Genetic screening and testing

UPDATES FOR OB/GYN AND PRIMARY CARE PROVIDERS
2020 EDITION

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OBJECTIVES

- Updates in carrier screening for OB/GYN patients
- Prenatal screening
- New advances in diagnostic testing
- Hereditary cancer syndromes: current testing and referral guidelines

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Carrier Screening

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Carrier screening for OB/GYN patients

- For **all** pregnant and preconception patients, offer carrier screening for:
  - Spinal muscular atrophy
  - Cystic fibrosis - 55 mutation panel
  - Hemoglobin traits (hemoglobin fractionation)

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Spinal Muscular Atrophy

- Symptoms include progressive muscle weakness and wasting
  - Phenotype ranges from severe (onset in early infancy leading to death from respiratory failure within the first 2 years of life) to milder, later onset forms
  - Leading genetic cause of infant death
- Incidence: ~1/6,000 -1/10,000 live births
- Inheritance: autosomal recessive
- Carrier frequency (most populations): 1/47 – 1/72
- Detection rate: 90% - 95%
- HC order: 81329C (Spinal muscular atrophy, SMN1 and SMN2, carrier testing)
  - SMA carrier screening accomplished through copy number analysis of SMN1 gene

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SMA – carrier screening

- Carrier screening results report out copy number of SMN1, SMN2, and presence of two polymorphisms: g.27134T>G and g.27706-27707delAT
- Most individuals have two copies of SMN1
  - Some individuals have more than two copies of SMN1
  - SMA carriers have one copy of SMN1
  - SMN2 copy number is not linked to SMA carrier status
- Individuals who have two copies of SMN1 and two polymorphisms may be non-carriers or possible "silent" carriers
  - The two polymorphisms may be associated with a risk of carrying two copies of SMN1 *in cis* (on the same chromosome) and can potentially identify silent carriers

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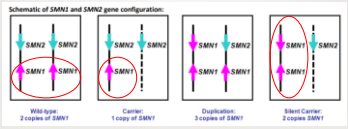
SMA carrier status and the SMN1 gene

- SMN1 genes may be in cis or in trans
- Silent carriers: have two or more copies of SMN1 on the same chromosome (in cis), and none on the opposite chromosome



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SMA - Silent carriers

- Risk of SMA silent carrier status is dependent on ethnicity
  - SMA results report out the status of two polymorphisms: *g.27134T>G and g.27706-27707delAT*
  - Individuals of Ashkenazi Jewish ancestry who carry the polymorphisms are considered to be SMA carriers
  - SMA results will be flagged if a patient is positive for the polymorphisms and is of AJ ancestry, or if the family history of SMA question is answered "yes" – refer these cases to Genetics
- SMA carrier screening order
  - Two questions have been added to help better ascertain SMA silent carriers
    - Is the patient of Jewish Ancestry? Yes/No
    - Does the patient have a family history of SMA? Yes/No

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SMA screening- residual risks

Ethnicity	Carrier Frequency	Detection Rate	Negative	Possible Silent Carrier
Ashkenazi Jewish	1:67	93%	1:918	Carrier
Asian	1:59	94%	1:907	1:61
African American	1:72	90%	1:375	1:39
Hispanic	1:68	93%	1:906	1:99
Caucasian	1:47	95%	1:921	1:69

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What should I do with SMA results?

- One copy of SMN1: patient is a carrier
  - Result is flagged. Inform the patient, and refer to Genetics
  - Partner/FOB screening should be ordered
- Two copies of SMN1, negative for polymorphisms
  - Result is not flagged. No referral to Genetics needed
  - Patient has a low risk of carrier status
- Two copies of SMN1, result flagged
  - Patient may have an increased risk of silent carrier status
  - Inform the patient, and refer to Genetics
  - Partner/FOB screening should be ordered

