Selecting the Right Genetic Test

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Overview

- Genetic testing
- Case examples
  - Step 1. Developing a differential diagnosis
  - Step 2. Selecting genetic test(s)

Common reasons for genetic testing

- Confirm a suspected genetic disorder in patients with signs or symptoms.
- Assess predisposition to a genetic disorder in patients with a concerning family history.
- Prenatal diagnosis to inform reproductive decisions, interventions before or after birth, and prepare for birth.
- Assess carrier status for a recessive condition to inform reproductive decisions.
- Inform response to treatment (pharmacogenetics).
- Inform prognosis of an inherited or acquired condition.

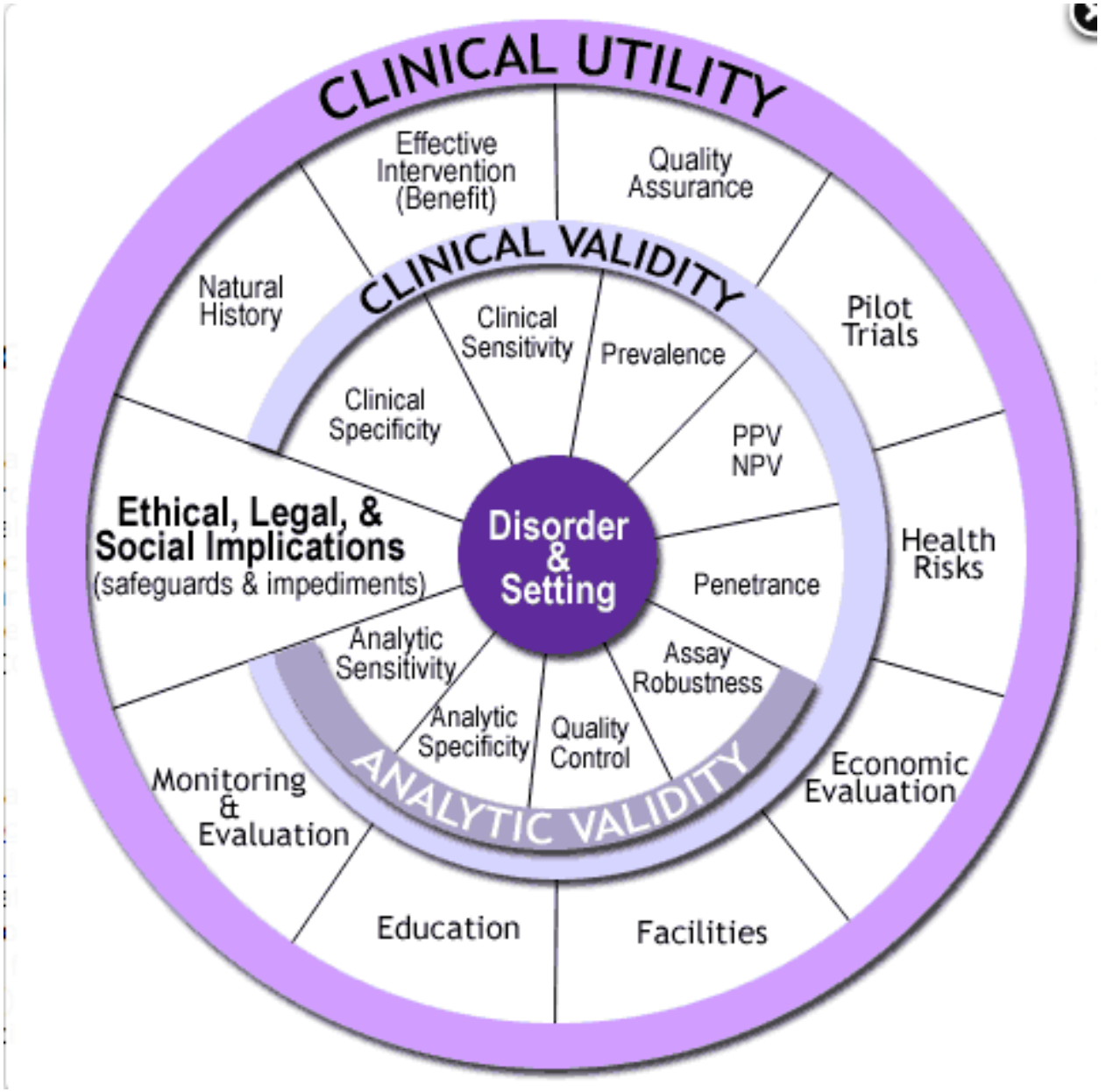
Genetic testing is fundamental to genetic diagnosis

