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Role of Haploinsufficency in Human Genetics: A Review with Emphasis on Brain Disorders

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Abstract

Haploinsufficency (HI), wherein a single functional copy of a gene is insufficient to maintain normal function, is a major cause of dominant disease. Human disease studies have identified several hundred haploinsufficient genes affecting a range of phenotypes. In this mini-review we review the topic of HI with reference to brain and human neuropsychiatric diseases, providing clinical, genetic, and pathological implications of the mechanism. Further,the genetic implications of HI ("stuck") genes from evolutionary and genetic lenses are reviewed along with potential clinical applications. In summary, the study of HI assumes significance since its spectrum extends to the realm of biophysics, gene expression, chromosome biology, quantitative traits, and evolutionary biology.

Keywords: Haploinsufficency, Dosage effects, Heterozygous mutations, Loss of functions, Fitness consequences.

INTRODUCTION

Haploinsufficency (HI) is defined as insufficiency of a gene to maintain a wild-type phenotype in the presence of mutant allele (Johnson et al., 2019) resulting in 50% of the active form of protein expression. In sexually reproducing eukaryotes, genes are present in 2-fold, a maternal and a paternal complement represented as the balanced karyotype such that the chromosomes are present in proportional amounts. In most cases there are two copies (alleles) of each autosomal gene located on the chromosome provided by the mother and the father. The majority of proteins in the cell are encoded by autosomal genes. HI leads to loss of function or partial or complete loss of expression of one allele. Apart from human beings, HI has been demonstrated in fungi, *Candida albicans* (Uhlet al., 2003), and plants (Quileset al., 2024). Classic and historical example in humans was described by Stearns and Botstein (1998), who noted a gene and allele-specific lethality when a mutation in beta-tubulin was combined with a cold-sensitive mutation in alpha-tubulin.

Laboratory methods coupled with model organisms such as *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila melanogaster* have proven instrumental in facilitating the biological studies of HI to address diverse questions in biology. The relative copy number of a gene in the genome is referred to as gene dosage. Altered dosage is reflected on RNA and protein products with potential to affect cellular processes (detrimental or beneficial) (Estupiñan *et al.*, 2023).

