Data Visualization in Reporting Next Generation Sequencing Results

[1]Center for Individualized Medicine, Mayo Clinic, Rochester MN, [2]University of Illinois at Urbana-Champaign, [3]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester MN

Background

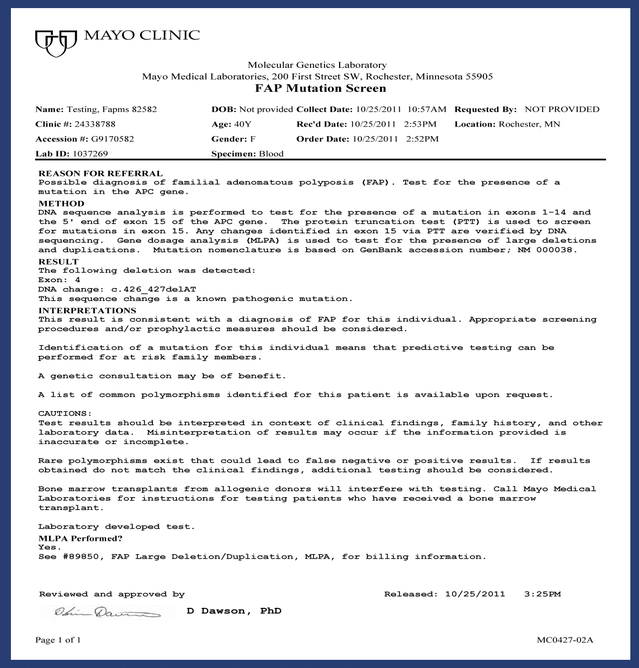
With the advent of next generation sequencing technologies, tremendous amounts of genomic data are being generated. It is becoming clear that the traditional "narrative" style reporting (reason for referral, method, result and interpretation) is too cumbersome for the amount of data generated by multi-gene panels, whole exome and whole genome sequencing. Additionally, physicians trained in fields other than genetics are playing a more central role in the ordering and reviewing of genetic test results. Previous studies, evaluating single gene reports, have suggested that patient care may be compromised as a consequence of poor communication between laboratory professionals and clinicians. Effective communication of results and the details of testing can promote appropriate clinical decision making and minimize the potential for patient harm. We believe that optimizing data visualization is critical in making NGS reports more understandable while reducing the chance of misinterpretation.

Study Aim

Our goal was to create a report capable of conveying NGS data in an easily understandable way to all clinicians. Ideally this report would be easily adapted to a multiple page static report or an interactive report accessible through a web-based portal.

Methods

To achieve this goal, we spoke with medical geneticists, genetic counselors and laboratory directors to determine the key information needed to support the decision-making process, and to identify where potential misunderstanding could occur. We worked through an iterative development process to formulate a hierarchy of answers that match the series of questions asked by the clinician. We conceptualized this hierarchy as content layers to efficiently guide the reader through the complex content. The prominent layer summarized answers to key questions first, then subsequent layers provided explanation, evidence, resources and reference. We explored multiple delivery techniques (interactive web portal, PDF, and print) to evaluate the feasibility of integrating into the clinician's environment.



MAYO CLINIC

Molecular Genetics Laboratory
Mayo Medical Laboratories, 200 First Street SW, Rochester, Minnesota 55905

FAP Mutation Screen

Name: Testing, Fapms 82582 **DOB:** Not provided **Collect Date:** 10/25/2011 10:57AM **Requested By:** NOT PROVIDED
Clinic #: 24338788 **Age:** 40Y **Rec'd Date:** 10/25/2011 2:53PM **Location:** Rochester, MN
Accession #: G9170582 **Gender:** F **Order Date:** 10/25/2011 2:52PM
Lab ID: 1037269 **Specimen:** Blood

REASON FOR REFERRAL
Possible diagnosis of familial adenomatous polyposis (FAP). Test for the presence of a mutation in the APC gene.

METHOD
DNA sequence analysis is performed to test for the presence of a mutation in exons 1-14 and the 5' end of exon 15 of the APC gene. The protein truncation test (PTT) is used to screen for mutations in exon 15. Any changes identified in exon 15 via PTT are verified by DNA sequencing. Gene dosage analysis (MLPA) is used to test for the presence of large deletions and duplications. Mutation nomenclature is based on GenBank accession number; NM 000038.

