Variant Classification and Reclassification
Introduction

This slide presentation covers several topics pertaining to Variant classification, reclassification and the Variant Classification Program (VCP). You may view the presentation in its entirety or click on one of the topics below to go directly to the relevant slides.

- **Variant Classification**
- **Variant Reclassification – Data Analyses**
- **The Variant Classification Program (For Family Testing)**
Myriad Genetic Laboratories (MGL) has identified thousands of DNA sequence variants

• The laboratory continues to find many new sequence variants per week

• Each of these new sequence variants must be classified before being reported to a healthcare provider
Current Variant Classifications

Variants are classified into one of five groups:

- **Polymorphism (PM)** – The change is not associated with an increased cancer risk. Because these variants are clinically benign, they are not reported.

- **Favor Polymorphism (FP)** – Evidence indicates with a high degree of certainty that the variant is not associated with an increased cancer risk.

- **Variant of Uncertain Significance (VUS)** – There is insufficient evidence to determine if the variant is associated with an increased cancer risk.

- **Suspected Deleterious (SD)** – Evidence indicates with a high degree of certainty that the variant is associated with significantly increased cancer risk.

- **Deleterious (DM)** – The variant is associated with a significantly increased cancer risk.
Current Variant Classifications

Why “Deleterious” and “Suspected Deleterious” if the clinical interpretations are the same?

• “Deleterious” mutations
  – Multiple strong lines of evidence
  – Expected to adversely affect protein production or function according to ACMG guidelines (Richards CS et al, Genet Med, 10:294-300, 2008)

• “Suspected Deleterious” mutations
  – Fewer strong lines of evidence
  – Clinical action still warranted
A similar distinction is made between “Polymorphisms” and “Favor Polymorphisms”

- **“Polymorphisms”**
  - Multiple strong lines of evidence
  - Not expected to adversely affect protein production or function according to ACMG guidelines (Richards CS et al, Genet Med, 10:294-300, 2008)

- **“Favor Polymorphisms”**
  - Fewer strong lines of evidence
  - High degree of certainty that the variant is not associated with an increased risk of cancer
Current Variant Classifications

Fictitious examples of typical variant classifications

• Deleterious Mutations
  – Nonsense – R39X
  – Frameshift – 239delAG
  – Large deletions – del exons 2-8
  – Initiation codon – M1V

• Variants of Uncertain Significance
  – Missense – R201N
  – In-frame deletions/insertions – K333del (999del3)
  – Intronic variants (not within the consensus splice site) – IVS2+12C>A
  – 5’UTR variants – 5’UTR-40A>G

• Polymorphisms
  – Silent – S235S
Variant Classification

Every new variant detected at MGL is evaluated by a team of experts prior to classification.

Committee members:
- Chief Medical Officer
- ABMG-certified Laboratory Directors
- Research Scientists
- Genetic Counselors
- Clinical Data Specialists
How New Variants are Classified

• Literature searches
  – Biochemical or functional analyses
  – Linkage/segregation analysis
  – Population studies (identification of a variant in normal, healthy control individuals who do not have a significant family history of cancer)

• Comparison of protein sequences with the sequences in other species

• Computerized splice site prediction using multiple models
Variant Reclassification

Once a variant is classified, this classification is used for future observations of the variant

MGL devotes substantial resources to establish the clinical significance of variants by monitoring emerging information from various sources:

- Scientific literature
- Family analysis
- Internal data
Types of Reclassification Data

For autosomal dominant diseases, having a deleterious mutation in both copies of the same gene is often either lethal or results in severe clinical manifestations

- **BRCA1** – Embryonic lethal
- **BRCA2** – Fanconi Anemia and early death; may be an embryonic lethal
- HNPCC genes – Neurofibromatosis (type 1) phenotype, early onset cancers and death
Types of Reclassification Data