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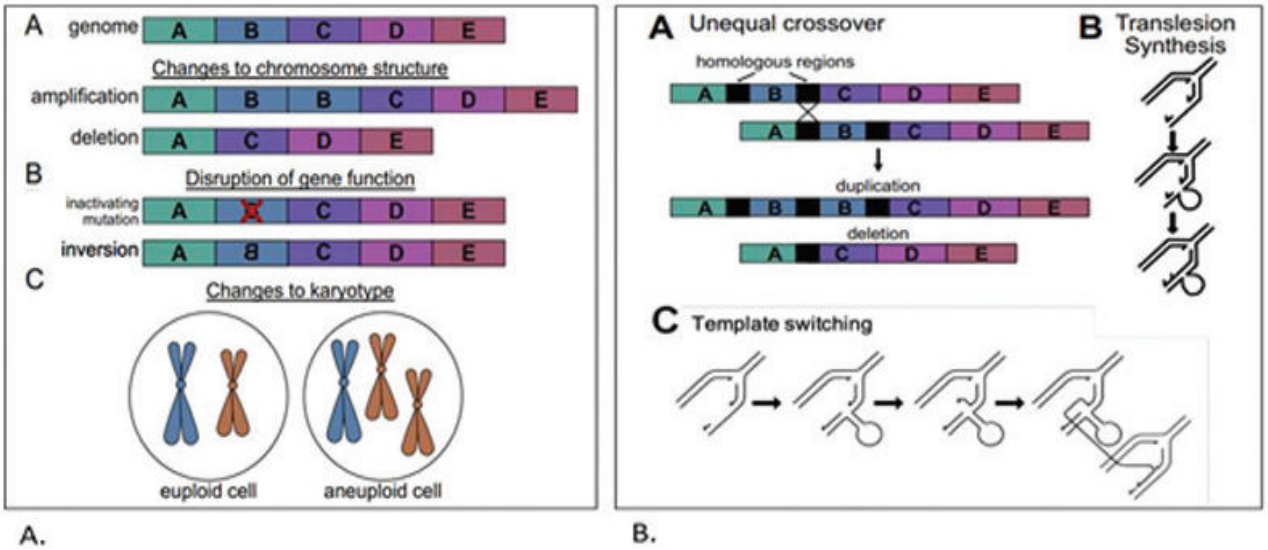
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Genes produce sufficient mRNA and protein from a single copy to prevent any loss of normal activity and function. HI occurs when loss of one copy of a diploid gene (hemizygosity) causes a phenotypic endpoint. Such changes can happen during DNA replication, DNA repair, or chromosome segregation and may result in both gains and losses of genetic information (Willemsen *et al.*, 2023; Potapova and Gorbsky 2017). Of the several HI causes, the copy number (CNVs) is frequently studied. The size of CNV range from few base pairs(bp) to millions of base pairs spanning one or several genes (Hujoel *et al.*, 2024). Three main sources of copy number alteration are changes to chromosome structure, disruption of gene function, or altered karyotype. All branches of life, from bacteria to humans, carry CNVs that cells and organisms accumulate throughout development and life term. As a result, chromosomes may contain amplifications and deletions, inactivated genes, or gains and losses of whole chromosomes. This could be a result of faulty repair of exogenous attacks on the genome (radiation, chemical) or endogenous (encountered during DNA replication and mitosis). The mutational event depends on the cellular process affected and location in the genome. For example, sub-chromosomal rearrangements are particularly likely to occur in repetitive areas of the genome, whereas whole chromosomal gains and losses are only likely to occur during cell division.

Mechanisms and effects

Broadly, these mechanisms are grouped into replicative mechanisms and non-replicative mechanisms. a. Replicative mechanisms—when normal DNA replication becomes blocked, attempts to continue DNA synthesis can lead to expansion or contraction at the site of replication fork stalling. Replication may become blocked as a result of DNA damage or the depletion of nucleotide pools (Zeman and Cimprich 2014). b. Non-replicative mechanisms cells can encounter a broad range of events that damage DNA throughout their lifetime through chemical or physical (carcinogenic agents or irradiation) or accumulation of oxidative stress (https://my.clevelandclinic.org/). DNA damage that results in a double-stranded break of the DNA helix is most relevant to the creation of CNVs. Non-replicative duplications and deletions occur during unequal crossover events in meiosis. Misalignments during crossing over result in alterations to chromosome structure. (https://www.nature.com/scitable/11) c. Mutations involve gene inactivation ranging from point mutations to single nucleotide insertions and deletions. Even though small in scale, they have a large effect on the gene product. A missense point mutation that changes a critical portion of the protein's structure could prevent it from binding with binding partners or disrupt the active site. Nonsense mutations introduce stop codons, truncating the protein products, often making them nonfunctional or degrading their mRNA or protein. Small insertions or deletions (few nucleotides) can cause a frameshift in the genetic code of a protein, leading to a change in shape or function (Encyclopedia of Dairy Sciences (Second Edition), 2011). d. Errors in mitosis or meiosis can result from genetic perturbation to the mitotic machinery or the surveillance mechanisms that monitor the chromosome attachments and tension. (Hosea *et al.*, 2024) e. Whole genome duplication throughout eukaryotic evolution, genomes undergoes an increase in ploidy (Levasseur and Pontarotti 2011). This increase in ploidy are typically unstable and are followed by widespread gene loss. The mechanisms are summarized in figure 1.



