CCTC Clinical Chemistry Trainee Council | AACC Better health through laboratory medicine.
PEARLS OF LABORATORY MEDICINE
www.traineecouncil.org

TITLE: Gene Dosage Analysis

PRESENTER: Ibi Aseyori

Slide 1:

Hello, my name is Dr. Ibi Aseyori. I am Assistant Lab Director at Immufood, an Instructor at University of the People, and a part-time consultant for Trinity Lab Services. Welcome to this Pearl of Laboratory Medicine on “Gene Dosage Analysis.”

Slide 2:

At the end of this presentation I hope:
• you will have learned about gene dosage effects
• understand the tests that are available for gene dosage analysis
• have a working knowledge on current trends in gene dosage research, treatment, and practice

Here’s the outline for this presentation – we’ll start
• with a general introduction and review some terms to gain an appreciation for understanding gene dosage

© 2016 *Clinical Chemistry*

•next we'll talk about how we test for gene dosage effects and how we perform gene dosage analysis by utilizing genome-wide approaches such as next generation sequencing and whole exome sequencing
• then we'll re**view current trends in research; for example, the majority of the literature on gene dosage seems to focus on cancer research and drug therapy**

Slide 3:

What are gene dosage effects?
A gene dosage effect occurs when the structural gene produces a proportional amount of product to its copy number.
There is an appropriate "dose" for every gene so too much or too little expression of a given gene or set of genes can result in cellular dysfunction and disease.
The more copies of a gene, the more gene product is expressed and vice versa (the less copies of a gene, the less that gene's product is going to be expressed or maybe no gene product will be expressed).

For example, in Down syndrome, the gene expression on chromosome 21 has increased by 50%, why? **Because the number of copies of chromosome 21 has increased from 2 to 3 or trisomy 21.**

Slide 4:

But is it really always that simple? Can we always follow the general rule of thumb?

Yes & no. It depends. It's complicated because:

• the number of genes may or may not have any dosage effects

• the number of a particular gene may cause a dosage effect on a different set of genes and their gene products

• the number of repeats varies between individuals (we call these polymorphisms)

© 2016 *Clinical Chemistry*

• different gene alterations result in different gene dosage effects, for example

• Single nucleotide variant or SNV as the name implies, is when a single nucleotide is substituted for another. The image shows the single nucleotide substitution of adenine to thymine.

- o SNVs can be rare in one population but common in another.
- **o** When a SNV is present in at least 1 % of the population then it is referred to as a single nucleotide polymorphisms or SNPs

As you can imagine, different types of mutations will result when one nucleotide is substituted for another. There are 3 kinds of SNV mutations or point mutations:

- o A synonymous mutation/silent mutation – is when a nucleotide substitution does not result in a change in amino acid.
- o A nonsynonymous mutation/missense – is when a nucleotide substitution leads to an amino acid substitution. This may or may not result in a pathogenic variant depending on the effect of the amino acid substitution on protein function and structure.

If missense does not result in a pathogenic variant then it is considered to be a conservative missense mutation where the function of a protein is only slightly changed and the properties of the amino acid remain the same (e.g., hydrophobic, hydrophilic, etc.)

In a non-conservative missense mutation, a completely different kind of amino acid is produced thus results in a completely different protein. For example, in sickle-cell anemia the β-globin gene on chromosome 11 suffers a non-conservative missense mutation where, as the image shows, adenine is switched with thymine and the GAG codon for glutamic acid is changed to GTG codon for valine

Hence sickle-cell anemia is an example of a gene dosage effect resulting from 2 abnormal copies of the β-globin gene – why? Because the inheriting 2 abnormal copies results in fewer or no copies of the normal β-globin gene

- o stop gain/start loss/or nonsense mutation – is when the nucleotide substitution results in a stop codon that consequently results in either premature protein truncation or a protein that is non-functional or a reduction in protein production or in the worst case scenario the elimination of protein production. For example, Cystic Fibrosis, similar to sickle-cell, is an example of gene dosage effects from

inheriting 2 mutated copies of the CFTR gene (or cystic fibrosis transmembrane conductance gene) on 7q31.2

Sometimes nonsense mutations are due to a deletion of one or more nucleotides and at other times nonsense mutations could be due to a single nucleotide polymorphism.

Slide 5:

Insertion/deletions – indels usually contain less than 50 base pairs of DNA that has either been inserted into or deleted from the genome.

• Indels always result in frameshift mutations

• The image shows an example of a frameshift mutation that occurred when adenine was inserted in between both CAT codons thus changing the reading frame from CAT CAT to CAT – ACA – TCA and so on.

Where indels are < 50 base pairs, structural mutations happen when genome rearrangement is more than 50 base pairs.

• Structural variations can be the result of indels, inversions, translocations, duplications, or a combination of all.