Same gene co-occurrences

• Homozygous or compound heterozygous observations (with a deleterious mutation) provide strong evidence that the variant is not deleterious

• This is one of the most effective ways to identify variants that are clinically insignificant for BRCA1, BRCA2 and the Lynch syndrome genes (MLH1, MSH2, MSH6, PMS2)
It must be determined if two variants are on opposite copies of the gene (\textit{in trans}) or the same copy of the gene (\textit{in cis})
In some cases, family analysis may be required to determine if the variant and the deleterious mutation lie on opposite chromosomes.
Family Analysis

Father Low Risk
Mother High Risk
Proband High Risk *in cis*

BRCA1
X Deleterious Mutation
● Benign Variant
Types of Reclassification Data

Other Gene Co-occurrence (OGC)

• Deleterious mutations in both *BRCA1* and *BRCA2* in the same individual are rare

• For example, *BRCA1* variants co-occurring with a deleterious mutation in *BRCA2* are less likely to be deleterious

• Statistical analysis is performed incorporating information about the clinical histories of the families in which the variants are found
Types of Reclassification Data

Segregation analysis is a traditional tool for establishing the clinical significance of genetic changes

- MGL’s long-standing MGA program was designed to gather segregation data

- Large families are most effective, but smaller families are also useful
Types of Reclassification Data

Phenotype analysis for the *BRCA1* and *BRCA2* genes

- Based on proband personal and family history

- Variants associated with weak clinical histories are less likely to be deleterious

- Variants associated with strong clinical histories are more likely to be deleterious
MGL’s efforts to reclassify *BRCA1* and *BRCA2* variants have been extraordinarily successful.
Variant Classification Program

MGL is committed to establishing the clinical significance of all variants in a timely and accurate manner.

The Variant Classification Program (VCP) relies on healthcare providers, patients, and family members to gather additional information about variants.
The VCP offers genetic testing at no cost to select patients and family members

Data is gathered for:

- Segregation/linkage analysis
- Establishing compound heterozygosity of variants with deleterious mutations
- Identifying other gene co-occurrences
Most patients with a variant result will receive:

- A Variant Information Sheet
- An invitation to participate in VCP
- A Clinical History Form
  - Completed by the patient at home or with the help of their Healthcare Provider (HCP)
  - Returned to the HCP
  - Signed and faxed by the HCP to a Clinical Variant Specialist at MGL
  - The HCP may provide a detailed pedigree in lieu of the Clinical History Form
MGL scientists will review the family history and determine if family testing could provide data useful for reclassification

- The HCP will be informed if family testing will be offered
- Family testing at no cost may include:
  - Single-site analysis for variants and/or deleterious mutations
  - Comprehensive sequencing and large rearrangement analyses
The HCP will be provided with an invitation for each family member designated by MGL

- The HCP gives the invitation(s) to the patient

- The patient gives the invitation(s) to the appropriate family member(s)
  - Family member invitations include a VCP authorization number that enables family members to receive testing at no cost
  - VCP invitations are person-specific and non-transferable
Authorized family members should contact their own healthcare provider to arrange testing

• The family member’s HCP can obtain a free test kit by calling MGL Customer Service

• Test kits may be available for both blood and buccal mouthwash samples, depending on the test authorized

• Genetic test results will be sent to the family member’s HCP
Variant Classification Program

Family test results are reviewed to determine if there is sufficient information to reclassify the variant

- If there is sufficient information to reclassify the variant, amended reports will be sent
- If there is insufficient information to reclassify the variant, MGL will continue to evaluate additional information as it becomes available
Summary

• Accurate variant classification at MGL is a top priority

• Based on extensive experience, MGL has developed a program, VCP, which enhances the ability to reclassify variants

• Participation by healthcare providers, patients, and family members is critical to the success of the VCP

• The probability of reclassifying a variant increases as program participation increases

• Reclassification of a single variant often affects multiple patients and their family members, allowing for more effective clinical management of these individuals