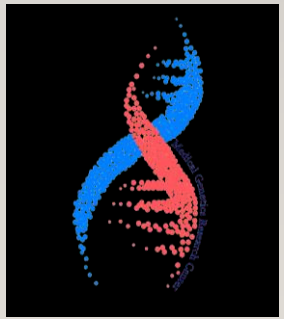
G.I. CANCERS AND GENETIC TESTING

M.R. Abbaszadegan, M.T. (ASCP), PhD., ABMG
Professor of Medical Genetics
Head, Dept of Medical Genetics & Molecular Medicine
Mashhad University of Medical Sciences
abbaszadeganmr@mums.ac.ir

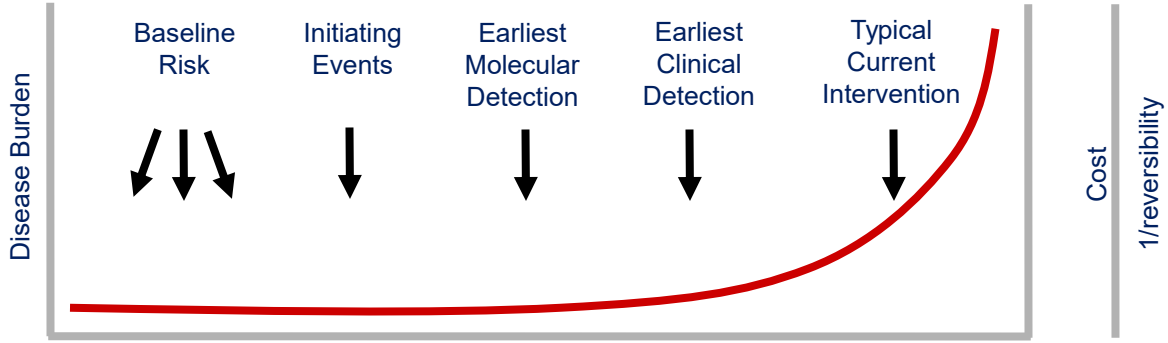


Tools Needed for Prediction and Personalized Care

THE CANCER GENOME ATLAS

Decision Support Tools:

Assess Risk | **Refine Assessment** | **Predict/Diagnose** | **Monitor Progression** | **Predict Events** | **Inform Therapeutics**



Baseline Risk | **Preclinical Progression** | **Disease Initiation and Progression** | **Therapeutic Decision Support**

Sources of New Biomarkers:

Stable Genomics: Single Nucleotide Polymorphisms (SNPs) Haplotype Mapping Gene Sequencing

Dynamic Genomics: Gene Expression Proteomics Metabolomics Molecular Imaging

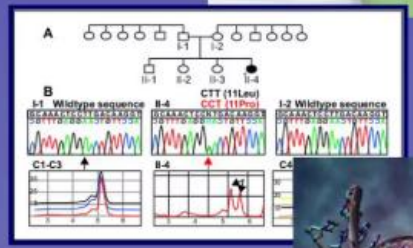
Genetic Cancer Risk Assessment (GCRA)

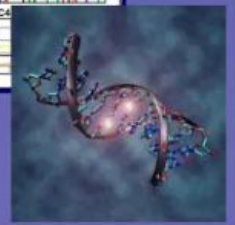
3

- Interdisciplinary specialty practice that uses genetic and genomic information to
  - Identify individuals with inherited cancer risk
  - Prescribe high-risk screening, preventive care, targeted treatment
- Increasing demands for GCRA services in community settings
- Guidelines/resources needed to help community-based clinicians
  - Recognize and refer patients
  - Provide GCRA services
  - Manage patients and families with cancer predisposition