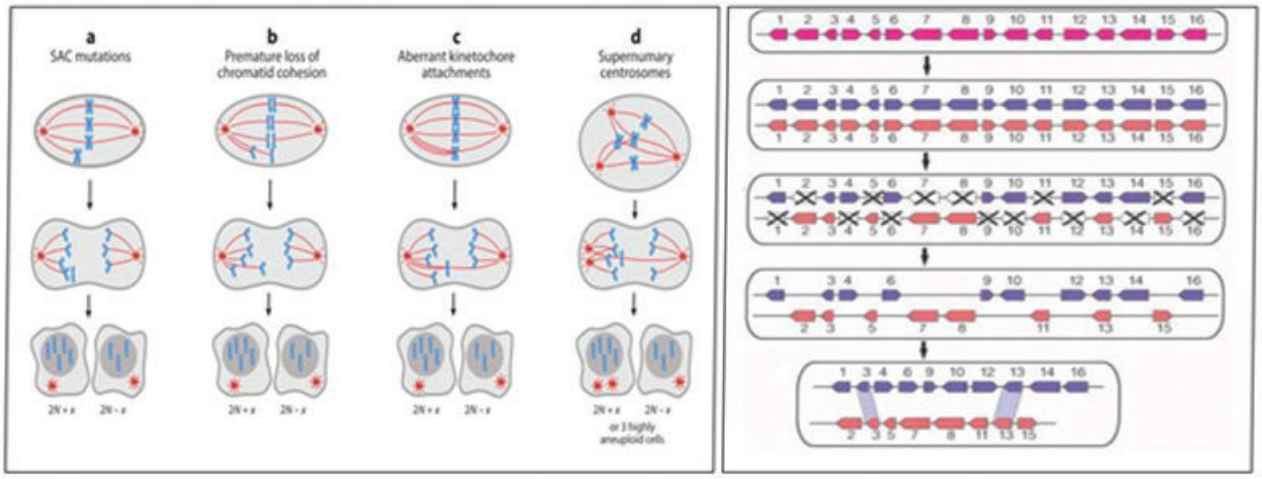


Figure-1.Various mechanims leading to copy number variation
A.Chromosomal mechanisms
B. Replication mechanisms
C. Chromosomal gain and loss.
D. Dosage imbalance following whole genome duplication in Saccharomyces cerevisiae.
Adapted from.MIT thesis submitted by Summer Ashlee Morrill

Gene expression in the human brain: anatomy, ontological, gene expression, and unique evolutionary features.

Receiving, processing, and carrying out the coordinated higher functions of perception, motion, and cognition are the tasks of the human brain, also known as the central nervous system (CNS). Neurons, glial cells, andneuropil, network of axons, dendrites, synapses, and extracellular matrix comprise the cellular components of the brain (Brain Atlas). Compared to non-human primates, the human brain exhibits a unique pattern of gene expression. An estimated one-third of the genes found in the human genome are active. Compared to other primates, this pattern gives humans distinct behavioral and cognitive abilities (Khrameeva*et al.*, 2020). Elevated levels of neuronal activity in different brain regions are indicative of this observation. This forms the basis for the diversity in the nervous system and associated physiology and functions reflecting higher expression levels of genes that carry out a wide range of cellular functions. An indicator of molecular evolution is the evolutionary rate of a protein. According to a study by Tamir Tuller *et al.*, (2008) report distinct human brain areas exhibit significant rates of evolution. Metazoan developmental gene expression profiles across species provide a window into the genes underlying organ development. Further, identifying the molecular programs underlying human brain development and how gene expression differs in various organs has implications for cellular biology and genomics of human diseases. According to a study by Moreir *et al.*, 2019) more than 200 genes linked to the brain, heart, and liver are found in orthologues in rhesus macaques, mice, rats, and rabbits. Brain expression compared to other organs and tissues implicates the highest expression in the brain (14 anatomically distinct regions, spinal cord, and corpus callosum) with 76% of all human proteins expressed in the brain. Regionally, the cerebellum has the most regionally enriched genes, followed by the cerebellum, hypothalamus, midbrain, and spinal cord (https://hupo.org/human-proteome-project).