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Cystic Fibrosis

- Symptoms include progressive obstructive lung disease and pancreatic insufficiency.
- Incidence: 1:2500 livebirths in European populations
- Inheritance: autosomal recessive
- Carrier frequency: 1/25 to 1/90
- Detection rate: 55% to 97%
- HC order: 81220A (Cystic Fibrosis, CFTR, Common mutations)
  - Targeted mutation analysis: 55 mutation panel

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Cystic fibrosis carrier screening

- Current CF carrier at KP accomplished through 55 mutation panel
  - Patients with prior negative 36 mutation panel can be offered an updated panel
  - Updated panel provides increased detection rates, primarily in Hispanic and African-American populations
- If patient is a carrier:
  - Refer to Genetics for consultation to review information and offer partner testing.
  - Genetics offers targeted mutation panel or CFTR sequencing to FOBs, or OB provider can order 55 mutation panel on FOB, if he is a member
- CFTR sequencing has a higher detection rate (~99%) across all ethnicities, but may yield uncertain results (variants of uncertain significance, lower penetrant mutations/ "mild" mutations)

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Cystic fibrosis – detection rates and residual risks

Ethnicity	Carrier Frequency	Detection Rate	Residual risk
Ashkenazi Jewish	1/25	97%	1/800
Asian	1/90	55%	1/200
African American	1/65	75%	1/250
Hispanic	1/46	75%	1/180
Caucasian	1/25	91%	1/240

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Hemoglobinopathies

- Incidence highest among individuals of African, Asian, Mediterranean, and Middle Eastern ancestries
- Inheritance: autosomal recessive
- Alpha globin diseases:
  - Alpha thalassemia: Hemoglobin Barts, Hemoglobin H disease
- Beta globin diseases:
  - Includes Sickle cell disease and other beta globin variants
  - Beta thalassemia

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Alpha Thalassemia

- Alpha thalassemia disease (Hemoglobin Bart hydrops fetalis syndrome)
  - Loss of all four alpha globin genes
- Hemoglobin H disease
  - Loss of three alpha globin genes
- Alpha thalassemia trait
  - Loss of two alpha globin genes, either in cis (αα/--) or in trans (-α/-α)
- Silent carrier
  - Loss of one alpha globin gene (αα/α-)
- Suspect alpha thalassemia trait when:
  - MCV <80 with normal ferritin/iron and normal Hb fractionation

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Beta globin diseases

- Sickle cell disease
  - Includes Hb SS, Hb SC, Hb SD, Hb S – beta thalassemia
- Beta thalassemia
  - Includes beta thalassemia major, beta thalassemia intermedia, Hb E – beta thalassemia
  - Order 83021B -hemoglobin fractionation, chromatography
- Suspect a beta globin variant when:
  - Hemoglobin fractionation result reveals a clinically significant beta globin variant (e.g., Hb S, Hb C, Hb D, Hb E identified on hemoglobin variant confirmation test)
  - Low MCV, elevated Hb A2 and occasionally elevated Hb F (suggestive of beta thalassemia trait)
  - Elevated Hb F (if rest of fractionation and MCV is normal) is likely benign/normal variant and testing of the partner is not necessary

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Hemoglobinopathy traits

- If patient has a low MCV (<80):
  - Checkchart for prior MCV values. If a prior MCV is >80, no referral to Genetics needed
  - Order 83021B -hemoglobin fractionation, chromatography
  - Order iron studies (ferritin, serum iron, TIBC, iron saturation)
- Low MCV, normal hemoglobin fractionation result, normal iron studies
  - Patient may have alpha thalassemia trait
  - FOB/partner testing indicated: order CBC and Hb fractionation
- Low MCV, elevated Hb A2 and/or elevated Hb F, normal iron studies
  - Patient may have beta thalassemia trait
  - FOB/partner testing indicated: order CBC and Hb fractionation
- Abnormal hemoglobin fractionation result (Hb AS, Hb AC, etc)
  - FOB/partner testing indicated: order CBC and Hb fractionation
- Refer to Genetics for consultation to review information and offer partner testing, **or** OB provider can order CBC and Hb fractionation on FOB
  - If couple is at risk for offspring with a hereditary hemoglobinopathy, Genetics offers molecular testing to identify alpha or beta globin gene mutations