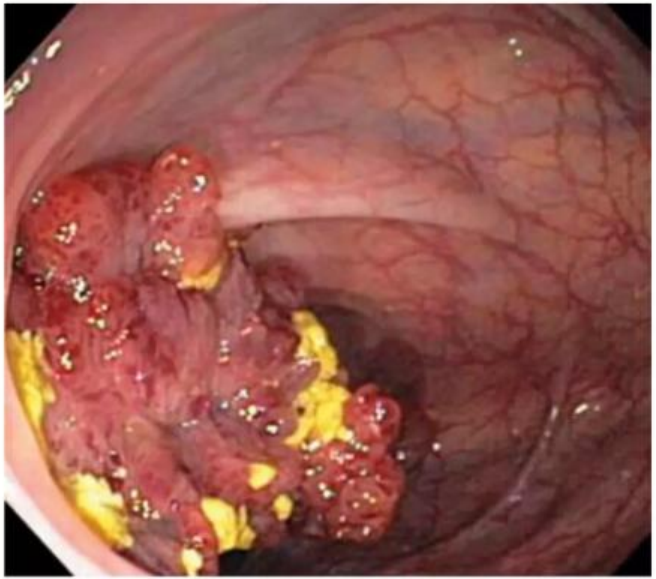






4

Molecular Diagnostics of Colorectal Cancer





Types of CRC

- **Sporadic =** 70% of cases
  - there is no family history, common above age of 50
- **Inherited** = *1 – 6%* of cases
  - Hereditary NonPolyposis CRC (HNPCC, Lynch syndrome)
  - Multiple polyps CRC
    - familial adenomatous polyposis (FAP)
    - hamartomatous polyposis syndromes (e.g. Peutz-Jeghers, juvenile polyposis)
    - MUTYH-associated polyposis (MAP)
- **Familial CRC = 25%** of cases
  - have a family history of CRC, but the pattern is not consistent and the risk is not as high as with the inherited syndromes.
    - single affected first-degree relative = 1.7 ↑^{ed} risk
    - two affected first-degree relatives or if Dx before age 55 = Further ↑ risk

Screening of CRC...

4.3 Fecal DNA Tests (Cologuard™)

- during apoptosis of normal colonic cell some DNA fragments of 180-200 bps long is released into stool.
- However, CRC cells show a decreased rate of apoptosis and DNA sequences with 1800-2400 bp are detected in stool samples
- CRC cells gene assay for
  - *APC* (mutated in up to 70% of specimens),
  - *Kras* (mutated in up to 60% of specimens) and
  - *p53* (mutated in up to 60% of specimens).
- for screening
  - specificity 95%; sensitivity 60 - 90%
  - not currently used – expensive, low sensitivity

MOLECULAR PATHOGENESIS

/Molecular tumorigenesis/

CRC can arise in more than one molecular pathways.

1. Chromosomal instability (CIN),
2. mismatch repair pathway
3. epigenetic gene silencing

1. The chromosomal instability (CIN/APC) pathway

- encompasses 80% to 85% of all CRC and adenoma
- Result in abnormal karyotypes, gross chromosomal abnormalities, such as aneuploidy, chromosome rearrangement, oncogene activation and loss of heterozygosity of tumor suppressor genes.
- Results "gain of function" mutations which may result in
  - loss or mutation of tumor suppressor genes such as *APC*, *TP53*
  - activation of oncogenes such as *KRAS*
  - apoptotic pathways
- **CRC caused by CIN usually have poor prognosis**

DCC (deleted in colorectal cancer)

- Tumor suppressor genes, affected as a result of 18q mutations
- DCC is an adhesion molecule of the immunoglobulin family, responsible for homotypic binding between cells.
- DCC protein loss can be examined by immunohistochemistry.

2. mismatch repair pathway /The mutator phenotype /

- accounts for 15% of CRC
- Mismatch repair corrects errors made when DNA is copied. For example, a C could be inserted opposite an A, or the polymerase could slip or stutter and insert two to five extra unpaired bases.
- If a mismatch or small loop is found, endonuclease (MSH2 protein recognizes, MSH6 or MSH3) cuts the strand bearing the mutation and exonuclease **(MLH1 and PMS2)** then digests this strand.
- Finally it will be filled in by normal cellular enzymes.

Microsatellite instability (MSI)