Finally, cell fate determination in the brain during development and homeostasis requires appropriate spatial and temporal expression of specific genes and coordination of transcription factors (TFs) and cofactors in the cis-regulatory elements (CREs). Many developmental disorders (DDs) arise from impaired gene regulation (loss-of-function mutations) in genes coding for TF (Fahrner and Bjornsson 2019; Van der Lee *et al.*, 2020). Few genes with dosage sensitivity and HI (HI) of transcriptional regulators are listed in Table 3. The emerging theme from several research studies converges on the gene regulatory network (GRN) based bi-stability as the underlying cause.

Instability is a crucial concept in biology that regulates cell-fate decisions that require both positive feedback and sensitivity, often achieved through TF cooperativity. As demonstrated in TWIST1 in Saethre–Chotzen syndrome (Firulli *et al.*, 2005), SIX1 in branchio-oto-renal syndrome (Ruf *et al.*, 2004), and several other TF as reviewed in (Roman Zug 2022).

Plausible contributors to haploinsufficiency in brain-expressed genes

In addition to conferring exceptional cognitive and physical skills, the evolution of the human brain may have made humans more vulnerable to a variety of neuropsychiatric and mental illnesses. The human brain is an immensely complex organ composed of billions of precisely interconnected neurons, thus defining humans as a species more unique than any other on the evolutionary event (Johnson *et al.*, 2009). Thus, it has long been postulated that changes in the transcriptional regulation of key developmentally expressed genes contributed significantly to the evolution of human brain uniqueness (Byoung-Il Bae *et al.*, 2015).The brain circuitry depends on the diversity and precise spatiotemporal regulation of its transcriptome. These changes are thought to have led to the creation of new combinatorial expression patterns from a relatively limited set of genes, resulting in the formation of distinct circuits. Alternative splicing (AS) is proposed as a mechanism for increasing the spatiotemporal complexity of the transcriptome, which generates multiple mRNA transcripts from a single gene (Jutzi and Ruepp 2022) The importance of specific AS programs to a number of neurodevelopmental and neurological processes has been recognized by several researchers (Licatalosi and Darnell, 2006; Reble *et al.*, 2018). Several studies have profiled transcription and translation in the developing human brain. Since translational regulation is a

Table 1: Brief list of human diseases associated with Haploinsufficency

Gene involved	Associated condition / brief description	Reference
Transcription factor 3 (TCF3)	Immunodeficiency/B-cell Defects and reduced serum immunoglobulin	Boast B *et al.*, 2023
Hedgehog interacting protein (HHIP)	Chronic obstructive pulmonary disease (COPD) Inhibited aerobic glycolysis and repressed cell proliferation.	Li Yet al., 2021
The serine-/arginine-rich splicing factor 2 (SRSF2)	Squamous cell carcinoma. DNA damage with cell cycle alterations. clonal expansion.	Frost, Erin L *et al.*, 2024
The arginine methyltransferase (PRMT5)	Inflammatory bowel disease and colorectal cancer. reduced mucosal defense	Hernandez JE *et al.*, 2023.
Filamin C (FLNC)	Cardiomyopathies,myofibrillarmyopathy, cardiac arrhythmias.	Job A J Verdonschot *et al.*, 2020
Paired like homeodomain 1(Pitx1)	Clubfoot, muscle hypoplasia	David M Alvarado *et al.*, 2010.