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Carrier screening for individuals of Jewish ancestry

- For patients of Jewish ancestry:
  - Tay Sachs screening – if only pt or FOB is of Jewish ancestry
    - HC order: 81255A (Tay Sachs disease, HEXA mutation panel)
- If both members of couple are of Ashkenazi Jewish ancestry or Persian Jewish ancestry:
  - An expanded carrier screening panel is recommended for conditions more common in Jewish populations
- If one member of the couple is Jewish and the other member is not:
  - Offer screening for Tay Sachs **on the individual who is Jewish**, if he/she is a KP member
  - If the KP member is not Jewish, but their partner is Jewish:
    - Recommend that partner have TS screening through their own insurance
    - Screen the non Jewish individual through enzyme analysis – refer to Genetics.

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Carrier screening in the Ashkenazi Jewish population

- If **both** patient and FOB are Ashkenazi Jewish:
  - Offer referral to Genetics to discuss AJ10 panel; this is a sendout test
- Expanded AJ panel:
  - Bloom syndrome
  - Canavan disease
  - Familial Dysautonomia
  - Fanconi Anemia type C
  - Gaucher Disease
  - Glycogen Storage disease, type 1A
  - Maple Syrup Urine disease, type 1A and 1B
  - Mucolipidosis, type IV
  - Niemann-Pick, type A and B
  - Tay Sachs disease
- Ashkenazi Genetic Panel 2 and Ashkenazi Genetic Panel 4 are not recommended
  - Although this is orderable in HC, this is run at Quest labs
  - Cystic fibrosis screening via AJ2 or AJ4 panel screens for fewer CFTR mutations

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Screening in the Persian Jewish population

- For couples who are both of Persian Jewish ancestry, offer an expanded panel for conditions common in the Persian Jewish population:
  - Tay-Sachs
  - Polyglandular autoimmune disease
  - Pseudocholinesterase deficiency
  - Inclusion body myopathy type 2
  - Corticosterone methyloxidase deficiency
- Refer to Genetics, as this is a send-out test

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Carrier screening follow-up: SMA and CF

- OB providers should inform their patients of their carrier screening results and refer all flagged or abnormal results to Genetics.
- SureNet follow-up initiated November 2019
- If no follow up on an abnormal CF or SMA result is documented in chart, SureNet will send a message similar to this to the ordering provider:
  - *Your patient has been identified by KP SureNet as having a positive Spinal Muscular Atrophy (or CF) carrier test result. Based on the recommendations of the SCPMG Genetic department and Obstetric regional chiefs, we have ordered a referral for genetic counseling. Please review the patient's chart and if you feel that this is appropriate, please sign and inform the patient of the results and of the genetic referral.*
- If the provider signs the referral for Genetics, it will automatically be sent to the local area Genetics office
- ***Other screening results (e.g., hemoglobin fractionation, Tay Sachs, etc) are not tracked by SureNet. OB providers should review all carrier screening results and refer for follow-up as needed.***

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Non-member testing

- If a pregnant patient is found to be a carrier of a genetic disorder, testing for the FOB is offered within KP to determine risk to the current pregnancy
- If FOB has not been a KP member previously, call the MRN Services at 8-279-3333 to obtain MRN for FOB
  - Will need name, sex, birthdate, SSN (optional), address, and phone number
  - Drop in message encounter: "Non member. Testing ordered on (date) covered under non-member genetic testing policy to determine risk to partner's pregnancy"
- Order appropriate test(s) under FOB's MRN and direct him to a KP lab (he will need a photo ID)

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Other carrier screening considerations

- Consanguinity – if couple is related by blood (i.e., first cousins):
  - Offer referral to Genetics for expanded carrier screening
- Expanded carrier screening panels (direct to consumer testing)
  - Not a covered benefit in most circumstances
  - Available as an out-of-pocket expense
- Patients who have a non-member partner who is a carrier for a genetic condition
  - A copy of non-member partner's carrier screening result is needed
  - If non-member partner is a carrier for a condition orderable in HC, please consider ordering test in HC (e.g., Hb fractionation, SMA)
  - Can refer to Genetics to assist with screening on patient