RESULT
The following deletion was detected:
Exon: 4
DNA change: c.426_427delAT
This sequence change is a known pathogenic mutation.

INTERPRETATIONS
This result is consistent with a diagnosis of FAP for this individual. Appropriate screening procedures and/or prophylactic measures should be considered.

Identification of a mutation for this individual means that predictive testing can be performed for at risk family members.

A genetic consultation may be of benefit.

A list of common polymorphisms identified for this patient is available upon request.

CAUTIONS:
Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

Rare polymorphisms exist that could lead to false negative or positive results. If results obtained do not match the clinical findings, additional testing should be considered.

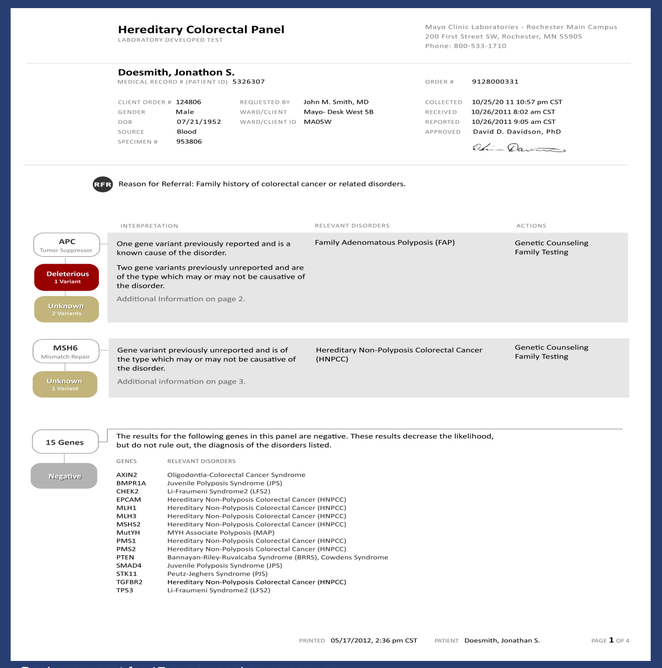
Bone marrow transplants from allogenic donors will interfere with testing. Call Mayo Medical Laboratories for instructions for testing patients who have received a bone marrow transplant.

Laboratory developed test.
MLPA Performed?
Yes.
See #89850, FAP Large Deletion/Duplication, MLPA, for billing information.

Reviewed and approved by D Dawson, PhD Released: 10/25/2011 3:25PM

Page 1 of 1 MC0427-02A

Sample report for a single gene in a typical narrative structure



Hereditary Colorectal Panel
LABORATORY DEVELOPED TEST

Mayo Clinic Laboratories - Rochester Main Campus
200 First Street SW, Rochester, MN 55905
Phone: 800-533-1710

Doesmith, Jonathon S.
MEDICAL RECORD # (PATIENT ID) 5326307

ORDER # 9128000331

CLIENT ORDER #	124806	REQUESTED BY	John M. Smith, MD	COLLECTED	10/25/20 11 10:57 pm CST
GENDER	Male	WARD/CLIENT	Mayo- Desk West 5B	RECEIVED	10/26/2011 8:02 am CST
DOB	07/21/1952	WARD/CLIENT ID	MA05W	REPORTED	10/26/2011 9:05 am CST
SOURCE	Blood			APPROVED	David D. Davidson, PhD
SPECIMEN #	953806				

