* 23, 652–663
36. Rahit, K.M.T.H. and Tarailo-Graovac, M. (2020) Genetic modifiers and rare mendelian disease. *Genes* 11, 239
37. Talbert, P.B. *et al.* (2019) Old cogs, new tricks: the evolution of gene expression in a chromatin context. *Nat. Rev. Genet.* 20, 283–297
38. Johnson, A.F. *et al.* (2019) Causes and effects of haploinsufficiency. *Biol. Rev.* 94, 1774–1785
39. El-Brolosy, M.A. and Stainier, D.Y.R. (2017) Genetic compensation: a phenomenon in search of mechanisms. *PLoS Genet.* 13, e1006780
40. Dang, V.T. *et al.* (2008) Identification of human haploinsufficient genes and their genomic proximity to segmental duplications. *Eur. J. Hum. Genet.* 16, 1350–1357
41. Huang, N. *et al.* (2010) Characterizing and predicting haploinsufficiency in the Human Genome. *PLoS Genet.* 6, e1001154
42. Petrovsk, i S. *et al.* (2013) Genic intolerance to functional variation and the interpretation of personal genomes. *PLoS Genet.* 9, e1003709
43. Steinberg, J. (2015) Haploinsufficiency predictions without study bias. *Nucleic Acids Res.* 43, e101
44. Ruderfer, D.M. *et al.* (2016) Patterns of genic intolerance of rare copy number variation in 59,898 human exomes. *Nat. Genet.* 48, 1107–1111
45. Shaikh, T.H. (2011) Genes and biological processes commonly disrupted in rare and heterogeneous developmental delay syndromes. *Hum. Mol. Genet.* 20, 880–893
46. Firth, H.V. *et al.* (2009) DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources. *Am. J. Hum. Genet.* 84, 524–533
47. Feenstra, I. *et al.* (2011) Disruption of teashirt zinc finger homeobox 1 is associated with congenital aural atresia in humans. *Am. J. Hum. Genet.* 89, 813–819
48. Spalinger, M.R. *et al.* (2015) Role of protein tyrosine phosphatases in regulating the immune system: implications for chronic intestinal inflammation. *Inflamm. Bowel Dis.* 21, 645–655
49. Brussino, A. *et al.* (2018) Spinocerebellar ataxia type 28. In *GeneReviews* (Adam, M.P. *et al.*, eds), University of Washington
50. Myers, K.A. *et al.* (2014) Deletion of *AFG3L2* associated with spinocerebellar ataxia type 28 in the context of multiple genomic anomalies. *Am. J. Med. Genet. A* 164, 3209–3212
51. Redon, R. *et al.* (2006) Global variation in copy number in the human genome. *Nature* 444, 444–454
52. Coe, B.P. *et al.* (2014) Refining analyses of copy number variation identifies specific genes associated with developmental delay. *Nat. Genet.* 46, 1063–1071
53. Bloomer, J.R. (1980) Characterization of deficient heme synthase activity in protoporphyria with cultured skin fibroblasts. *J. Clin. Invest.* 65, 321–328

54. Gouya, L. *et al.* (2002) The penetrance of dominant erythropoietic protoporphyria is modulated by expression of wildtype *FECH*. *Nat .Genet.* 30, 27–28
55. Pinto, D. *et al.* (2010) Functional impact of global rare copy number variation in autism spectrum disorders. *Nature* 466, 368–372
56. Chiang, P.-W. *et al.* (2008) Synergistic interaction of the *OCA2* and *OCA3* genes in a family. *Am. J. Med. Genet.* 146A, 2427–2430
57. Coba, M.P. *et al.* (2018) Dlgap1 knockout mice exhibit alterations of the postsynaptic density and selective reductions in sociability. *Sci. Rep.* 8, 2281
58. Gazzellone, M.J. *et al.* (2016) Uncovering obsessive-compulsive disorder risk genes in a pediatric cohort by high-resolution analysis of copy number variation. *J. Neurodev. Disord.* 8, 36
59. Kirov, G. *et al.* (2012) *De novo* CNV analysis implicates specific abnormalities of postsynaptic signalling complexes in the pathogenesis of schizophrenia. *Mol. Psychiatry* 17, 142–153