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Member Education

- Member education handouts for pre-test and post-test screen positive
  - English and Spanish available through Member Health Ed
  - SMA Carrier Screening information
  - SMA Carrier handout
- New "infographic style" for general carrier screening pamphlet also available in English and Spanish
  - Genetic Carrier Screening
- Additional topics are available on Genetics website:
  - http://kp.org/scal/genetics

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Considerations in carrier screening

- Always inform your patient of their carrier screening result, and refer to Genetics if a result is abnormal or flagged
- If a patient has had a prior negative carrier screening result, re-testing is not necessary, unless testing has been updated
  - Example: prior to 2017, cystic fibrosis screening was a 36 mutation panel
- Questions about carrier screening results?
  - Contact your local Genetics office or send a Doctor Advice message

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Prenatal Screening

California Prenatal Screening Program and NIPT

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Prenatal Screening

- **California Prenatal Screening Test**
  - Offer to all pregnant women
  - Screens for Down syndrome, Trisomy 18, neural tube defects, and Smith-Lemli-Opitz syndrome
  - Screening accomplished through analysis of serum analytes and nuchal translucency ultrasound
- **Non-Invasive Prenatal Testing (NIPT)**
  - Only available to women at increased risk for a pregnancy with aneuploidy
  - Screens for Down syndrome, Trisomy 18, Trisomy 13, and sex chromosome aneuploidy
  - Screening accomplished through analysis of cell-free fetal DNA

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California Prenatal Screening Test

- 1st trimester blood draw: 10 weeks and 13 weeks 6 days
- Nuchal translucency ultrasound: 11 weeks 2 days and 14 weeks 2 days
- 2nd blood draw: 15 weeks and 20 weeks
- Detection rates:
  - Down syndrome: 90%
  - Trisomy 18: 81%
  - Neural tube defects: 80%
  - Abdominal wall defects: 85%
  - Smith-Lemli-Opitz syndrome: 60%

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Non Invasive Prenatal Testing (NIPT)

- Blood draw between 10 weeks 0 days and 24 weeks
  - Analysis of circulating cell free fetal DNA in maternal blood
- Detection rates:
  - Down syndrome: 94% - 99%
  - Trisomy 18: 97%-99%
  - Trisomy 13: 87%- 92%
  - Sex chromosome aneuploidy: 94%

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NIPT Indications

- **Advanced Maternal Age**
  - ≥ 35 years at EDC for singleton gestation.
- **Previous Trisomy**
  - History of a previous fetus/child with an autosomal trisomy.
- **Parental Translocation**
  - Parental balanced Robertsonian translocation with increased risk of fetal trisomy 21 or trisomy 13.
- **Screen Positive**
  - California Prenatal Screen positive result for trisomy 21, trisomy 18, or Large NT (≥ 3.0 mm).
- **Ultrasound Anomaly**
  - Structural fetal ultrasound anomaly indicating an increased risk for aneuploidy (excluding isolated soft markers).
- ***NIPT is not indicated for average risk pregnancies, and we do not facilitate screening***

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Considerations in ordering NIPT

- AMA is the only indication for NIPT ordered through the OB/GYN department
- Isolated ultrasound soft markers are not indications for NIPT
  - Soft markers defined as: CPC, EIF, SUA, echogenic bowel and pyelectasis).
  - Two or more soft markers: NIPT can be offered
- Vanishing twins
  - Increased possibility of false positive results
  - Results could be discordant for fetal sex
  - Accuracy/reliability of NIPT is not the same as for singleton gestations

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Lab orders and results

- California Prenatal Screening
  - First trimester: HC test code 227020
  - Second trimester: HC test code 227021
  - Results are scanned into the Media tab
- NIPT
  - HC test code 81420E - "Trisomy 21,18,13 aneuploidy analysis"
  - Results are available in HC (lab tab)
  - Patients active on kp.org get results via oPAP
    - Patients who do not have kp.org access should be informed of NIPT result by OB provider
- Abnormal CPSP and NIPT results are referred to Genetics