Table 2: Human clinical syndromes associated with haploinsufficency

Disease	Gene involved	Organ involved / Major anomalies	Associated pathology	Reference
Xia-Gibbs Syndrome	AT-hook DNA binding motif containing 1AHDC1)	Intellectual disability, muscular hypotonia, brain anomalies.	Foot deformity, skin and connective tissue abnormalities.	Bertrand M *et al.*, 2024
Nedlaad disorder	Cell cycle associated protein 1(CAPRIN1)	Dysmorphic facial and digital Hand anomalies feature. Respiratory difficulties. Central nervous system.	Delay in walking, Language delay, anomalies mainly of the corpus callosum, Mild hearing loss.	Pavinato L et al., 2024
Von recklinghausen disease	NF1 (Neuro fibromin gene)	Skin Lesions and afé-au-la spots on skin. Lisch Nodules on Iris.	Fibromatous Tumors on skin,Forms Benign and Malignant tumor of skin.	William *et al* 2009

Autoimmune lymhoproliferative disease Autoimmune lymhoproliferative disease	cytotoxic T-lymphocyte associated protein 4(CTLA4)	Abnormal lymphocytic Infiltration of Non Lymphoid organs include Brain,Lung and Gastrointestinal tract.	Inflamatory Lung Lesions. Lymphocytic enteropathy.Seizures and Headache.	Lopez nevado *et al.*, 2021
Leri-weill dyschondrosteosis	SHOX (homeobox SHOX)	Skeletal dysplasia of Bone and cartilage,medulung wrist deformity includes proximal carpal bones.	Tibiofibular disproportion. Problems on limbs.	Ross et al., 2005

Table 3: A brief list of genetic defects associated with TF factor mutations.

Genes/ implicated	Disorder	Reference
GATA4, NKX2-5, TBX5	Congenital heart defects (CHDs)	Bruneau, 2008
HOXD13, RUNX2, SOX9	Holoprosencephaly	Zuniga et al., 2012
PAX3, SOX10, TBX1	Neurocristopathies	Vega-Lopez et al., 2018
NR2F2, NR5A1, SOX9	Disorders of sex development (DSD)	Ono and Harley, 2013;
MED13L, SETBP1, TBR1	Intellectual disability (ID)	Visserset al., 2016

unique mechanism, especially in the brain, a study of 73 human prenatal and adult cortexes suggests identification and annotation of several unknown translation events, small ORFs that give rise to microproteins (Duffy *et al.*, 2022). In neurological and neuropsychiatric diseases, different brain regions are affected, and differences in gene expression patterns could potentially explain this mechanism. Identifying and understanding the specific genes and gene expression patterns in each brain region may provide crucial insights into the underlying mechanisms. A study by Shimada (2024) on three different brain regions of the same individuals the cerebellum, hypothalamus, and temporal cortex implicate novel isoforms of genes expressed in multiple samples across multiple regions. Also, the study implicates, in part, DNA methylation as a driver of different isoform expressions in different regions.

Pathology studies of the brain have suggested that a number of factors, including changes in neurotransmitters, protein homeostasis, and energy demand, may be involved in this selectivity. Other changes that cannot be measured by histological techniques, such as synaptic connections or gene expression patterns, may also be involved. Finally, several genetic models (additive, epistatic) and protein regulation and still unknown mechanisms contribute to the final phenotypic heterogeneity. Therefore, from a genetics lens, HI serves as a potential contributing factor. Cumulatively, the above observations implicate unique gene expression in brain and regions paralleled with distinct protein expression. Also, several undiscovered regulatory and evolutionary mechanisms setting the stage for HI.

Haploinsufficiency in neuropsychiatric diseases

The spectrum of human diseases affected by HI includes organs and organ systems and affects diverse cellular and metabolic pathways.For a detailed and updated list, the reader is referred to OMIM and PubMed. Table-1 is a brief list of human diseases associated with haploinsufficiency. Several genes and networks involved in several human disorders, such as asthma, diabetes, cancer, and several other disorders are also affected by HI. Few genes belonging to this category are listed in Table 2.