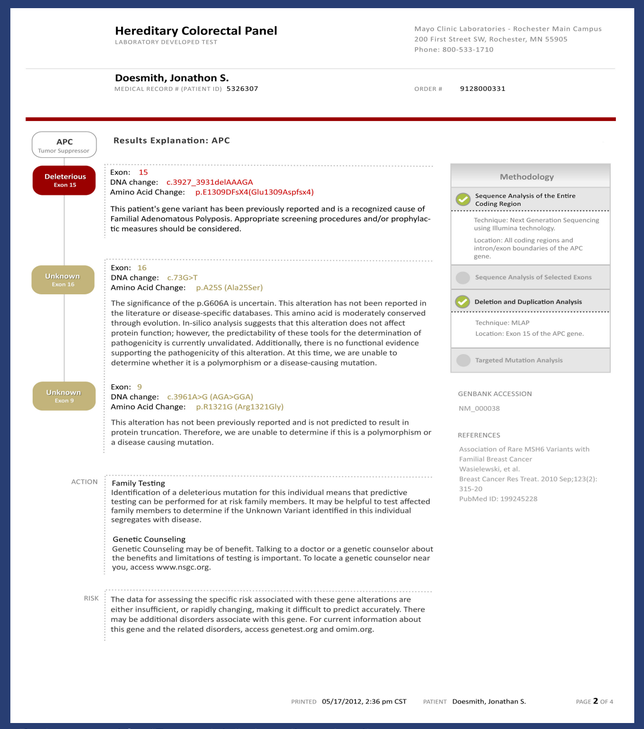
RFR Reason for Referral: Family history of colorectal cancer or related disorders.

	INTERPRETATION	RELEVANT DISORDERS	ACTIONS
APC (Tumor Suppressor) — Deleterious: 1 Variant; Unknown: 2 Variants	One gene variant previously reported and is a known cause of the disorder. Two gene variants previously unreported and are of the type which may or may not be causative of the disorder. Additional Information on page 2.	Family Adenomatous Polyposis (FAP)	Genetic Counseling Family Testing
MSH6 (Mismatch Repair) — Unknown: 1 Variant	Gene variant previously unreported and is of the type which may or may not be causative of the disorder. Additional information on page 3.	Hereditary Non-Polyposis Colorectal Cancer (HNPCC)	Genetic Counseling Family Testing
15 Genes — Negative	The results for the following genes in this panel are negative. These results decrease the likelihood, but do not rule out, the diagnosis of the disorders listed.		

GENES	RELEVANT DISORDERS
AXIN2	Oligodontia-Colorectal Cancer Syndrome
BMPR1A	Juvenile Polyposis Syndrome (JPS)
CHEK2	Li-Fraumeni Syndrome2 (LFS2)
EPCAM	Hereditary Non-Polyposis Colorectal Cancer (HNPCC)
MLH1	Hereditary Non-Polyposis Colorectal Cancer (HNPCC)
MLH3	Hereditary Non-Polyposis Colorectal Cancer (HNPCC)
MSHS2	Hereditary Non-Polyposis Colorectal Cancer (HNPCC)
MutYH	MYH Associate Polyposis (MAP)
PMS1	Hereditary Non-Polyposis Colorectal Cancer (HNPCC)
PMS2	Hereditary Non-Polyposis Colorectal Cancer (HNPCC)
PTEN	Bannayan-Riley-Ruvalcaba Syndrome (BRRS), Cowdens Syndrome
SMAD4	Juvenile Polyposis Syndrome (JPS)
STK11	Peutz-Jeghers Syndrome (PJS)
TGFBR2	**Hereditary Non-Polyposis Colorectal Cancer (HNPCC)**
TP53	Li-Fraumeni Syndrome2 (LFS2)

PRINTED 05/17/2012, 2:36 pm CST PATIENT Doesmith, Jonathon S. PAGE 1 OF 4

Design concept for 17 gene panel summary page



Hereditary Colorectal Panel
LABORATORY DEVELOPED TEST

Mayo Clinic Laboratories - Rochester Main Campus
200 First Street SW, Rochester, MN 55905
Phone: 800-533-1710

Doesmith, Jonathon S.
MEDICAL RECORD # (PATIENT ID) 5326307

ORDER # 9128000331

APC (Tumor Suppressor) — Deleterious: Exon 15

Results Explanation: APC

Exon: 15
DNA change: c.3927_3931delAAAGA
Amino Acid Change: p.E1309DFsX4(Glu1309Aspfsx4)

This patient's gene variant has been previously reported and is a recognized cause of Familial Adenomatous Polyposis. Appropriate screening procedures and/or prophylactic measures should be considered.