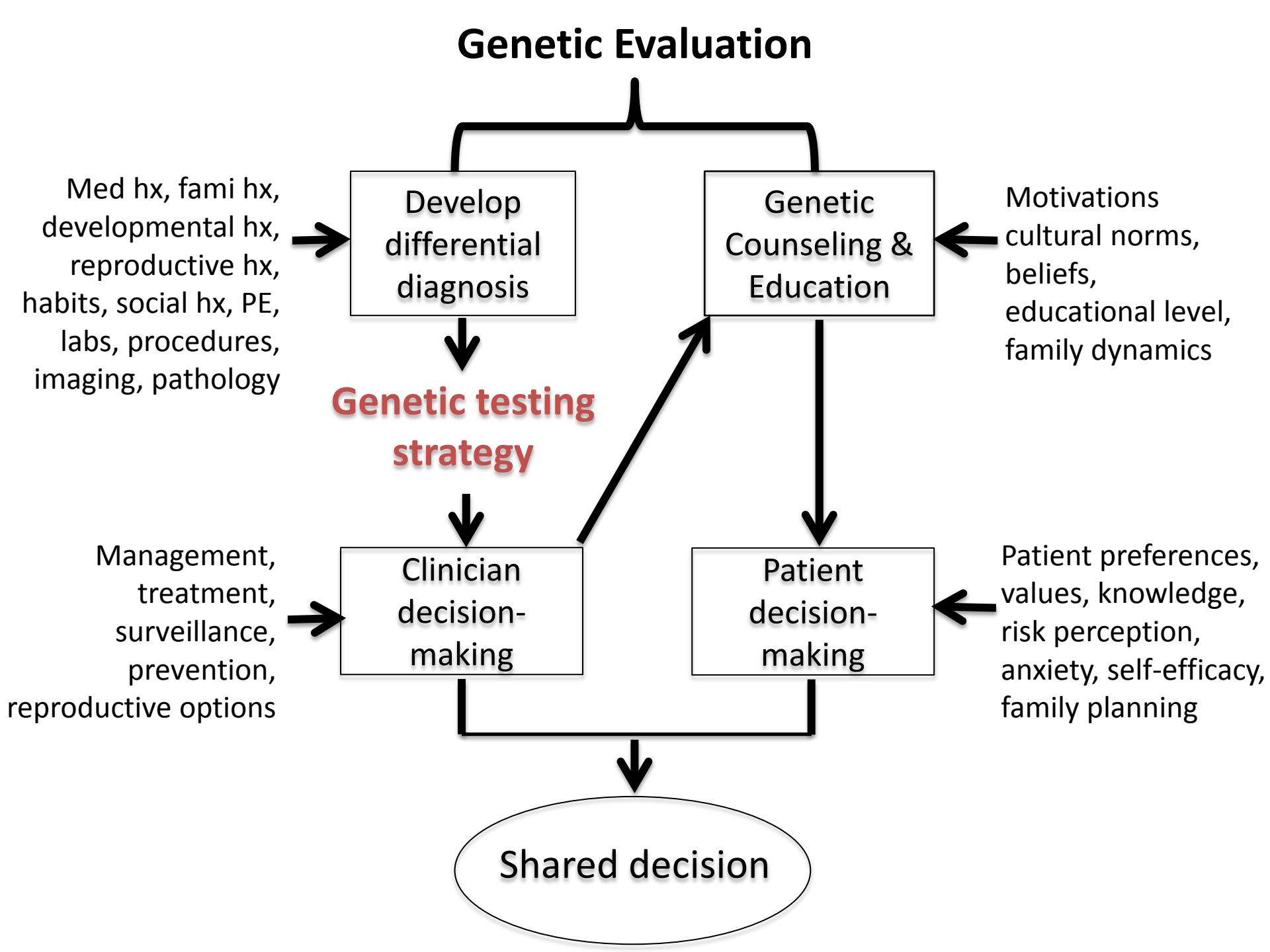
Analysis of:		To assess:
DNA and RNA	→	Genotypes
Chromosomes	→	Karyotypes
Proteins, other metabolites	→	Phenotypes