60. Tanaka, K.I. *et al.* (2018) FAM210A is a novel determinant of bone and muscle structure and strength. *Proc. Natl. Acad. Sci. U. S. A.* 115, E3759–E3768
61. Allen, H.L. *et al.* (2011) GATA6 haploinsufficiency causes pancreatic agenesis in humans. *Nat. Genet.* 44, 20–22
62. Suzuki, S. *et al.* (2014) A case of pancreatic agenesis and congenital heart defects with a novel GATA6 nonsense mutation: evidence of haploinsufficiency due to nonsense-mediated mRNA decay. *Am. J. Med. Genet. A* 164A, 476–479
63. Gostyńska, K.B. *et al.* (2016) Carriers with functional null mutations in LAMA3 have localized enamel abnormalities due to haploinsufficiency. *Eur. J. Hum. Genet.* 25, 94–99
64. Motazacker, M.M. *et al.* (2016) A loss-of-function variant in OSBPL1A predisposes to low plasma HDL cholesterol levels and impaired cholesterol efflux capacity. *Atherosclerosis* 249, 140–147
65. Ohkubo, N. *et al.* (2014) Abnormal behaviors and developmental disorder of hippocampus in zinc finger protein 521 (ZFP521) mutant mice. *PLoS One* 9, e92848
66. Isrie, M. *et al.* (2015) Mutations in either TUBB or MAPRE2 cause circumferential skin creases Kunze type. *Am. J. Hum. Genet.* 97, 790–800
67. Howng, S.Y. *et al.* (2010) ZFP191 is required by oligodendrocytes for CNS myelination. *Genes Dev.* 24, 301–311
68. Filges, I. *et al.* (2011) Reduced expression by SETBP1 haploinsufficiency causes developmental and expressive language delay indicating a phenotype distinct from Schinzel-Giedion syndrome. *J. Med. Genet.* 48, 117–122
69. Hahn, S.A. *et al.* (1996) DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science* 271, 350–353
70. van Diepen, M.M. *et al.* (2011) A 797 kb *de novo* deletion of 18q21.31 in a patient with speech delay, mental retardation, sleeping problems, facial dysmorphism, and feet anomalies. *Eur. J. Med. Genet.* 54, 86–88
71. Demeyer, A. *et al.* (2019) MALT1-deficient mice develop atopic-like dermatitis upon aging. *Front. Immunol.* 10, 2330
72. Ng, D. *et al.* (2009) Neto1 is a novel CUB-domain NMDA receptor-interacting protein required for synaptic plasticity and learning. *PLoS Biol.* 7, e41
73. Miller, W. (2012) The syndrome of 17,20 lyase deficiency. *J. Clin. Endocrinol. Metab.* 97, 59–67
74. Martin, M. *et al.* (2006) Myelin deficiencies visualized *in vivo*: visually evoked potentials and T2-weighted magnetic resonance images of shiverer mutant and wild-type mice. *J. Neurosci. Res.* 84, 1716–1726
75. Xing, Y. *et al.* (2012) Age-related changes of myelin basic protein in mouse and human auditory nerve. *PLoS One* 7, e34500
76. Smets, K. *et al.* (2014) Partial deletion of AFG3L2 causing spinocerebellar ataxia type 28. *Neurology* 82, 2092–2100
77. Glas, J. *et al.* (2012) PTPN2 gene variants are associated with susceptibility to both Crohn's disease and ulcerative colitis supporting a common genetic disease background. *PLoS One* 7, e33682
78. Herlin, M.K. *et al.* (2019) Whole-exome sequencing identifies a GREB1L variant in a three-generation family with Müllerian and renal agenesis: a novel candidate gene in Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome. A case report. *Hum. Reprod.* 34, 1838–1846
79. Hernández-Ramírez, L.C. *et al.* (2017) Loss-of-function mutations in the *CABLES1* gene are a novel cause of Cushing's disease. *Endocr. Relat. Cancer* 24, 379–392
80. Bremova-Ertl, T. *et al.* (2020) Clinical, ocular motor, and imaging profile of Niemann-Pick type C heterozygosity. *Neurology* 94, e1702–e1715