• copy number variation or CNV is a type of structural variation that changes the number of copies of specific regions of DNA, these regions can either be deleted or duplicated

  o the number of copies of a particular gene varies from one individual to the next

  o about 2/3rds of the human genome are repeats, out of those repeats about 4.8 to 9.5% are classified as CNV

  oCNVs are defined as segments of DNA 1 kb or greater and are present in a variable copy number compared to a reference genome

  o CNVs can arise due to homologous recombination between repeated sequences or nonhomologous recombination mechanisms that occur throughout the genome.

Slide 6:

• CNV can be rare (that is it occurs in less than 1% in the population) or common (where variants are observed in greater than 5% of the population)

• When CNVs aren't inherited, they are de novo meaning they spontaneously arise in the population and may or may not contribute to the familial transmission (for example autism spectrum disorder and increased age are associated with higher risk of CNV mutations that arise de novo)

• CNV structural gains can be

  o duplications or insertional transpositions

• CNV structural losses or deletions can be

  o heterozygous (with only one copy missing)

  o homozygous (with both copies missing)

  o or hemizygous where only 1 copy is present

• Interestingly, CNV variation can also be observed in individuals with a normal phenotype

• So in essence CNV is the cause and gene dosage is the effect. Sometimes those copy number variants include several normal genes or dosage-sensitive genes.

Bottom-line:

CNV and dosage influence a wide range of traits, are associated with disease risk (low and high), and explains some disorders resulting from de novo mutations.

Slide 7:

Research is showing that disease risk is commonly associated with CNV and dosage sensitive genes. For example, maladaptive CNV's and gene dosage effects are associated with:

• Psychiatric Disorders (for example autism spectrum disorder, attention-deficit/hyperactivity disorder (ADHD), intellectual disability, as well as schizophrenia)

• cardiomyopathies

• amyotrophic lateral sclerosis (ALS) -In addition to single-nucleotide polymorphisms (SNPs) which account for only a limited number of ALS cases, a consistent number of common and rare CNVs have also been associated with ALS.
• Huntington disease or HD is a dominantly inherited neurodegenerative disorder
  - o CAG repeats more than 36 times in Huntington gene
  - o Meaning that the smallest CAG repeat length associated with the Huntington phenotype is 36, but repeat lengths of 36–39 CAG have been found in asymptomatic elderly individuals
  - o individuals with 27–35 CAG repeats are unaffected by the disease
  - o these unaffected individuals (with 27–35 CAG repeats) have increased CAG tract sizes compared to the general population

Slide 8:

The normal dosage of a gene is two because most genes present in the human genome are found in two copies.
So if there are two functional copies of a gene there is normal dosage of the gene and normal gene product expression.
If there are more than two functional copies of a gene then the gene is amplified and gene product becomes overexpressed, resulting in cellular abnormalities and possible disease progression for example cancer.
Gene dosage can also be affected by a loss of one functional gene copy so the gene product is under expressed or not expressed at all, for example, disorders such as intellectual disability and developmental delay.
Dosage compensation is a way to equalize or balance the expression of genes.
For example, dosage compensation can be seen in sex-chromosomes:
Female cells have double the number of X chromosomes as male cells. Therefore, female cells should express twice the amount of X chromosome genes than male cells BUT - they DON’T.
Male and female cells express X chromosome genes at the same level.

How? Because of dosage compensation which can happen either by:
A) Random X inactivation where females shut off expression from one of the two Xs, matching
male single X expression OR
B) 2-fold transcription of an X where males could increase expression of some genes on their one X-chromosome OR
C) Decreased transcription of both X's by either males or females for example, one of the two
X chromosomes is selected to be shut down early in development in a process known as X chromosome inactivation.

Slide 9:

Types of dosage and compensation:
(A) the copy number of the structural gene is proportional to the amount of product produced
(B) There are also direct transacting effects, in which a gene is modulated in expression in direct correlation with a different chromosomal dosage
(C) dosage and compensation can be indirect
(D) Dosage compensation can also be the result of when the expression of a gene is inversely correlated with the dosage of another chromosomal region. In this case dosage compensation would not change or affect expression because

- an inverse dosage effect of an aneuploid region includes genes that are also on the altered chromosome and
- the combined structural gene and inverse dosage can produce nearly equal expression in all chromosomal doses
- so the two effects, the structural gene on the altered chromosome and inverse dosage, combined together cancel to produce nearly equal expression in all chromosomal doses

Slide 10:

In summary, gene dosage effects are not as simple as having more or less of one gene copy or that multiple gene copies results in phenotypic dysfunction.
The phenomena of seeing certain individuals with a few damaging mutations who are severely diseased compared to others harboring multiple potentially deleterious mutations who appear phenotypically normal, complicates gene dosage effects.
For example, Parkinson's disease. Evidence has shown that genetic causes can vary depending on the geographic and ethnic backgrounds of certain studied populations.
Gene product expression varies – could be more or less depending on the variation disease & disease phenotype expression varies.

