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PEARLS OF LABORATORY MEDICINE

Gene Dosage Analysis

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Gene Dosage Analysis

Learning objectives

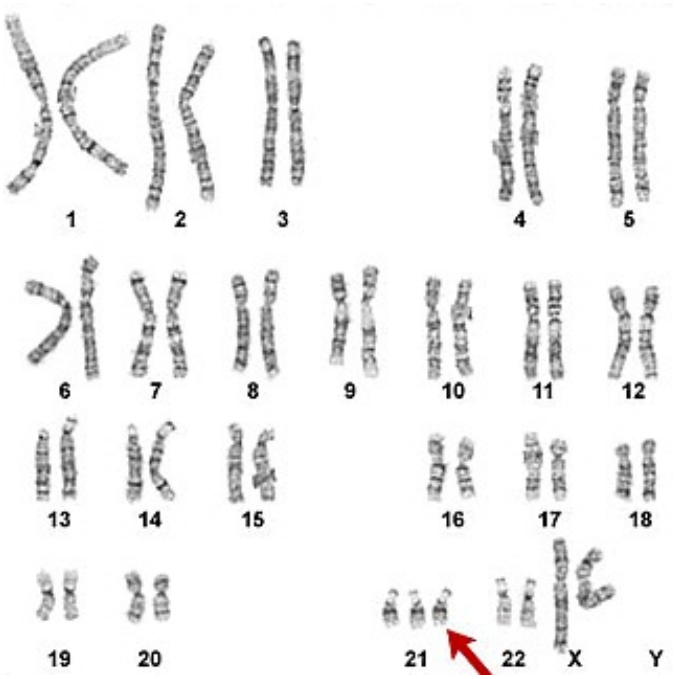
- Understand gene dosage effects
- Identify testing available for gene dosage analysis
- Discuss current trends in gene dosage analysis

Outline

- Introduction and definitions – gene dosage effects
- Testing – genome-wide approaches using next generation sequencing (NGS) and whole exome sequencing (WES)
- Current trends – cancer, drug therapy, and research

What are gene dosage effects?

- Copies of a gene in a genome
- Amount of gene product that can be expressed
- A gene dosage effect occurs when the structural gene produces a proportional amount of product relative to its copy number
- More copies of a gene = more gene product expressed
- Less copies = less product expressed
- Down syndrome (Trisomy 21)



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https://embryology.med.unsw.edu.au/embryology/index.php?title=File:Karyotype_Down_syndrome.gif

Does copy number always affect gene dose?

- **Depends**
  - Gains or losses may not always affect phenotype or dose
  - A gene copy number may affect the dosage of another gene
  - Copy number variability among individuals (polymorphisms)
  - Type of genetic alternation can effect gene doses differently

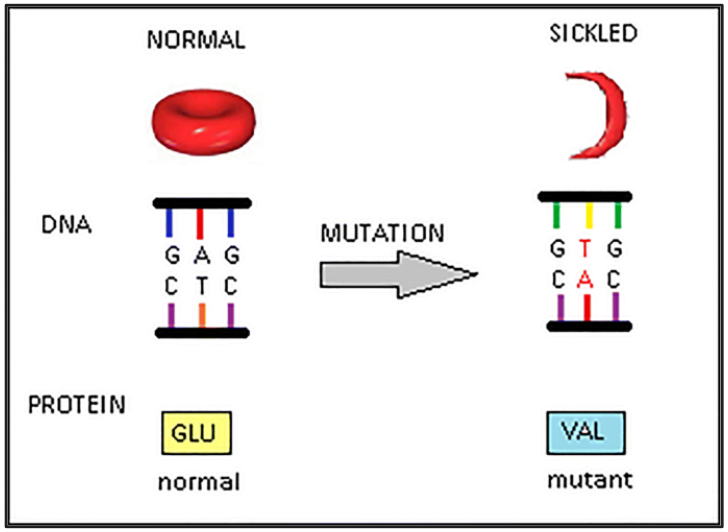
General rule of thumb:

Normal dosage = 2 = 2 copies

< 2 = deficiency (loss)

> 2 = excess (gain)