81. Nomura, T. *et al.* (2018) Loss-of-function mutation in DSG1 underlies focal palmoplantar keratoderma. *J. Eur. Acad. Dermatol. Venereol.* 33, e137–3138
82. Halgren, C. *et al.* (2012) Haploinsufficiency of CELF4 at 18q12.2 is associated with developmental and behavioral disorders, seizures, eye manifestations, and obesity. *Eur. J. Hum. Genet.* 20, 1315–1319
83. Yang, B. *et al.* (2005) Urea and urine concentrating ability: new insights from studies in mice. *Am. J. Physiol. Renal. Physiol.* 288, F881–F896
84. Gallea, C. *et al.* (2011) Congenital mirror movements: a clue to understanding bimanual motor control. *J. Neurol.* 258, 1911–1919
85. Zhu, H. *et al.* (2012) Thymidylate synthase polymorphisms and risk of conotruncal heart defects. *Am. J. Med. Genet. A* 158A, 2194–2203
86. Lemmers, R. *et al.* (2012) Digenic inheritance of an SMCHD1 mutation and an FSHD-permissive D4Z4 allele causes faciocapulohumeral muscular dystrophy type 2. *Nat. Genet.* 44, 1370–1374
87. Cox, A.J. and Ferguson, P.J. (2018) Update on the genetics of nonbacterial osteomyelitis in humans. *Curr. Opin. Rheumatol.* 30, 521–525
88. San Agustin, J.T. *et al.* (2016) Genetic link between renal birth defects and congenital heart disease. *Nat. Commun.* 7, 11103
89. Najmanadi, H. *et al.* (2011) Deep sequencing reveal 50 novel genes for recessive cognitive disorders. *Nature* 478, 57–63
90. Shaffer, J.R. *et al.* (2013) GWAS of dental caries patterns in the permanent dentition. *J. Dent. Res.* 92, 38–44
91. Billington Jr., C.J. *et al.* (2014) Impact of retinoic acid exposure on midfacial shape variation and manifestation of holoprosencephaly in Twsg1 gastrulation mutant mice. *Dis. Model. Mech.* 8, 139–146
92. Awasthi, S. *et al.* (2005) RLIP76 is a major determinant of radiation sensitivity. *Cancer Res.* 65, 6022–6028
93. Haliloglu, G. *et al.* (2016) Recessive PIEZO2 stop mutation causes distal arthrogryposis with distal muscle weakness, scoliosis and proprioception defects. *J. Hum. Genet.* 62, 497–501
94. Vemula, S.R. *et al.* (2013) Role of Gα(olf) in familial and sporadic adult-onset primary dystonia. *Hum. Mol. Genet.* 22 2510-1519
95. Khan, M.M. *et al.* (2019) Gnal haploinsufficiency causes genomic instability and increased sensitivity to haloperidol. *Exp. Neurol.* 318, 61–70
96. de Jesus, A.A. *et al.* (2019) Novel proteasome assembly chaperone mutations in PSMG2/PAC2, cause the autoinflammatory interferonopathy, CANDLE/PRAAS4. *J. Allergy Clin. Immunol.* 143, 1939–1943
97. Hassan, M.J. *et al.* (2008) A syndromic form of autosomal recessive congenital microcephaly (Jawad syndrome) maps to chromosome 18p11.22-q11.2. *Hum. Genet.* 123, 77–82
98. Guran, T. *et al.* (2016) Rare causes of primary adrenal insufficiency: genetic and clinical characterization of a large nation-wide cohort. *J. Clin. Endocrinol. Metab.* 284, 92
99. Qvist, P. *et al.* (2011) CtIP mutations cause Seckel and Jawad syndromes. *PLoS Genet.* 7, e1002310
100. Ayhan, O. *et al.* (2014) Truncating mutations in TAF4B and ZMYND15 causing recessive azoospermia. *J. Med. Genet.* 51, 239–244
101. Ayub, M. *et al.* (2009) A homozygous nonsense mutation in the human desmocollin-3 (DSC3) gene underlies hereditary hypotrichosis and recurrent skin vesicles. *Am. J. Hum. Genet.* 85, 515–520
102. Lorenzon, A. *et al.* (2015) Homozygous desmocollin-2 mutations and arrhythmogenic cardiomyopathy. *Am. J. Cardiol.* 116, 1245–1251

103. Cohen-Barak, E. *et al.* (2018) Desmoglein 4 mutation underlies autosomal recessive keratosis pilaris atrophicans. *Acta Derm. Venereol.* 98, 809–810