Also, gene dosage mechanisms vary.
For example, gene dosage effects could occur due to
1. Structural genetic aberrations like – Deletions, duplications, indels, and so on
2. or variations in length
   - You have can have Short bi-nucleotide repeats like A-C-A-C-A-C- or short trinucleaotide repeats like C-A-G- in Huntington disease
   - Or Long repeats where entire genes repeated

3. gene dosage effects could also be the result of LOF mutations.
To add another layer of complexity, Dosage Compensation mechanisms vary (like direct trans effect, indirect, and so on)

Slide 11:

How do we test for gene dosage effects? How do we perform gene dosage analysis?
What are genome-wide approaches for testing?
• under Cytogenetic and molecular testing
  o we have Karyotyping – which is looking at chromosome images to identify abnormal chromosome number or structure
  o FISH - fluorescence in situ hybridization (first molecular method able

to detect submicroscopic genomic CNVs, but it is a time-consuming method that requires prior knowledge of the regions of interest for probing so FISH is not used for genome-wide analyses)

o on the other hand Microarray is a genome-wide approach that lets us scan the entire genome for abnormalities in particular CNV's

o CGH - comparative genomic hybridization

o SNP - single-nucleotide polymorphisms

both are Microarray technologies currently used to identify CNVs but they cannot detect short CNV

• Sequencing on the other hand can detect low-frequency variants, 2 sequencing methods are

o NGS – next generation sequencing

o WES – whole-exome sequencing

• PCR – polymerase chain reaction

o qPCR - quantitative real-time PCR (is an efficient method for screening CNV-targeted genomic regions. However, this technique does not allow the simultaneous amplification and quantification of a large number of targets in a single reaction)

o mPCR-RETINA - multiplex PCR-based real-time invader assay

• MLPA - multiplex ligation-dependent probe amplification is an alternative targeted PCR-based approach that allows simultaneous analysis of multiple targets (up to 40 targets) with one primer pair, reducing the probability of obtaining spurious qPCR results due to different reaction conditions.

Slide 12:

Other notable approaches include:

paralogue ratio test (PRT)

molecular copy-number counting (MCC)

multiplex PCR-based approaches, such as
multiplex amplifiable probe hybridization (MAPH)
quantitative multiplex PCR of short fluorescent fragments (QMPSF)
and multiplex amplicon quantification (MAQ)

Slides 13/14:

- Current trends in research look at gene dosage variation and how the associated changes in gene expression influence a wide variety of traits in humans, plants, disease, and so on
- CNVs can affect phenotype through gene dosage or disrupting functional genes. Therefore, CNVs can be exposed to selection pressure during evolution
- In addition to SNPs, a consistent number of common and rare CNVs are associated with disease
    - CNV has been recognized as a predominant source of genetic variation among human individuals.

A lot of gene dosage research seems to focus on cancer and drug therapy

Slide 15:

Drug Therapy

- As studies relating CNV to diseases expand, our understanding of human diversity, the causes and development of complex diseases, and disease resistance will grow accordingly, which will allow the development of improved diagnostic and treatment strategies and more personalized medicine in the form of Pharmacogenetics
- Pharmacogenetics aims to study the gene variants associated with drug metabolism enzymes, transporters, drug receptors and relieve the burden of sickness caused by interindividual differences in drug response or vulnerabilities to drug toxicity, e.g.
    - 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.

© 2016 *Clinical Chemistry*

• Genome editing is a way of making changes to specific parts of a genome - adding, removing, or altering at particular locations in the genome

  o Currently, most research on genome editing is done to understand diseases using cells and animal models. Scientists are still working to determine whether this approach is safe and effective for use in people. It is being explored in research on a wide variety of diseases, including single-gene disorders such as cystic fibrosis, hemophilia, and sickle cell disease.

Gene Therapy

- is a type of treatment that uses genetic material to hopefully change the course of a disease
- 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)

Slide 16:

Gene therapy research begun over 40 years ago, our understanding combined with technological advancements has greatly advanced the field.
In 2017, after extensive research in labs and in human clinical trials around the world, the first gene therapies were approved by the Food and Drug Administration (FDA) for use in the United States.
As of January 2020, the FDA has approved 2 gene therapy products.
Additionally, two gene based cellular immunotherapies were approved by FDA as of Jan 2020.
To date, the FDA has received more than 900 applications to investigate gene therapy in clinical trials.
This is part of a new age of medicine whose foundations lay in gene dosage effects.

Slide 17: References

Slide 18: Disclosures

Slide 19: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “Gene Dosage Analysis.”