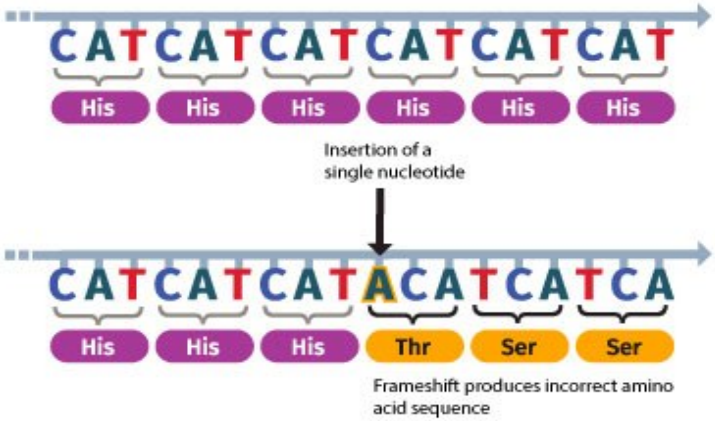
- Single nucleotide variant (SNV)
  - ≥ 1% = single nucleotide polymorphisms (SNPs)
  - synonymous/silent mutation
  - nonsynonymous/missense
    - conservative missense mutation
    - non-conservative missense mutation
      e.g. sickle-cell anemia
  - stop gain, start loss, or nonsense mutation
    - e.g. cystic fibrosis
    - deletion
    - SNP



Laurentino, M. R., Parente Filho, S. L. A., Parente, L. L. C., da Silva Júnior, G. B., Daher, E. D. F., & Lemes, R. P. G. Non-invasive urinary biomarkers of renal function in sickle cell disease: an overview. Annals of hematology 2019; 1-8. (*Adapted with permission*)

Other types of genetic alterations

- Insertion or deletions = Indel (< 50 base pairs)
  - Mutation type: Frameshift
  - Occurs during translation



- Structural variations (> 50 base pairs)
  - Mutation types: deletions, insertions, inversions, translocations, duplications, or combination of all
  - **Copy number variation (CNV)**
    - structural variation that changes DNA copy number either del or dup
    - CNV varies among individuals
    - about 4.8 to 9.5% of the repeat sections in the genome are classified as CNV
    - TEXTBOOK = ≥1 kb and present in a variable copy number vs. reference genome
    - CNVs arise via either homologous recombination or nonhomologous recombination mechanisms

Rifai, N., Horvath, A. R., Wittwer, C. T., & Park, J. (Eds.). Principles and Applications of Molecular Diagnostics. Elsevier 2018. (*Adapted with permission*)

CNV

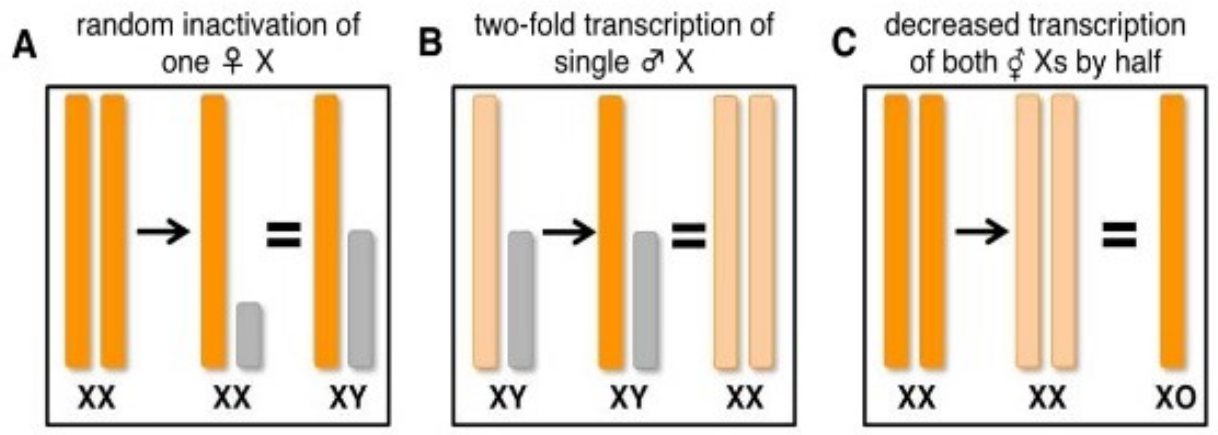
- Rare (<1%) or common (>5%) variants observed in the population
- Inherited or *de novo* (e.g. autism spectrum disorder, increased age)
- Structural gains can be
  - Duplications
  - Insertional transpositions
- Structural losses or deletions can be
  - heterozygous (with only one copy missing),
  - homozygous (with both copies missing), or
  - hemizygous (e.g. X chromosome in males, cancer)
- CNV is normal. E.g. a person could have four copies instead of the usual two, and somebody else has three, and somebody else has five, all normal
- **CNV and dosage influence a wide range of traits, are associated with disease risk (low and high), and explains some *de novo* disorders**