104. Lin, Y. *et al.* (2018) Compound and heterozygous mutations of DSG2 identified by whole exome sequencing in arrhythmogenic right ventricular cardiomyopathy/dysplasia with ventricular tachycardia. *J. Electrocardiol.* 51, 837–843
105. Malfait, F. *et al.* (2013) Defective initiation of glycosaminoglycan synthesis due to B3GALT6 mutations causes a pleiotropic Ehlers-Danlos-syndrome-like connective tissue disorder. *Am. J. Hum. Genet.* 92, 935–945
106. Cullup, T. *et al.* (2013) Recessive mutations in EPG5 cause Vici syndrome, a multisystem disorder with defective autophagy. *Nat. Genet.* 45, 83–87
107. Chitu, V. *et al.* (2012) PSTPIP2 deficiency in mice causes osteopenia and increased differentiation of multipotent myeloid precursors into osteoclasts. *Blood* 120, 3126–3135
108. Betancur, C. *et al.* (2009) The emerging role of synaptic cell-adhesion pathways in the pathogenesis of autism spectrum disorders. *Trends Neurosci.* 32, 402–412
109. Sulem, P. *et al.* (2015) Identification of a large set of rare complete human knockouts. *Nat. Genet.* 47, 448–452
110. Shalev, S.A. *et al.* (2013) Microcephaly, epilepsy, and neonatal diabetes due to compound heterozygous mutations in IER3IP1: insights into the natural history of a rare disorder. *Pediatr. Diabetes* 15, 252–256
111. Osipovich, A.B. *et al.* (2008) Dyggve-Melchior-Clausen syndrome: chondrodysplasia resulting from defects in intracellular vesicle traffic. *Proc. Natl. Acad. Sci. U. S. A.* 105, 16171–16176
112. Ruemmele, F.M. *et al.* (2010) Loss-of-function of MYO5B is the main cause of microvillus inclusion disease: 15 novel mutations and a CaCo-2 RNAi cell model. *Hum. Mutat.* 31, 544–551
113. Perles, Z. *et al.* (2012) A human laterality disorder associated with recessive CCDC11 mutation. *J. Med. Genet.* 49, 386–390
114. Van Gele, M. *et al.* (2009) Griscelli syndrome: a model system to study vesicular trafficking. *Pigment Cell Melanoma Res.* 22, 268–282
115. El-Sayed, W. *et al.* (2009) Mutations in the beta propeller WDR72 cause autosomal-recessive hypomaturation amelogenesis imperfecta. *Am. J. Hum. Genet.* 85, 699–705
116. Männik, K. *et al.* (2015) Copy number variations and cognitive phenotypes in unselected populations. *JAMA* 313, 2044–2054
117. Jacquemin, E. (2012) Progressive familial intrahepatic cholestasis. *Clin. Res. Hepatol. Gastroenterol.* 1, S26–S35
118. Chassaing, N. *et al.* (2014) Molecular findings and clinical data in a cohort of 150 patients with anophthalmia/microphthalmia. *Clin. Genet.* 86, 326–334
119. Zhen, Q. *et al.* (2019) Genetic study on small insertions and deletions in psoriasis revealed an important impact on complex human diseases. *J Invest. Dermatol.* 139, 2302–2312
120. Ye, Z. *et al.* (2019) Phenotype and genotype of a cohort of Chinese children with early-onset protein-losing enteropathy. *J. Pediatr.* 208, 38–42
121. Alessandri, J.L. *et al.* (2018) Recessive loss of function PIGN alleles, including an intragenic deletion with founder effect in La Réunion Island, in patients with Fryns syndrome. *Eur. J. Hum. Genet.* 26, 340–349
122. Kapur, R.P. *et al.* (2004) Malignant autosomal recessive osteopetrosis caused by spontaneous mutation of murine Rank. *J. Bone Miner. Res.* 19, 1689–1697
123. Boyden, L.M. *et al.* (2017) Mutations in KDSR cause recessive progressive symmetric erythrokeratoderma. *Am. J. Hum. Genet.* 100, 978–984
124. Kubo, A. *et al.* (2013) Mutations in SERPINB7, encoding a member of the serine protease inhibitor superfamily, cause Nagashima-type palmoplantar keratosis. *Am. J. Hum. Genet.* 93, 945–956