Common Molecular Techniques

- Sequence analysis
  - Sanger sequencing
  - Next generation sequencing
- Deletion/duplication testing
  - Single gene (e.g., MLPA)
  - Array genomic hybridization
- Targeted mutation analysis
  - Familial mutation
  - Common mutations (i.e., based on ancestry)

Evaluating a genetic test: the ACCE framework




Genetic Evaluation
Med hx, fami hx, developmental hx, reproductive hx, habits, social hx, PE, labs, procedures, imaging, pathology
Develop differential diagnosis
Genetic Counseling & Education
Motivations cultural norms, beliefs, educational level, family dynamics
Genetic testing strategy
Management, treatment, surveillance, prevention, reproductive options
Clinician decision-making
Patient decision-making
Patient preferences, values, knowledge, risk perception, anxiety, self-efficacy, family planning
Shared decision

Genetic testing errors

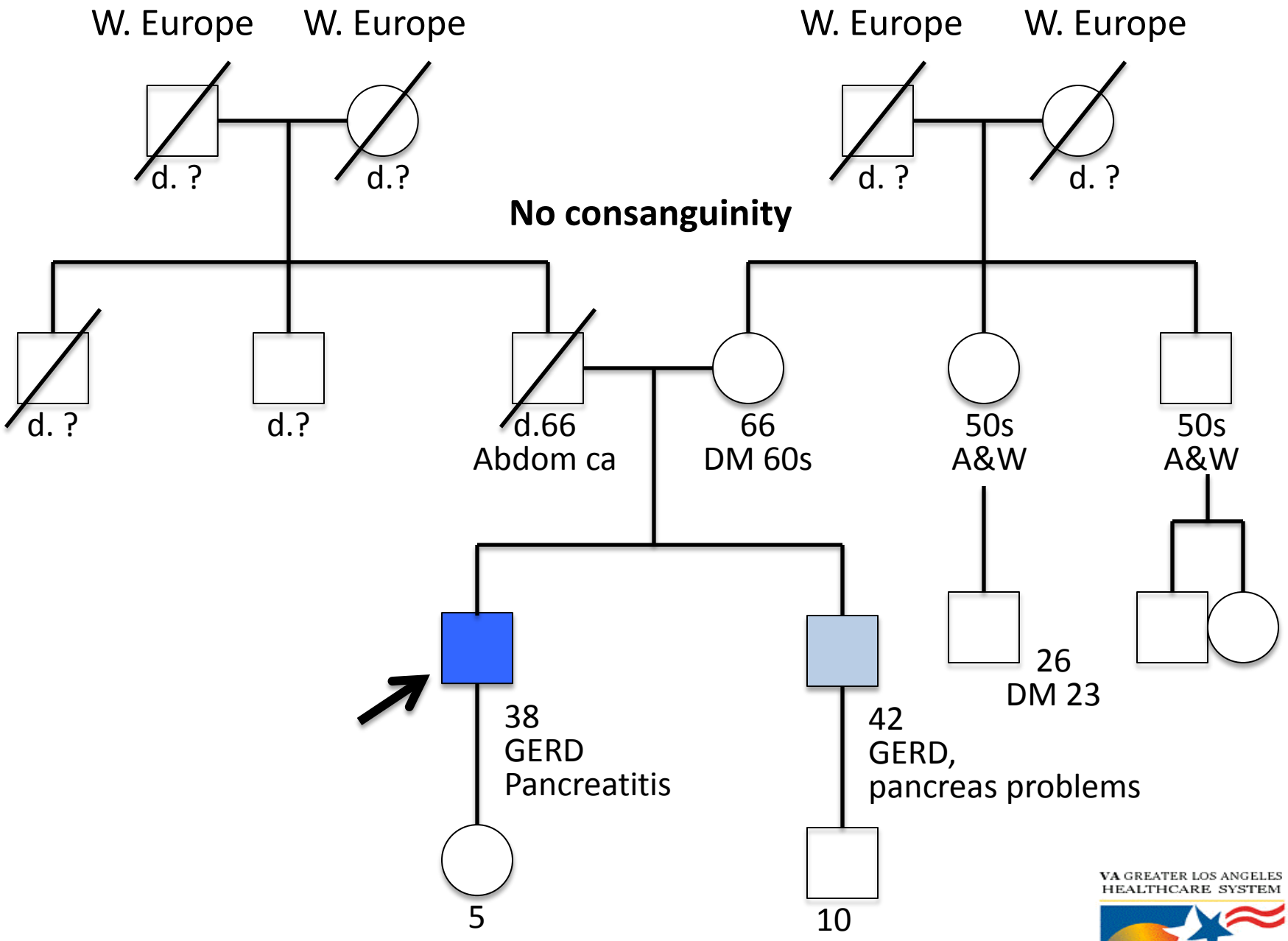
- Due to:
  - Insufficient personal and family history needed to inform a differential diagnosis and test selection.
  - Lack of knowledge about genetic principles, testing methods and their limitations.
- Results in inappropriate test selection:
  - Compromised informed consent process.
  - Overutilization of tests that are not indicated → misdiagnosis and adverse outcomes.
  - Underutilization of indicated tests → delayed or missed dx.