Unknown — Exon 16

Exon: 16
DNA change: c.73G>T
Amino Acid Change: p.A25S (Ala25Ser)

The significance of the p.G606A is uncertain. This alteration has not been reported in the literature or disease-specific databases. This amino acid is moderately conserved through evolution. In-silico analysis suggests that this alteration does not affect protein function; however, the predictability of these tools for the determination of pathogenicity is currently unvalidated. Additionally, there is no functional evidence supporting the pathogenicity of this alteration. At this time, we are unable to determine whether it is a polymorphism or a disease-causing mutation.

Unknown — Exon 9

Exon: 9
DNA change: c.3961A>G (AGA>GGA)
Amino Acid Change: p.R1321G (Arg1321Gly)

This alteration has not been previously reported and is not predicted to result in protein truncation. Therefore, we are unable to determine if this is a polymorphism or a disease causing mutation.

ACTION

Family Testing
Identification of a deleterious mutation for this individual means that predictive testing can be performed for at risk family members. It may be helpful to test affected family members to determine if the Unknown Variant identified in this individual segregates with disease.

Genetic Counseling
Genetic Counseling may be of benefit. Talking to a doctor or a genetic counselor about the benefits and limitations of testing is important. To locate a genetic counselor near you, access www.nsgc.org.

RISK

The data for assessing the specific risk associated with these gene alterations are either insufficient, or rapidly changing, making it difficult to predict accurately. There may be additional disorders associate with this gene. For current information about this gene and the related disorders, access genetest.org and omiim.org.

Methodology

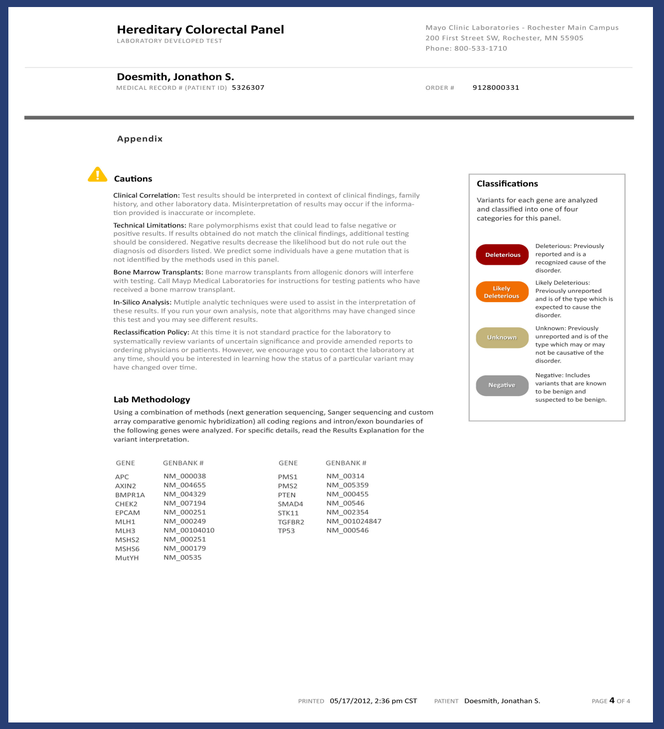
- Sequence Analysis of the Entire Coding Region
  - Technique: Next Generation Sequencing using Illumina technology.
  - Location: All coding regions and intron/exon boundaries of the APC gene.
- Sequence Analysis of Selected Exons
- Deletion and Duplication Analysis
  - Technique: MLAP
  - Location: Exon 15 of the APC gene.
- Targeted Mutation Analysis

GENBANK ACCESSION
NM_000038

REFERENCES
Association of Rare MSH6 Variants with Familial Breast Cancer
Wasielewski, et al.
Breast Cancer Res Treat. 2010 Sep;123(2): 315-20
PubMed ID: 199245228

PRINTED 05/17/2012, 2:36 pm CST PATIENT Doesmith, Jonathon S. PAGE 2 OF 4

Design concept for 17 gene detailed results explanation page.