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NIPT orders

- OB providers should place NIPT order at the time of first OB visit.
  - When ordered by OB, the sole indication for NIPT is AMA
- Future vs regular orders
  - If pt is <10 weeks at time order is placed, NIPT should be a **future** order
    - Activation date should be 11 weeks 0 days, by ultrasound dating. Note: the system allows for orders to be activated 6 days prior to activation date
    - Expiration date should be 3 months post activation date..
  - If patient is already 10 weeks gest age: place as a regular order

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NIPT- unable to obtain results

- Approximately 4% - 6% of specimens may yield a "no call" result (unable to obtain result).
- First No Call

  If the patient's only indication is AMA:
  - OB provider should inform patient and reorder NIPT, if pt elects a redraw
  - About 70% of 1st failures provide a result
  - If there are any additional risk factors such as enlarged NT or soft ultrasound marker, etc., consult with MFM or Genetics
- Second No Call

  Refer to Genetics
  - Diagnostic Testing will be offered
  - If the patient elects screening, offer California Prenatal State Screening
  - A follow-up anatomic survey with perinatology is recommended
- No 3rd sample is authorized for NIPT

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Advances in diagnostic testing

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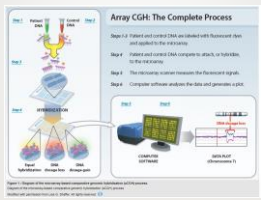
Microarray analysis



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Karyotypes vs. Microarrays

KARYOTYPE

- Detects aneuploidy
- Can detect structural rearrangements, balanced or unbalanced
- Can detect chromosomal mosaicism
- Cannot detect small deletions or duplications

MICROARRAY

- Detects copy number variants, including aneuploidy, deletions, and duplications not detectable on a routine karyotype analysis
- Can detect some cases of uniparental disomy
- Cannot detect balanced chromosome rearrangements
- May not detect low levels of mosaicism (<20%)
- Results can include variants of uncertain significance
- Parental blood specimens needed

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Prenatal chromosomal microarrays

- As of November 2019, chromosomal microarrays became the first tier test for prenatal diagnostic specimens.
- Prenatal microarrays are being performed at an outside lab
  - Results can be found in the Media tab under "Laboratory Result - KP Reference Lab" (Document Type – Description)
- The Regional Genetic Testing lab will keep a backup culture and will reflex to a karyotype if indicated.

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Products of conception (POC analysis)

- If POC analysis is requested for diagnostic evaluation/confirmation, this can be accomplished via a microarray analysis
  - Complete a Genetics TRF (Genetic Test Request Form) and the outside lab form (ARUP labs, call local Genetics office for form)
  - Send forms along with the specimen.
  - Send a maternal blood specimen (EDTA) along with the specimen.
- If multiple tests are ordered, please indicate if the request is to have these performed concurrently, or as reflex testing
- All specimens should be placed in sterile saline. DO NOT place specimens in formalin.
- Do not send entire fetuses.
- Place paperwork in the paperwork pocket of the specimen bag; please do not place paperwork in with the specimen itself.
- Call local Genetics office or Genetics lab with any questions

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Cancer Genetics

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When to refer or suspect a hereditary cancer syndrome?