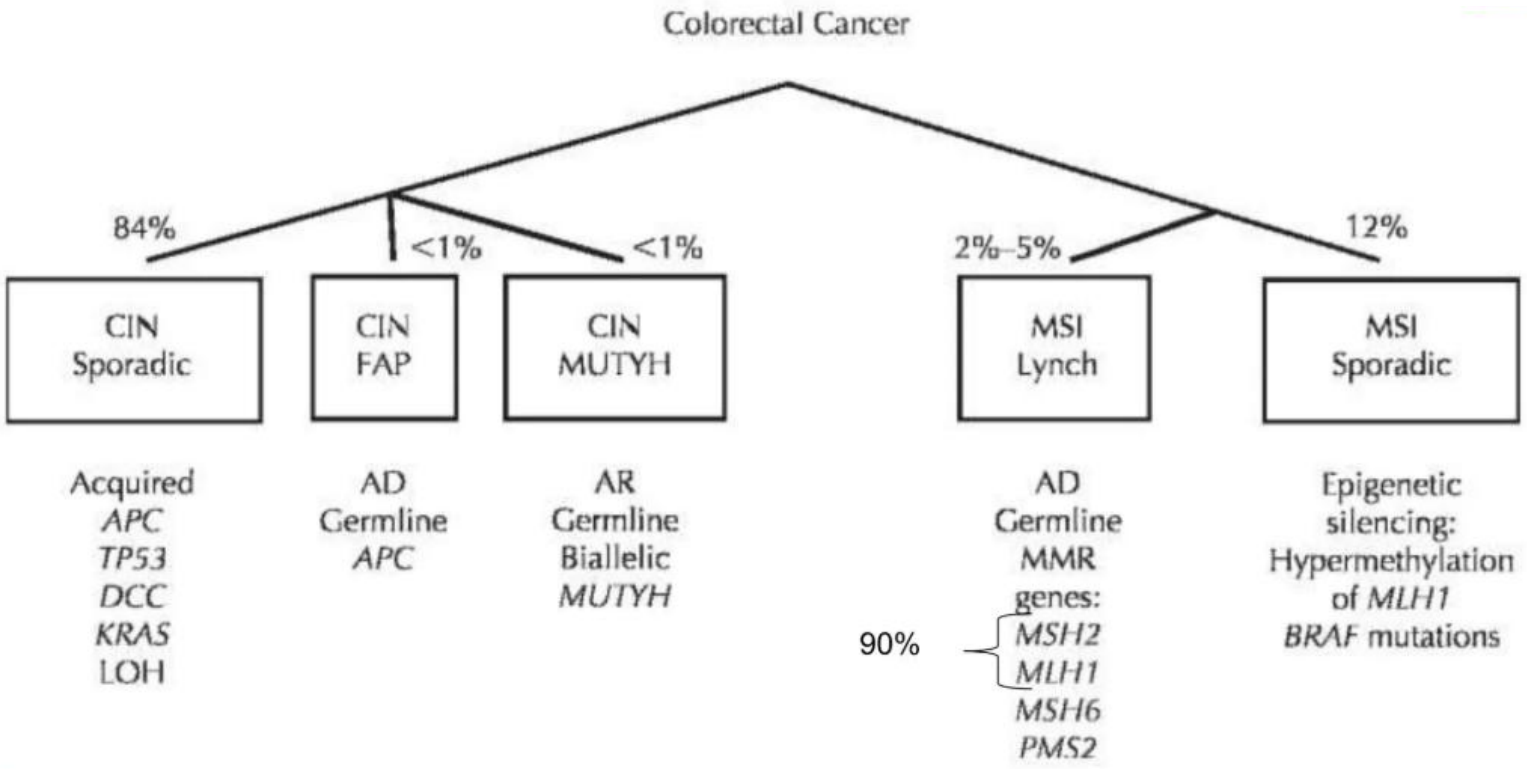
- due to the presence of the mismatch repair system, in vivo microsatellite mutation rates range from 10^{-6} to 10^{-2} per generation.
- Mutation of **both alleles** of mismatch repair (MMR) system → DNA strand (**microsatellites**) might be displaced, and realigns out of register creating small loop of unpaired DNA → unable to remove the loops → **microsatellite increases or decrease in size** due to either insertion or deletion of repeating units when **compared to the normal cell's** = **Microsatellite instability (MSI)**
- MSI is implicated in 50–60% of inherited condition HNPCC(Lynch syndrome) → germline mutations

3. epigenetic gene silencing
(Hypermethylation phenotype (CIMP+)) pathway

- DNA methylation is involved in normal cellular control of gene expression
  - (CpG island) are usually found in the regions close to promoters
- characterized by methylation of a number of genes rich in CpG islands → silencing of multiple genes or promoter region → loss of gene function or transcriptional inactivation (e.g. tumor-suppressor genes, APC)
- If MMR system is silenced→ **MSI**
- CRCs with high frequency of methylation of some CpG islands are referred to as CpG island methylation phenotype (CIMP) positive tumors