125. Pigors, M. *et al.* (2016) Loss-of-function mutations in SERPINB8 linked to exfoliative ichthyosis with impaired mechanical stability of intercellular adhesions. *Am. J. Hum. Genet.* 99, 430–436
126. Chao, R. *et al.* (2010) A male with unilateral microphthalmia reveals a role for TMX3 in eye development. *PLoS One* 5, e10565
127. Shamseldin, H. *et al.* (2015) RTTN mutations cause primary microcephaly and primordial dwarfism in humans. *Am. J. Hum. Genet.* 97, 862–868
128. Peters, V. *et al.* (2018) Carnosinase, diabetes mellitus and the potential relevance of carnosinase deficiency. *J. Inherit. Metab. Dis.* 41, 39–47
129. Kambouris, M. *et al.* (2014) Mutations in zinc finger 407 [ZNF407] cause a unique autosomal recessive cognitive impairment syndrome. *Orphanet. J. Rare Dis.* 9, 80
130. Wieczorek, D. *et al.* (2014) Compound heterozygosity of low-frequency promoter deletions and rare loss-of-function mutations in TXNL4A causes Burn-McKeown syndrome. *Am. J. Hum. Genet.* 95, 698–707
131. Kottyan, L.C. *et al.* (2014) Genome-wide association analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic disease. *Nat. Genet.* 46, 895–900
132. Lal, D. *et al.* (2015) Burden analysis of rare microdeletions suggests a strong impact of neurodevelopmental genes in genetic generalised epilepsies. *PLoS Genet.* 11, e1005226
133. Zhao, X. *et al.* (2010) Examination of copy number variations of CHST9 in multiple types of hematologic malignancies. *Cancer Genet. Cytogenet.* 203, 176–179
134. Tenorio, J. *et al.* (2014) A new overgrowth syndrome is due to mutations in RNF125. *Hum. Mutat.* 35, 1436–1441
135. Krumm, N. *et al.* (2013) Transmission disequilibrium of small CNVs in simplex autism. *Am. J. Hum. Genet.* 93, 595–606
136. Corominas, R. *et al.* (2014) Protein interaction network of alternatively spliced isoforms from brain links genetic risk factors for autism. *Nat. Commun.* 5, 3650
137. O'Roak, B.J. *et al.* (2012) Sporadic autism exomes reveal a highly interconnected protein network of *de novo* mutations. *Nature* 485, 246–250
138. Zaidi, S. *et al.* (2013) *De novo* mutations in histone-modifying genes in congenital heart disease. *Nature* 498, 220–223
139. Du, R. *et al.* (2015) Integrative mouse and human studies implicate ANGPT1 and ZBTB7C as susceptibility genes to ischemic injury. *Stroke* 46, 3514–3522
140. Papangeli, I. and Scambler, P.J. (2013) Tbx1 genetically interacts with the transforming growth factor-β/bone morphogenetic protein inhibitor Smad7 during great vessel remodeling. *Circ. Res.* 112, 90–102
141. Allan, A.M. *et al.* (2008) The loss of methyl-CpG binding protein 1 leads to autism-like behavioral deficits. *Hum. Mol. Genet.* 17, 2047–2057
142. Sivaprasad, U. *et al.* (2015) SERPINB3/B4 contributes to early inflammation and barrier dysfunction in an experimental murine model of atopic dermatitis. *J Invest. Dermatol.* 135, 160–169
143. Onengut-Gumuscu, S. *et al.* (2015) Fine mapping of type 1 diabetes susceptibility loci and evidence for colocalization of causal variants with lymphoid gene enhancers. *Nat. Genet.* 47, 381–386
144. Reinards, T.H. *et al.* (2015) CD226 (DNAM-1) is associated with susceptibility to juvenile idiopathic arthritis. *Ann. Rheum. Dis.* 74, 2193–2198
145. Aliprantis, A.O. *et al.* (2010) NFATc1 in inflammatory and musculoskeletal conditions. *Adv. Exp. Med. Biol.* 658, 69–75
146. Shin, J. *et al.* (2011) Genetic predisposition in degenerative lumbar scoliosis due to the copy number variation. *Spine* 36, 1782–1793