Examples of maladaptive CNVs

- Psychiatric Disorders
- cardiomyopathies - cardiac diseases associated with sudden cardiac death
- amyotrophic lateral sclerosis (ALS) -In addition SNPs, a consistent number of common (>5%) and rare (<1%) CNVs have been associated to ALS
- Huntington disease or HD ≥ 36 CAG repeats in the HD gene (*HTT*)
  - individuals with 27–35 CAG repeats are unaffected by the disease
  - these unaffected individuals (with 27–35 CAG repeats) have increased CAG tract sizes relative to the general population

Example of dosage compensation

- Male and female cells express X chromosome genes at the same level
- Female cells have double the number of X chromosomes as male cells



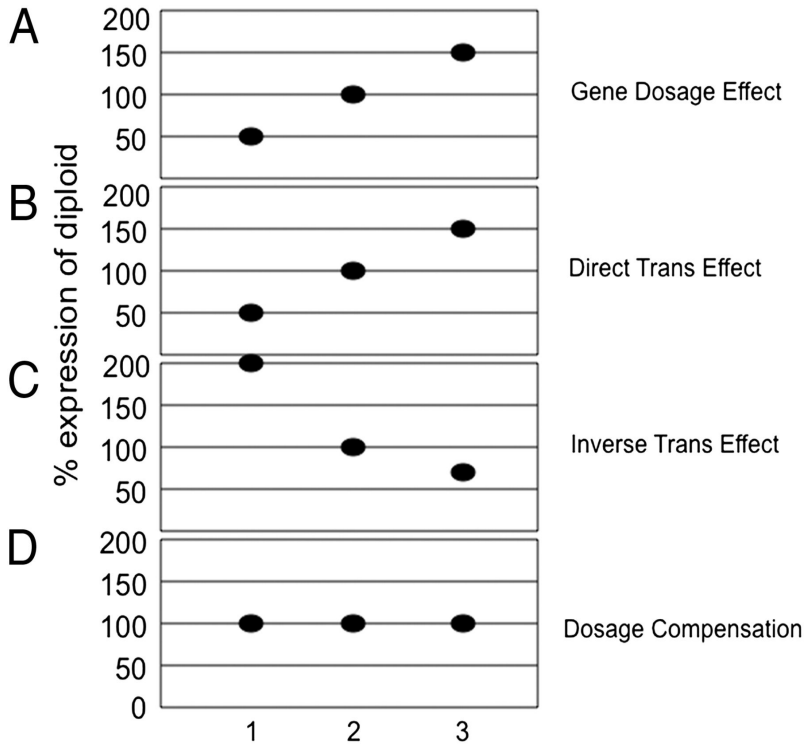
Types of dosage and compensation

(A) Structural gene copy number = amount of product

(*B*) Direct transacting effects when gene expression of one gene is affected by the gene dosage of another gene

(*C*) Expression of a gene is inversely correlated with the dosage of another chromosomal region

(*D*) Dosage does not change or affect expression
- an inverse dosage effect of an aneuploid region includes genes that are also on the altered chromosome
- Combined structural gene and inverse dosage can produce nearly equal expression in all chromosomal doses



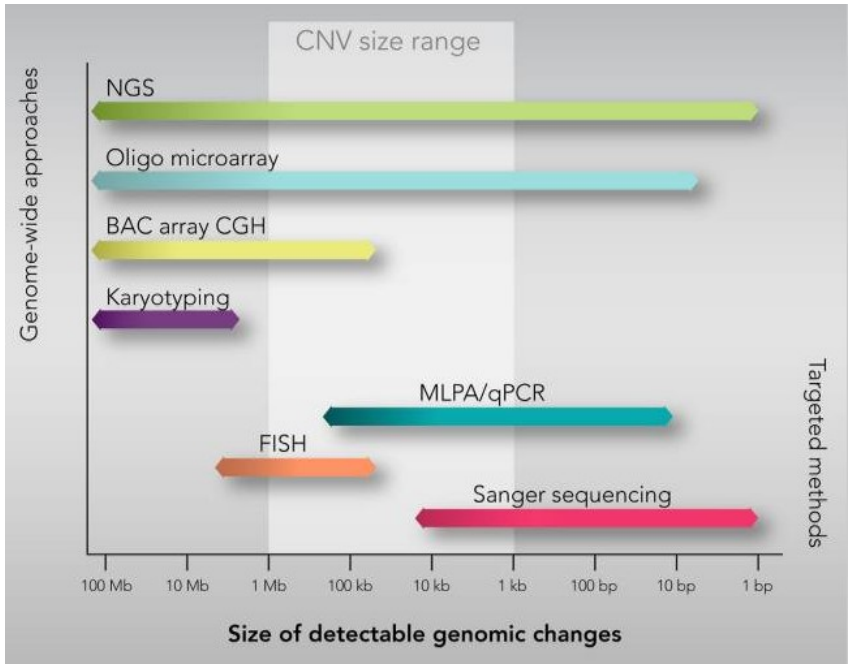
Birchler, J. A., & Veitia, R. A. Gene balance hypothesis: connecting issues of dosage sensitivity across biological disciplines. Proceedings of the National Academy of Sciences 2012; 109:37, 14746-14753. (*Adapted with permission*)