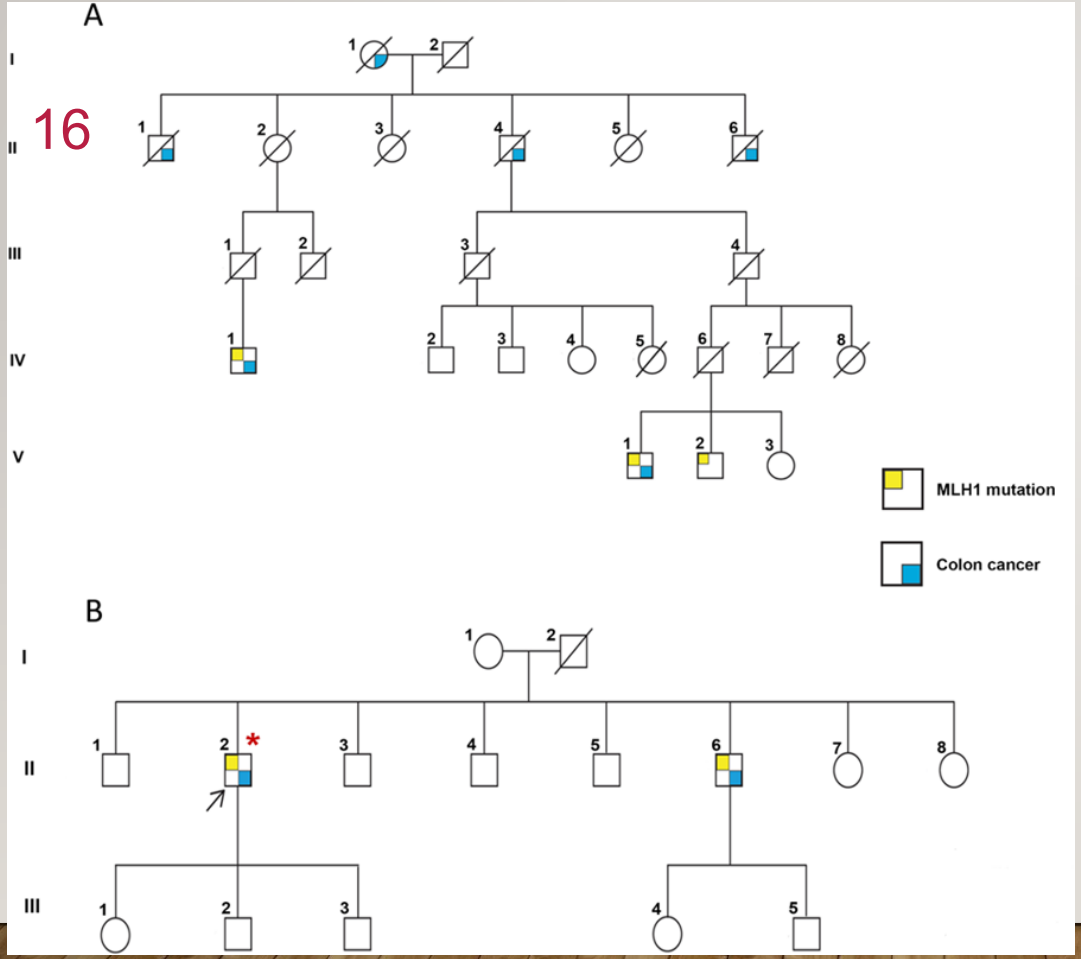
3. epigenetic gene silencing...

- encompasses 35%-40% of sporadic CRC
- begin with serrated polyps, especially sessile serrated adenomas (SSAs) bearing an activating mutation in the BRAF gene.
- Epigenetic changes are potentially reversible by drugs.
- CIMP+ CRCs
  - microsatellite stable (MSS) cancer (60% of CIMP+) or
  - MSI-H (40% of CIMP+)
    - Methylation occur in hMLH1
  - methylation may occur in tumor suppressor genes


Colorectal Cancer
84%
<1%
<1%
2%–5%
12%
CIN Sporadic
CIN FAP
CIN MUTYH
MSI Lynch
MSI Sporadic
Acquired
APC
TP53
DCC
KRAS
LOH
AD
Germline
APC
AR
Germline
Biallelic
MUTYH
AD
Germline
MMR
genes:
90%
MSH2
MLH1
MSH6
PMS2
Epigenetic
silencing:
Hypermethylation
of MLH1
BRAF mutations