- **Women or men whose family history fits the 3-2-1 rule:**
  - Any family history of **3 or more** cancers, in at least **2 generations on the same side** of the family, and **at least 1 person** with:
    - Two cancer primaries
    - Cancer diagnosis ≤50 years
    - Unusual or rare cancer

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Tips for Identifying High Risk Cancer Families – The Rule of Two/Too

- Two:
  - Two or more cancers in the same person (of the same type or related types):
    - Examples include (but are not limited to) breast & ovarian, colorectal & endometrial, colorectal & ovarian, prostate & pancreas, gastric & breast, or bilateral breast
  - Two or more cancers on the same side of the family: same or related types, see above
  - Two or more risk factors: Ashkenazi Jewish ancestry & family or personal history of breast, ovarian, prostate (Gleason score ≥7) or pancreatic cancer
  - Two or more generations with cancer on the same side of the family: suggests autosomal dominant inheritance
- Too:
  - Too young: diagnosis of cancer at young age (<50 or younger for breast or colorectal cancer)
  - Too many: cases of cancer on the same side of the family, ≥10 colorectal polyps
  - Too unusual: male breast cancer, adrenocortical carcinoma, transitional cell carcinoma of the ureter

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Referrals for genetic counseling

- Hereditary breast and ovarian cancer
  - Referral guidelines for hereditary breast/ovarian cancer are on Clinical Library:
    Breast And Ovarian Cancer: Referral To Genetic Counseling For Inherited Susceptibility
  - Referral recommendations updated in 2019. Changes include:
    - Women diagnosed at ≤50 years
    - Triple negative breast cancer (ER-, PR -, Her2Neu-), diagnosed at any age
    - Ashkenazi Jewish women, diagnosed with breast cancer (invasive or DCIS) at any age
    - Epithelial carcinoma of the ovary, fallopian tube or peritoneal cancer at any age (excluding LMP tumors)
    - Individuals with pancreatic cancer, at any age
    - Men with prostate cancer, diagnosed at age ≤65 years with a Gleason score of 7 or more

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Referrals for genetic counseling

- Lynch syndrome screening (hereditary non-polyposis colon cancer)
  - All colorectal and endometrial cancer specimens screened by immunohistochemical analysis (IHC) for mismatch repair proteins (MMR) in patients of any age
  - IHC results appear in pathology report; some abnormal results lead to reflex testing
  - Abnormal results, suggestive of increased risk for Lynch syndrome– refer to Genetics
- Questions about family or personal histories? Send a Dr. Advice message to Genetics

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Genetic testing

- Current testing offered is a 31 gene panel of genes associated with high and moderate risks of developing cancer:
  - APC, ATM, AXIN2, BAP1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDKN2A, CHEK2, EPCAM, FH, MLH1, MSH2, MSH6, MUTYH, NBN, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, SCG5/GREM1, SMAD4, STK11, TP53, and VHL
- Updated testing for individuals with prior negative genetic test results (e.g., BRCA1/BRCA2 testing performed prior to implementation of gene panels – generally prior to 2014):
  - Send a Dr Advice message or call Genetics
- Positive results (pathogenic variant detected):
  - Increased lifetime risks of developing specific cancers, increased screening/surveillance recommended. In some cases, risk reducing surgeries and chemoprevention may be offered.
- Negative results (no pathogenic variants detected):
  - Reduces likelihood of a hereditary cancer syndrome, but does not eliminate risk. Cancer screening should be based on personal and family history factors.
- Uncertain results (variants of uncertain significance):
  - Approximately 32% of patients tested have at least one variant of uncertain significance

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Management

- Management recommendations for some cancer-risk genes are outlined by NCCN
- Newer genes do not have management recommendations yet due to limited data and insufficient evidence
- Management for patients should be based on personal and family history factors
- Some medical centers have specialty clinics for patients with hereditary cancer syndromes (LAMC, Baldwin Park, Orange County)

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Questions? Contact us!

- Baldwin Park: 626-851-5920; tie line 370
- Downey: 562-657-4842; tie line 327
- Fontana: 909-427-3089; tie line 250
- Los Angeles: 323-783-5612; tie line 363
- Orange County: 714-254-2704; tie line 230
- Panorama City: 818-375-2073; tie line 350
- Riverside: 951-353-3494; tie line 258
- San Diego: 619-516-6484; tie line 240
- South Bay (Carson): 310-660-2577; tie line 317
- West Los Angeles: 323-857-2074; tie line 390
- Woodland Hills: 818-719-3367; tie line 348
- Genetic Screening Services: 626-564-3322; tie line 331

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