Hereditary Colorectal Panel
LABORATORY DEVELOPED TEST

Mayo Clinic Laboratories - Rochester Main Campus
200 First Street SW, Rochester, MN 55905
Phone: 800-533-1710

Doesmith, Jonathon S.
MEDICAL RECORD # (PATIENT ID) 5326307

ORDER # 9128000331

Appendix

Cautions

Clinical Correlation: Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

Technical Limitations: Rare polymorphisms exist that could lead to false negative or positive results. If results obtained do not match the clinical findings, additional testing should be considered. Negative results decrease the likelihood but do not rule out the diagnosis od disorders listed. We predict some individuals have a gene mutation that is not identified by the methods used in this panel.

Bone Marrow Transplants: Bone marrow transplants from allogenic donors will interfere with testing. Call Mayo Medical Laboratories for instructions for testing patients who have received a bone marrow transplant.

In-Silico Analysis: Mutiple analytic techniques were used to assist in the interpretation of these results. If you run your own analysis, note that algorithms may have changed since this test and you may see different results.

Reclassification Policy: At this time it is not standard practice for the laboratory to systematically review variants of uncertain significance and provide amended reports to ordering physicians or patients. However, we encourage you to contact the laboratory at any time, should you be interested in learning how the status of a particular variant may have changed over time.

Lab Methodology

Using a combination of methods (next generation sequencing, Sanger sequencing and custom array comparative genomic hybridization) all coding regions and intron/exon boundaries of the following genes were analyzed. For specific details, read the Results Explanation for the variant interpretation.

GENE	GENBANK #	GENE	GENBANK #
APC	NM_000038	PMS1	NM_00314
AXIN2	NM_004655	PMS2	NM_005359
BMPR1A	NM_004329	PTEN	NM_000455
CHEK2	NM_007194	SMAD4	NM_00546
EPCAM	NM_000251	STK11	NM_002354
MLH1	NM_000249	TGFBR2	NM_001024847
MLH3	NM_00104010	TP53	NM_000546
MSHS2	NM_000251		
MSH56	NM_000179		
MutYH	NM_00535		

Classifications

Variants for each gene are analyzed and classified into one of four categories for this panel.

- Deleterious: Deleterious: Previously reported and is a recognized cause of the disorder.
- Likely Deleterious: Likely Deleterious: Previously unreported and is of the type which is expected to cause the disorder.
- Unknown: Unknown: Previously unreported and is of the type which may or may not be causative of the disorder.
- Negative: Negative: Includes variants that are known to be benign and suspected to be benign.

PRINTED 05/17/2012, 2:36 pm CST PATIENT Doesmith, Jonathon S. PAGE 4 OF 4

Design concept for 17 gene panel appendix page.

Discussion

Using the feedback we obtained, we created a report that provides the information in a "layered" format. This can be implemented as a multiple page static report or more desirably as an interactive report. The results (gene name and mutation "score") are color coded and located prominently on the first page. If the clinician wants more detailed information on the gene or the specific details of a mutation they can click on the appropriate box to access the information. Likewise additional information can be obtained about the specific methods and references.

Unfortunately at this time simply creating a static report that is adaptable to most laboratory information systems and electronic medical records (EMR) is complicated. Ideally this type of report could be accessed by providers and potentially patients through a dynamic web-based reporting portal to optimize the interactive features. The EMR has the potential to transform the way clinicians view and understand complex genetic reports but we must be aware of the limitations as we move forward. It is critical that laboratories and clinicians work together to overcome these challenges.

References

1. Lubin IM, McGovern MM, Gibson Z, et al. Clinician perspectives about molecular genetic testing for heritable conditions and development of a clinician-friendly laboratory report. J Mol Diagn. 2009 Mar;11(2):162-71.
2. Lubin IM, Caggana M, Constantin C, et al. Ordering molecular genetic tests and reporting results: practices in laboratory and clinical settings. J Mol Diagn. 2008 Sep;10(5):459-68.
3. Ronquillo JG, Li C, Lester WT. Genetic testing behavior and reporting patterns in electronic medical records for physicians trained in a primary care specialty or subspecialty. J Am Med Inform Assoc. 2012 Jul-Aug;19(4):570-4.