Burke W, et al. Am J Med Genet A 2009;149A(3):349-356; Brierley KL, et al. Conn Med 2010;74(7):413-423; Levy DE, et alGenet Med. 2011;13(4):349-355; Dhar SU, et al. Breast Cancer Res Treat 2011;129(1):221-227; Plon SE, et al. Genet Med 2011;13(2):148-154.

Getting the differential diagnosis right

Case 1: Mr. WK

- 38 year old male
- Referred from gastroenterology clinic
- “Genetic testing desired. D/W biliary attending. Intermittent sharp, stabbing RUQ pain 3-4 yrs with N/V. H/o GERD. Non-smoker. 1 beer/mo. 130 pounds, 5’9”. Possibly having attacks of pancreatitis and now with absent body/tail of pancreas and chronic pancreatitis changes in the head. May have hereditary pancreatitis. Genetic testing of pancreas panel (PRSS1, SPINK1, CFTR, CTRC).”



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Labs and Imaging

- CBC, Electrolytes wnl
- eGFR 50; Cr 1.4 – 1.6
- LFTs, amylase and lipase wnl

- CT Abdomen – agenesis of the dorsal pancreas; prominent pancreatic head with calcifications and cystic lesions. Common bile duct nl, no intrahepatic biliary ductal dilatation. No gall stones seen. Multiple bilateral renal cysts. Liver, spleen, adrenals unremarkable.

Assessment

38-year old male with:

- Dorsal agenesis of the pancreas, evidence of chronic pancreatitis.
- Bilateral renal cysts with renal insufficiency.
- Maternal family history of diabetes with early-onset.

Could any/all of the above be related to a single gene disorder?

Genetic differential diagnosis

- Renal cysts and diabetes syndrome, also known as MODY5 due to heterozygous *HNF1B* gene mutation.
  - Renal disease is highly variable: renal cysts, kidney malformation, abnl of genital tract.
  - Diabetes before age 25 c/w MODY
  - Dorsal agenesis of pancreas with pancreatitis
- Agenesis of the dorsal pancreas – can be associated with diabetes, abdominal pain, pancreatitis; polysplenia and heterotaxy syndrome
- Hereditary pancreatitis – due to mutation in the *PRSS1, SPINK1, CFTR,* or *CTRC* genes unrelated to renal cysts, renal insufficiency, diabetes.