Several genes play crucial roles in the development and regulation of cellular functions and in the maintenance of homeostasis in the brain. HI in these genes has the potential to perturb these processes. Initially, HI was investigated in autism, epilepsy, and major depressive disorders, with several reports in other neuropsychiatric syndromes such as frontotemporal dementia (FTD), schizophrenia (SCZ), and bipolar disorder (bp). In the following paragraphs we describe a few representative (HI) genes in major psychiatric disorders. HI has been reported in other psychiatric disorders like depression. Riga *et al.*, 2024, OCD Baig *et al.*, 2018, anxiety Purple *et al.*, 2020. They have not been covered in this short review in detail. The readers are referred to OMIM and PubMed for detailed descriptions. Sex-dependent effects of histone methyltransferase (*Setd1a*) gene HI are reported in development and adult behavior. Loss of function mutations affecting the *Setd1a* gene are implicated in the etiology of a range of neurodevelopmental disorders, including scz. Male mice displayed lower body weight and enhanced growth. Embryonic

whole brain RNA-seq analysis revealed expression changes enriched for mitochondria-related genes and anxiogenic behavior in both sexes(Bosworth *et al.*,2024). A study by Hara et al., 2024 report loss-of-function variants in the scz risk gene *Setd1a* alters neuronal networks in human neurons through the cAMP/PKA pathway. Another gene affected is the Disrupted-in-schizophrenia 1 (*DISC1*) gene. Misassembled protein impairs cognitive flexibility and social behaviors in a transgenic rat model. Cellular effects include increased corticosterone levels (Wang et al., 2022). Behavioral and molecular characterization of the rat model of the Dlg2 gene HI implicates genetic risk for psychiatric disorders. HI of knockout rat demonstrated abnormal behavioral phenotypes. Further, reductions in PSD-93 messenger RNA and protein were observed in the absence of compensation by other related genes or proteins (Waldron et al., 2022).Studies shownreelin gene (*RELN*) is associated with bp.Reelin is a glycoprotein mainly secreted by the Cajal-Retzius cells and a subpopulation of GABAergic interneurons where it is involved in signaling and synaptic functions. HI causes cognitive impairment in rodents (Ishii et al., 2016). Impaired pre-pulse inhibitions (PPI) are associated with several psychiatric disorders. Behavioral abnormalities of 22q11 deletion syndrome (22q11DS) includes Diverge and velo-cardiofacial syndromes, develops overlapping psychiatric disorders, mainly scz and bp. HI of two TF genes, *Tbx1 and Gnb1lcauses*PPI in either gene or both. Mice model of 22q deletion mimic PPI (Payloret al., 2016). Another gene the ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 2 *(ATP2A2),* is implicated in bp. The heterozygous loss-of-function of the endoplasmic reticulum (ER) Ca2+ pump responsible for Darier's disease is characterized by behavioral abnormalities and a hyper-dopaminergic state. ATP2A2 aliases SERCA2)which encodes the Ca2+ pump. The ER membranes of the knockout mouse brain showed decreased Ca2+ uptake activity. Neurons display decay of cytosolic Ca2+ levels after depolarization; also, the mice showed alterations to behavioral responses suggestive of enhanced dopamine signaling (Nakajima et al., 2021). HI in autism and autism spectrum disorders(ASD) are also reported, an example include the *MYT1Lgene.* The TF MYT1L promotes appropriate neuronal differentiation and maturation. HIincludes aberrant hypothalamic function and aberrant regulation of the neuropeptide system. Zebrafish knockdown recapitulate these changes (Maloney et al., 2024).Another gene implicated is the dual specificity tyrosine phosphorylation regulated kinase 1A (*Dyrk1a*) gene. Mice with HI exhibit traits associated with ASD and neurodevelopmental conditions such as developmental delays, intellectual impairment (ID), and autism spectrum disorders (ASD). Knock-in mice mutations displayed microcephaly, synaptic deficits, and altered phosphor-proteomic patterns suggestive of signaling/synaptic changes (Daniel Rohet al., 2024). In another example ASD risk is closely linked to disruptive variations in the chromodomain helicase *CHD8* gene, which regulates transcription throughout neurodevelopment. The etiology is proposed to be influenced by the disruption of gene regulatory networks in the developing brain caused by loss of CHD8 activity. In the embryonic and juvenile wild-type and Chd8 +/- mouse brains dysregulated expression of genes in chromatin remodeling synaptic signaling organization and synaptogenesis was observed (Yimetal., 2024).