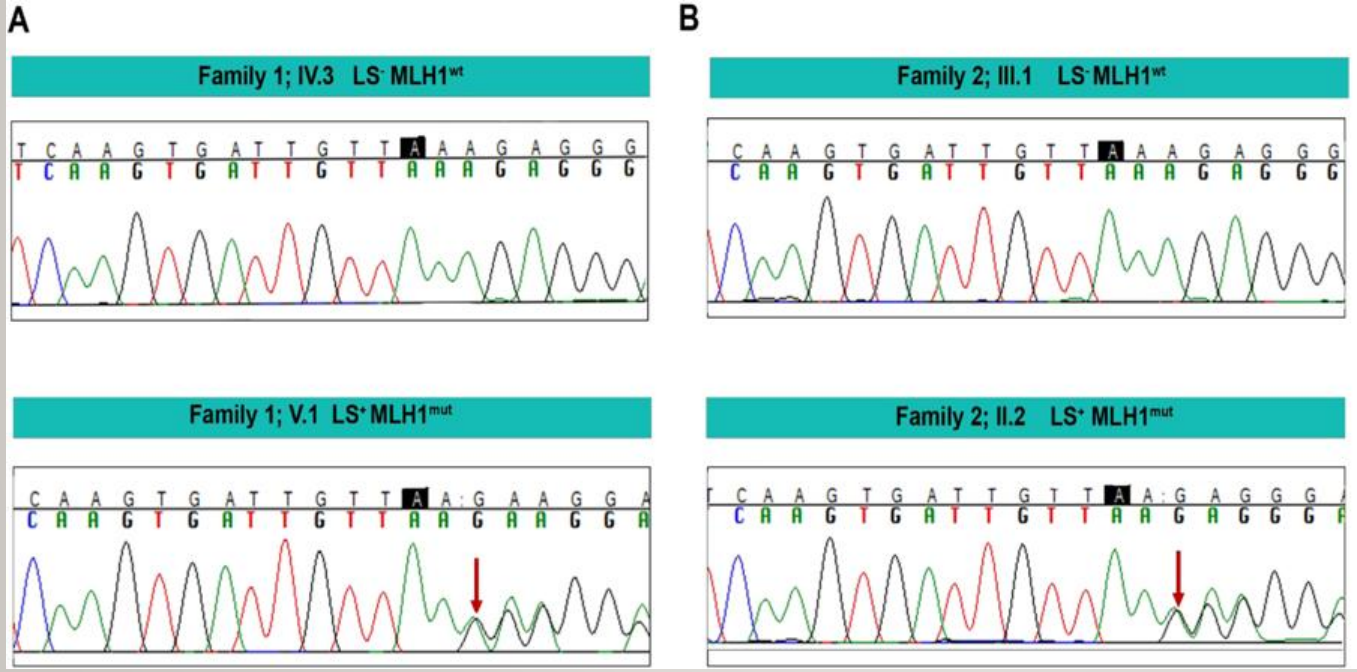
Bethesda guidelines

- Bethesda guidelines can be used as a screening tool for HNPCC.
  - (1) CRC in a patient younger than 50 years of age;
  - (2) synchronous or metachronous colorectal or other HNPCC-related tumors,
  - (3) CRC with histologic features associated with MSI-H status in a patient younger than 60 years of age;
  - (4) CRC in 1 or more first-degree relatives with an HNPCC-associated tumor, with one of the patients being diagnosed before age 50 years; and
  - (5) CRC in 2 or more first- or second-degree relatives with HNPCC-related tumors, regardless of age.



MLH1 gene mutation
diagnosed with colorectal cancer

IDENTIFCATION OF A COMMON GERMLINE MUTATION IN THE *MLH1* GENE

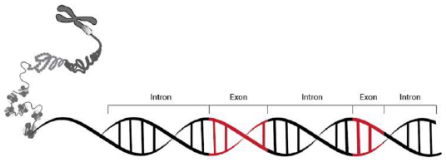


c.154delA Cuasitive mut

آزمایشگاه پاتوبیولوژی و ژنتیک پردیس
آدرس : بلوار احمد آباد - مقابل بیمارستان قائم - ساختمان ۷۳ تلفن : ۳۷۷۰-۰۵۱
www.pardislab.com
ISO 15189 - ISO 45001
دکتر محمدرضا عباس زادگان دکتر عبدالرضا پارسته دکتر نوربه شریفی دکتر امیررضا جلیلیان دکتر محمدامیر بنائی دکتر فرزانه خزیمه دکتر سمانه مهجوری دکتر سمانه وجدانی