Summary: Gene dosage effects & Dosage compensation

- Gene dosage effects varies and varies between individuals
  - Example: Parkinson's disease. Evidence has shown that genetic causes can vary depending on the geographic and ethnic backgrounds of the studied populations
- Variation of gene expression products
- Variation of disease and disease phenotype
- How gene dosage occurs varies
  - structural - duplication or deletion that affects a number of base pairs
  - length
    - Short repeats = bi-nucleotide repeats (like A-C-A-C-A-C-) or trinucleaotide repeats (like CG in Huntington disease)
    - Long repeats = entire genes repeated
  - Loss of Function (LOF) mutations
- Variation of compensation mechanisms

How do we perform gene dosage analysis? Genome-wide approaches?

- Cytogenetic & molecular testing
  - Karyotyping
  - FISH - fluorescence in situ hybridization
  - Microarray
    - CGH - comparative genomic hybridization
    - SNP - single-nucleotide polymorphisms
- Sequencing
  - NGS – next generation sequencing
  - WES - whole-exome sequencing
- PCR – polymerase chain reaction
  - qPCR - quantitative real-time PCR
  - mPCR-RETINA - multiplex PCR-based real-time invader assay
  - MLPA - multiplex ligation-dependent probe amplification



Morello, G., Guarnaccia, M., Spampinato, A. G., La Cognata, V., D'Agata, V., & Cavallaro, S. Copy number variations in amyotrophic lateral sclerosis: piecing the mosaic tiles together through a systems biology approach. Molecular neurobiology 2018; 55:2, 1299-1322. (*Adapted with permission*)

Other notable approaches

- Paralogue ratio test (PRT)
- Molecular copy-number counting (MCC)
- Multiplex PCR-based approaches, i.e.
  - multiplex amplifiable probe hybridization (MAPH)
  - quantitative multiplex PCR of short fluorescent fragments (QMPSF)
  - multiplex amplicon quantification (MAQ)

Current trends in gene dosage effects

Trends in Genetics
Volume 36, Issue 10, October 2020, Pages 764-776

Review

The Consequences of Abnormal Gene Dosage: Lessons from Chromosome 18

Jannine DeMars Cody [1, 2, 3, 4]

Macromolecular crowding links ribosomal protein gene dosage to growth rate in *Vibrio cholerae*

Alfonso Soler-Bistué[1,2], Sebastián Aguilar-Pierlé[1], Marc Garcia-Garcerá[3,4,5], Marie-Eve Val[1], Odile Sismeiro[6], Hugo Varet[6], Rodrigo Sieira[7], Evelyne Krin[1], Ole Skovgaard[8], Diego J. Comerci[2], Eduardo P. C. Rocha[3,4] and Didier Mazel[1*]

Maximizing antibody production in a targeted integration host by optimization of subunit gene dosage and position

Joe Carver, Domingos Ng, Michelle Zhou, Peggy Ko, Dejin Zhan, Mandy Yim, David Shaw, Brad Snedecor, Michael W. Laird, Steven Lang, Amy Shen, Zhilan Hu

First published: 21 January 2020 | https://doi.org/10.1002/btpr.2967 | Citations: 3

Research article | Open Access | Published: 09 November 2019

Copy number variation is highly correlated with differential gene expression: a pan-cancer study

Xin Shao, Ning Lv, Jie Liao, Jinbo Long, Rui Xue, Ni Ai, Donghang Xu & Xiaohui Fan

BMC Medical Genetics **20**, Article number: 175 (2019) | Cite this article

5096 Accesses | **7** Citations | Metrics

Current research in gene dosage effects

Kidney Int Rep. 2020 May; 5(5): 575–576.
Published online 2020 Apr 10. doi: 10.1016/j.ekir.2020.03.007
PMCID: PMC7210744
PMID: 32406422