Genetic Testing Strategy

- MODY5 testing (*HNF1B* gene), if normal
- Testing for hered pancreatitis with sequencing and del/dup (*PRSS1, SPINK1, CFTR,* and *CTRC* genes*)*

HNF1B genetic test results

- Heterozygous EX1_3'UTRdel pathogenic mutation
- A gross deletion spans coding exons 1 through the 3' UTR .
- Consistent with MODY5.

Implications and Management

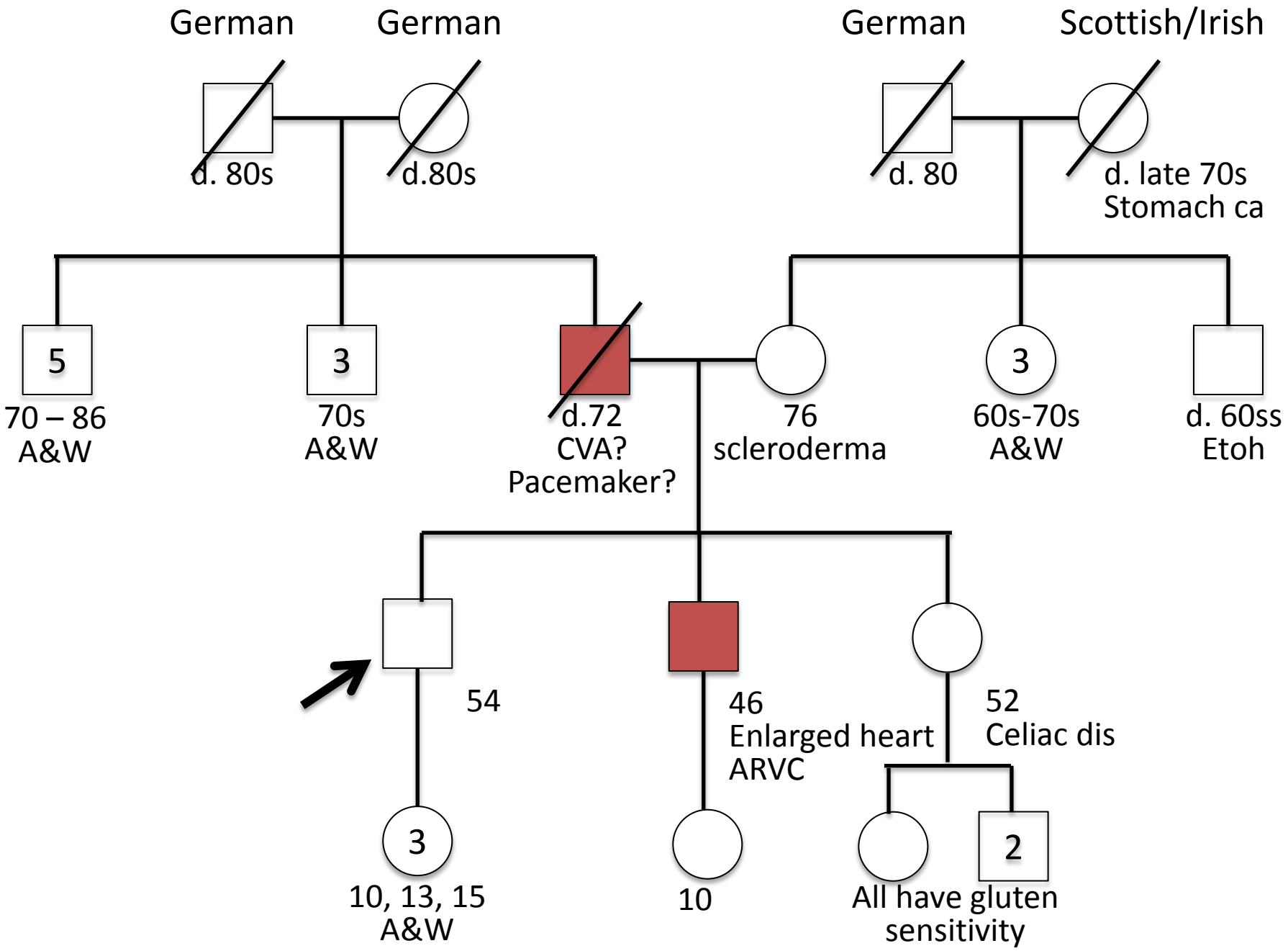
- Chronic pancreatitis due to *HNF1B* mutation (MODY5).
- Risk for diabetes – referred to endocrine clinic, monitor
- Risk for exocrine pancreatic dysfunction – monitor for malabsorption/weight, check fat soluble vitamins, followed by GI clinic, requesting referral to dietician.
- Renal cysts with renal insufficiency – referred to nephrology; avoid nephrotoxic agents.
- Likely maternal transmission. Family members at risk can test for familial mutation.