شماره پذیرش:M04-2028	نام بیمار:جناب آقای [redacted]	جنسیت :مذکر 67
تاریخ پذیرش: 1404/03/11	نام پزشک :جناب آقای دکتر احسان حسن زاده	تاریخ گزارش :1404/05/26

Whole Exome Sequencing

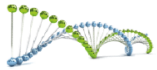




Sample: Peripheral Blood
Clinical History: A 67-year-old symptomatic male with colon cancer reffered for screening variation on MSH2, MSH6 genes.

Methods: Whole Exome Sequencing (WES) utilizes DNA-enrichment methods and massively parallel nucleotide sequencing to identify disease-associated variants throughout the human genome. DNA was extracted from whole blood. Human whole exome enrichment was performed using Agilent SureSelect V6 Target Enrichment Kit, followed by Next Generation Sequencing using Illumina HiSeq4000 platform to yield an average coverage depth of ~100X. All exons and flanking 10bp were detected and analyzed. Detected variations include: single point mutations, small Indels (within 20bp). The DNA sequence was mapped to, and analyzed in comparison with, the published human genome build (UCSC hg19 reference sequence).

Results:



Genes	RefSeq	Nucleic Acid Alteration	Amino Acid Alteration	Zygosity	Ch. Location	Mutation
MSH2	NM_000251.3	c.1076+1G>T	---	Heterozygous	Chr 2	**pathogenic**

Variant classification according to the ACMG Guidelines:

Class 1: Pathogenic
Class 2: Likely pathogenic

19

Interpretations summary:

1) Heterozygous pathogenic c.1076+1G>T variant on *MSH2* gene has been detected. ***MSH2*** gene is associated with autosomal dominant of Lynch syndrome and Muir-Torre syndrome.

- **Lynch syndrome,** also known as hereditary nonpolyposis colorectal cancer, is an autosomal dominant condition linked to impaired DNA mismatch repair activity. It predisposes individuals to a range of cancers, primarily affecting the colorectum and endometrium. Lynch syndrome is the most common form of inherited colorectal cancer in the Western world. It is characterized by a familial predisposition to early-onset colorectal carcinoma and extra-colonic tumors in various organs. The syndrome can be clinically divided into two types: Type I involves a hereditary predisposition to colorectal cancer with a young age of onset, while Type II is associated with an increased risk of cancers in tissues such as the uterus, ovary, breast, stomach, small intestine, skin, and larynx, in addition to the colon. Diagnosis is based on the Amsterdam criteria, which include specific criteria related to family history and age of onset of colorectal cancer. Lynch syndrome is associated with heterozygous mutations in the MSH2 gene on chromosome 2p21-p16.

-Muir-Torre syndrome is an autosomal dominant disorder characterized by sebaceous gland tumors and skin cancers, including keratoacanthomas and basal cell carcinomas. Affected individuals may also develop internal malignancies such as colorectal, endometrial, urologic, and upper gastrointestinal neoplasms. The syndrome is considered a subtype of Lynch syndrome, with mutations in DNA mismatch repair genes MLH1, MSH2, and MSH6. Skin lesions can precede or follow the diagnosis of internal cancers, with the gastrointestinal tract being the most commonly affected internal site.

Recommendations: Sanger sequencing to validate the variant described above is recommended.

Limitations: Absence of a plausible explanation for the reported phenotype by exome sequencing does not exclude a genetic basis of the patient's condition. Some types of genetic abnormalities, such as copy number changes, may not be detectable with the technologies performed by this exome analysis test. It is possible that the genomic region where a disease causing mutation exists in the proband was not captured using the current technologies and therefore was not detected. Additionally, it is possible that a particular genetic abnormality may not be recognized as the underlying cause of the genetic disorder due to incomplete scientific knowledge about the function of all genes in the human genome and the impact of variants in those genes. Only variants in genes associated with the medical condition, or thought to be clinically relevant potentially for the proband's medical condition, are reported here

Disclaimer:

- WES has been proposed for diagnostic use in individuals who present with complex genetic phenotypes suspected of having a rare genetic condition, who cannot be diagnosed by standard clinical workup, or when features suggest a broad differential diagnosis that would require evaluation by multiple genetic tests.
- WES is limited to the DNA sequence of coding regions (exons) and flanking intronic regions of the genome, which is estimated to contain 85% of heritable disease-causing variants
- WES is typically not an appropriate first-tier test, but may be appropriate if initial testing is unrevealing, or if there is no single-gene or panel test available for the particular condition, or if a rapid diagnosis for a critically-ill child is indicated.
- The exome data also contains a number of other variants with unknown clinical significance at this time. Future investigation is ongoing to determine their clinical significance. As soon as any clinical relevance get documented, a supplementary report will be issued.
- There are still many limitations in whole exome sequencing itself and also in interpretation of obtained data. Among them is the possibility of pathogenic variants, present in the genome but not detected by the technique. Therefore this report is not able to rule out the presence of other pathogenic variants in the genome.
- At the present time, WES typically fails to detect certain classes of disease-causing variants, such as structural variants (e.g., translocations, inversions), Copy Number Variations (CNVs), abnormal chromosome imprinting or methylation, some mid-size insertions and deletions (ca. 10-500 bp), trinucleotide repeat expansion mutations, deeper intronic mutations, and low-level mosaicism.
- Large duplication and deletion, balanced translocation, inversions, ploidy changes, uniparental disomy and methylation alterations cannot be detected by this test.
- Prenatal diagnosis by genomic (i.e., next-generation whole-exome or whole-genome) sequencing has significant limitations. The current technology does not support short turnaround times, which are often expected in the prenatal setting. There are high rates of false positives, false negatives, and variants of unknown clinical significance. These can be expected to be significantly higher than seen when array CGH is used in prenatal diagnosis.

With Cooperation of partner laboratory

M.R. Abbaszadegan, MT(ASCP), Ph.D., ABMG
Professor of Medical Genetics
Clinical Director, Molecular &Cytogenetics Lab

S. Vejdani, Ph.D.
Medical Geneticist
Clinical Director, Molecular & Cytogenetics Lab

MOLECULAR DIAGNOSTICS METHODS

Detection of gene mutations with known positions and base changes

- Sanger Sequencing
- Allele Specific PCR

Name/Method Target	Intended Use	Detected Property	Source Material	Molecular Method	Use /Availability
KRAS	therapeutic decision EGFR targeted therapy	KRAS mutations	FFPE or snap frozen tissue	Sequencing	clinical routine
BRAF	chemotherapeutic susceptibility	BRAF mutations	>> >>	sequencing, Real-time PCR	clinical routine
BRAF	chemotherapeutic susceptibility	BRAF mutations	>> >>	Digital PCR, COLD-PCR	studies
MSI status PCR	chemotherapeutic susceptibility	MSI status	>> >>	PCR	clinical routine
MSI status IHC	chemotherapeutic susceptibility	MSI status	>> >>	IHC	clinical routine
TP53 mutation	screening	p53 mutation analysis	>> >>	sequencing	clinical routine
TP53 mutation	screening	>> >>	>> >>	oligonucleotide microarray	studies
CIMP	probable screening /staging	methylation	>> >>	methylation microarray	studies
miRNA assay for blood/stool	screening	miRNA expression level	plasma, stool	micro array	studies
Multitarget stool DNA test	screening, increasing sensitivity for colonoscopy	KRAS mutation, NDRG4, BMP3 methylation, hemoglobin immunoassay	stool	mutation and methylation analysis, immunoassay	under approval for clinical use
Epi proColon early detection assay	screening, increasing sensitivity for colonoscopy	Septin 9 DNA methylation assay	blood plasma	Real-time PCR	available for clinical use

CIMP = CpG island methylator phenotype, FFPE = formalin fixed paraffin embedded, IHC = immunohistochemistry, miRNA = micro ribonucleic acid,MSI = microsatellite instability, PCR = polymerase chain reaction. ARMS= amplification refractory mutation system

PCR Identification of MSI-H ...