Genetic implications of haploinsufficiency

(HI) loci have intriguing implications for studies of genome evolution, gene dosage, genetic redundancy, stability of protein complexes, and gene expression. For over a century, scientists have pondered on answering the question: Why do genes exhibit an abnormal phenotype upon deletion when the majority of genes do not? Early theories considered HI to be an artifact of diploidy, a failure of the wild-type allele to maintain protective dominance, ultimately disproven by the observation that equivalent rates of HI are present in organisms that primarily exist in the haploid state. Later theories explained the mechanism through physiological explanations to explain changes in dosage. More recent studies suggest that the context of gene function is also important. (Morriland and Amon.2019). High-throughput screens, metadata analyses, and computational predictions have been applied to define which genes arehaploinsufficient. Approximately 300 genes are known to be haploinsufficient, contributing to a wide range of human diseases, including neurodevelopmental disorders, psychiatric disorders, hematological disorders, and cancer. Another important observation is the conservation of the mechanism from yeast to humans, indicating that strong selective forces are in play in the mechanism (Michaela de Clare et al., 2011). Theoretical studies of HI and sensitivity to increased gene dosage explain why HI has persisted over evolutionary time. As suggested previously in the "dosage-stabilizing" hypothesis which proposes that HI persists in organisms

over evolutionary time because a balance must be struck between a gene products being limiting for a biological process while avoiding the toxicity of its overproduction (Yang et al., 2024).This theory offers a plausible explanation for the persistence of the mechanism throughout the evolution of the eukaryotic genome. Elaborate cellular mechanisms enable maintenance of the correct chromosome content over generations during evolution. With the rare errors often leading to cell death, cell cycle arrest, or impaired proliferation. At the same time, aneuploidy can provide a growth advantage under selective conditions in a stressful, frequently changing environment, such as aneuploidy commonly found in cancer cells, where it correlates with drug resistance, malignancy, and poor prognosis (Simonetti et al., 2018).

Given the relatively high frequency of gene-inactivating mutations over the lifespan of an organism and cell-to-cell variability in gene expression, HI represents a significant barrier to organismal fitness. Studies indicate over time to accommodate fluctuations in gene dosage fitness penalty is associated with both downregulating and upregulating of HI gene expression (JBirchler and Veitia 2012). Thus, HI genes represent a unique class of genes that must carefully balance their expression, ensuring that the cost of overproduction does not outweigh the potential benefit of maximizing growth. Changes to gene dosage can lead to imbalances in protein complex stoichiometry that adversely affect cellular fitness. Such as alterations in developmental disorders such as mental retardation (*DYRK1A*), leukemia (*ETS2*), and the premature aging and neurodegenerative features (*SOD1* and *APP*)(Basilicata, et al., 2012) and chromosomal alterations in *Myc, MAPK13,* and *MAPK14* genes in cancer that confer drug resistance (David & Amon 2020). Advantage of this mechanism at the cellular level is the stress-responsive chaperone Hsp90 gene which impacts development and adaptation from microbes to humans through fitness consequences of regulatory variation (Jakobson et al., 2023).Another example is the *EGR1* gene in tumor development. HI confers a fitness advantage to hematopoietic stem cells following chemotherapy (Stoddart et al., 2022). The observation that the fraction of haplosufficient genes is the highest among the genes that encode enzymes is compatible with the physiological theory. From an evolutionary perspective, HI genes, on average, have more paralogs than haplosufficient genes, supporting the idea that gene dosage could be important for the initial fixation of duplications. Thus, HI of a gene and its propensity for duplication might have a common evolutionary basis (Kondrashov and Koonin 2004).