Case 2: Mr. SH

- 54 yo asymptomatic male
- Referred by primary care
- “Requesting genetic testing, brother recently screened for defects due to arrhythmia. His information: Arrhythmogenic right ventricular cardiomyoapthy. PVCs, over 16000 a day. Sleep apnea. The genetic test was performed by [lab] and my accession or proband number is [xxx]. If any family member wants to get a DNA test they will need this number.”

More history

- SH has no complaints concerning for cardiomyopathy or arrhythmia. Active and exercises 4 times a week. No CP, no DOE, no palpitations, syncope or near-syncope, no PND, no edema.
- Pt has not had echo. Has normal EKG.
- “My brother was diagnosed with a large heart. My uncle said we all have it because we were all athletes, wrestlers…. My brother’s doctor recommended an ICD after genetic testing. Then he saw a specialist at Johns Hopkins and he was told something different.”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A&W
d.72
CVA?
Pacemaker?
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scleroderma
3
60s-70s
A&W
d. 60ss
Etoh
54
46
Enlarged heart
ARVC
52
Celiac dis
3
10, 13, 15
A&W
10
2
All have gluten
sensitivity

Brother's genetic test results

- ARVC NGS panel: *RYR2, TMEM43, DSP, PKP2, DSG2, DSC2* and *JUP*
- Heterozygous for a variant of uncertain significance in the RYR2 gene, c.410G>A (p.Arg137Gln).

Genetic testing strategy for SH

- Explained brother's test results do not confirm ARVC; not enough evidence to assess pathogenicity.
- No indication to test for *RYR2* VUS.

Recommendations

- Brother h/o “enlarged heart” and arrhythmia. Need to better understand brother’s phenotype; request brother’s medical records.
- Ordered echo given fam hx of possible cardiomyopathy.
- Follow up in genetics 2-3 months.

Selecting the right test depends on the right diagnosis

Requires synthesis of medical history, family history, social history, exposures, habits, physical exam, review of labs, procedures, imaging, and pathology reports.