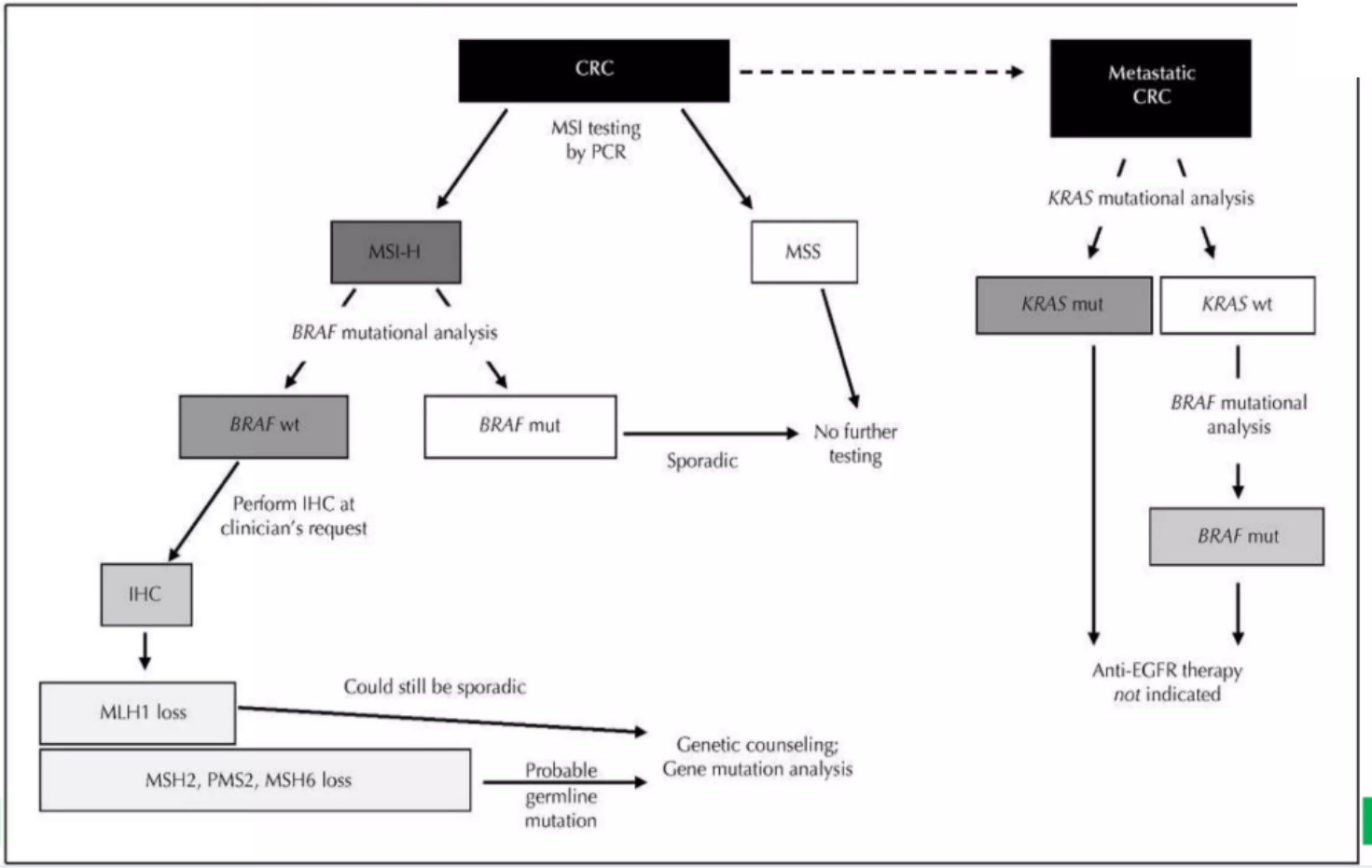
4. Analyze 5 loci (2 mononucleotide repeats and 3 dinucleotide repeats) for change of any length

1. microsatellite-high(MSI-H) = instability ≥2 loci
   - 5-fluorouracil is not effective in treating
   - betterprognostic factor
   - sporadic (~12%) or Lynch syndrome–associated (~3%)
2. microsatellite-low (MSI-L) = instability at 1 locus
   - If that locus is dinucleotide → additional analysis of one mononucleotide repeat with BAT-40) is recommended
3. microsatellite-stable (MSS) = no microsatellite mutations

microRNAs in CRC

- are a class of small non-coding single-stranded RNAs with important posttranscriptional regulatory functions by binding to their target mRNAs.
- E.g. involvement of miRNA-133a in regulating the EGFR pathway

Personalized Cancer Medicine
/Targeted therapy/



DIVISION OF HUMAN GENETICS, AVICENNA RES. INSTITUTE, MUMS



THANKS FOR YOUR ATTENTION