A unique finding in medical genetics is that not all carriers of mutation actually develop the disease, and express the disease phenotype in different degrees known as incomplete penetrance and variable (Rebecca Kingdom and Caroline F Wright et al., 2013). Mutations that interfere with buffering mechanisms (genetic redundancy, parallel feedback) can expose otherwise buffered variability are the prevalent explanations for the mechanism. Parallel arrangements of positive feedback loops are a general feature of cell-to-cell variability during cell fate a major contributor to variable expressivity (Ahrends et al., 2014; Dey and Barik, 2017).The stochastic fluctuation in gene expression best explains the incomplete penetrance.

Study by Agarwal et al., 2023 report loss-of-function (LOF) variants for Mendelian and severe complex diseases implicate enrichment in 'mutation intolerant' genes. Notable findings of the study such as the enrichment of de novo mutations in highly constrained genes which have implications for human diseases.*Saccharomyces cerevisiae* (yeast) offers alternative cellular models to study gene properties and genome scale HI phenotypes. A study by Norris et al., 2013, compared the HI profiles against 23 gene properties and found genes with higher levels of connectivity (degree) in a protein protein interaction network, genetic interaction, sequence conservation, and protein expression were significantly more likely to be haploinsufficient. Additionally HI showed negative relationships with cell cycle regulation and promoter sequence conservation. The stoichiometric principle has implications for the control of gene expression and on the constraints for various regulatory genes. Gene balance effects are hypothesized to result from stoichiometric differences among gene members of macromolecular complexes, interactome and signaling pathways(Birdet al., 2023). These consequences could have implications in biophysics,quantitative traits and could at least in part explain multi-genic inheritance. Within populations, this principle governs the fate of natural variants that alter the quantity of regulatory molecules as well as the actual gene number. The evolutionary consequence of gene dosage balance results in retention of classes of genes depending on whether they are duplicated by whole genome (WGD) or segmentally (Basilicata, et al., 2021). Subtle variations can exist for regulatory genes, which have the potential to affect any one trait. With appropriate strong selection variants contributing to more phenotypic extremesaccumulate. Thus,

anchoring observations in cellular models, biophysics and human population genetic models and evolution enables to study fitness effects of mutations (HI) identified by different mapping strategies for different human traits.

Applications of haploinsufficiency and conclusions

In the context of human diseases, identifying the mechanism of action of drugs (novel) and medications is a crucial step toward improving clinical treatment. HI may have important consequences in pharmacogenomics (individual treatment) and variable drug toxicity observed in human populations. Identify drug targets through screening of genome-wide deletion involves deletion of key molecular components involved in a drug's mechanism of actionreferred to as haploinsufficency profiling (HIP) (Evans et al., 2024). HI can be used in prenatal diagnosis to identify genetic disorders when one allele is mutant and the other is wild-type, resulting in insufficient function. Few examples include *SHOX*, a skeletal disorder where *in utero* genetic techniques and prenatal ultrasounds is carried out. Another example is the *SETBP1* anautosomal dominant disorder, detected using prenatal and preimplantation genetic testing. Another area of genetics where HI has applications is the area of genetic interaction. Wang and Peng 2017 demonstrate this in a GIT (Genetic Interaction Network-Assisted Target Identification)network analysis for drug target identification. With the drug-induced phenotypic fitness defect of the deletion of a gene, GIT incorporates the defects of the gene's neighbors in the GIT in HI profiling (HIP) and homozygous profiling (HOP) screens. Anexample is the study of *Candida albicans*, an important fungal human pathogen. Glazier et al., 2023 identifyHI-based genetic interaction in TF in each stage of biofilm formation.In summary, the study of HI traverses several areas of human biomedical research, from human genetic diseases, biophysics, developmental biology, and quantitative genetics to biomedical applications. The author acknowledges that several areas of relevance to human biology, genetics, and brain disorders have not been included in the review.

No human consent or animal ethics was involved in the study. No conflict of interest.

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