Copy Number Variation: A New Genetic Form of Polycystic Kidney and Liver Disease

Takuya Fujimaru[1] and Eisei Sohara[1,*]

A comprehensive genomic scan reveals gene dosage balance impacts on quantitative traits in *Populus* trees

Héloïse Bastiaanse, Matthew Zinkgraf, Courtney Canning, Helen Tsai, Meric Lieberman, Luca Comai, Isabelle Henry, and Andrew Groover

PNAS July 2, 2019 116 (27) 13690-13699; first published June 18, 2019 https://doi.org/10.1073/pnas.1903229116

Edited by James A. Birchler, Division of Biological Sciences, University of Missouri, Columbia, MO, and approved May 24, 2019 (received for review February 22, 2019)

Research article | Open Access | Published: 29 March 2020

Sensitivity to gene dosage and gene expression affects genes with copy number variants observed among neuropsychiatric diseases

Maria Yamasaki, Takashi Makino, Seik-Soon Khor, Hiromi Toyoda, Taku Miyagawa, Xiaoxi Liu, Hitoshi Kuwabara, Yukiko Kano, Takafumi Shimada, Toshiro Sugiyama, Hisami Nishida, Nagisa Sugaya, Mamoru Tochigi, Takeshi Otowa, Yuji Okazaki, Hisanobu Kaiya, Yoshiya Kawamura, Akinori Miyashita, Ryozo Kuwano, Kiyoto Kasai, Hisashi Tanii, Tsukasa Sasaki, Makoto Honda & Katsushi Tokunaga

BMC Medical Genomics **13**, Article number: 55 (2020) | Cite this article
694 Accesses | **11** Altmetric | Metrics

Plant Epigenetics and Epigenomics pp 161-171 | Cite as

The Gene Balance Hypothesis: Epigenetics and Dosage Effects in Plants

Authors Authors and affiliations

Xiaowen Shi, Chen Chen, Hua Yang, Jie Hou, Tieming Ji, Jianlin Cheng, Reiner A. Veitia, James A. Birchler

Drug Therapy & Gene Therapy

- Drug Therapy
  - Pharmacogenetics
  - Example: *CYP2D6*, (chromosome 22) a key drug-metabolizing gene, which not only harbors multiple genetic variants known to affect enzyme function but also shows a broad range of copy-number and hybrid alleles in various patient populations

- Gene editing
  - changes specific parts of a genome
  - CRISPR-Cas9
  - ongoing research to determine whether this approach is safe and effective for use in people

- Gene therapy
  - It is a therapeutic approach that is being investigated for the treatment of multiple diseases
  - a popular vector/envelope is Adeno-associated virus or AAV
  - Though many gene therapies are currently in early research or clinical trials, some have already been approved by the US Food and Drug Administration (FDA)

FDA approved gene therapies

APPROVED GENE THERAPIES

Type of Therapy	Disease State	Year Approved
Gene Addition		
Adeno-associated virus vector, *in vivo*	Inherited retinal dystrophy[5]	2017
Adeno-associated virus vector, *in vivo*	Spinal muscular atrophy[6]	2019

APPROVED CAR T-CELL THERAPIES

Type of Therapy	Disease State	Year Approved
CAR T		
Lentiviral vector, *ex vivo*	Acute lymphoblastic leukemia (ALL)[7]	2017
Retroviral vector, *ex vivo*	Relapsed or refractory large B-cell lymphoma[3]	2017

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1. Birchler, J. A., & Veitia, R. A. Gene balance hypothesis: connecting issues of dosage sensitivity across biological disciplines. Proceedings of the National Academy of Sciences 2012; 109:37, 14746-14753.
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4. Morello, G., Guarnaccia, M., Spampinato, A. G., La Cognata, V., D'Agata, V., & Cavallaro, S. Copy number variations in amyotrophic lateral sclerosis: piecing the mosaic tiles together through a systems biology approach. Molecular neurobiology 2018; 55:2, 1299-1322.
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7. Zhang, F., Gu, W., Hurles, M. E., & Lupski, J. R. Copy number variation in human health, disease, and evolution. Annual review of genomics and human genetics 2009; 10, 451-481.

Disclosures/Potential Conflicts of Interest

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- **Stock Ownership:** No disclosures
- **Honoraria:** No disclosures
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- **Patents:**No disclosures

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