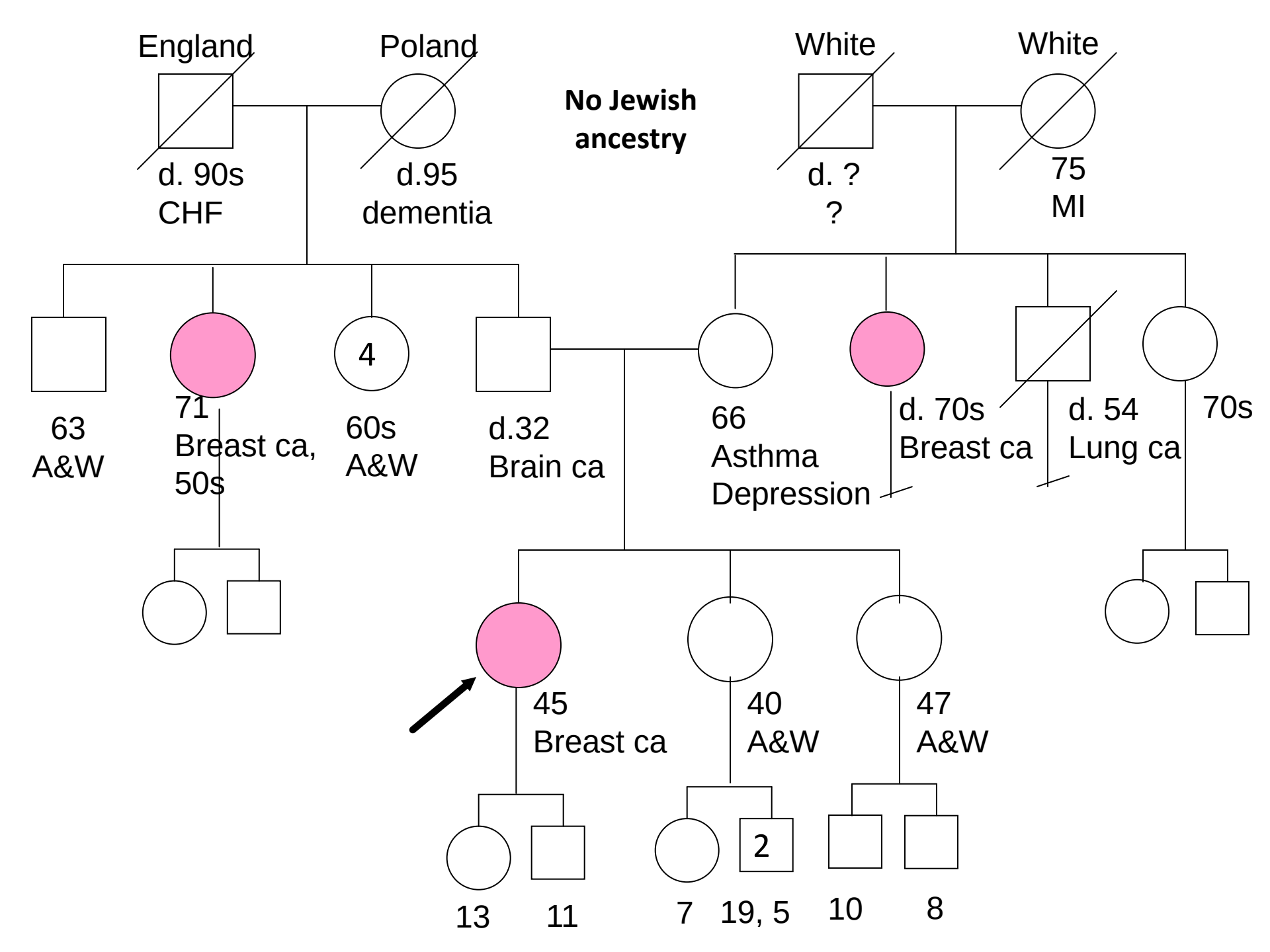
Selecting the right test

Case 3: Ms. T

- 47 yo female
- Referred by primary care
- Newly diagnosed with invasive lobular carcinoma of right breast. Mat aunt had breast cancer.

More history

- Diagnosed with invasive lobular adenoca right breast on biopsy – 15 mm with 2 foci of cancer.
- s/p bilateral mastectomy with reconstruction; sentinel node negative and tumor ER/PR+, her2neu-.
- “If it’s positive, then I would get everything taken out. I have bleeding (dx of adenomyosis), it would be better to get everything out.”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8

DDX and Genetic testing strategy

- Early age at onset and multifocal disease suggests possible in inherited breast cancer predisposition from paternal or maternal lineage.
- Genetic heterogeneity for inherited breast ca: *BRCA1, BRCA2, PALB2, PTEN, TP53, CDH1, STK11, etc.*
- s/p bilateral mastectomy; thus, testing will impact risk for other cancers, e.g., ovarian, gastric and other cancers, and subsequent management.

Genetic test results

- *BRCA2* gene, heterozygous for c.1929delG, a pathogenic mutation.
  - Frameshift with alternate stop codon.
  - Observed in families with breast, male breast, ovarian and prostate cancers
  - Likely founder mutation in individuals from northwest England, 1.4% prevalence in br/ov families

Implications and Recommendations

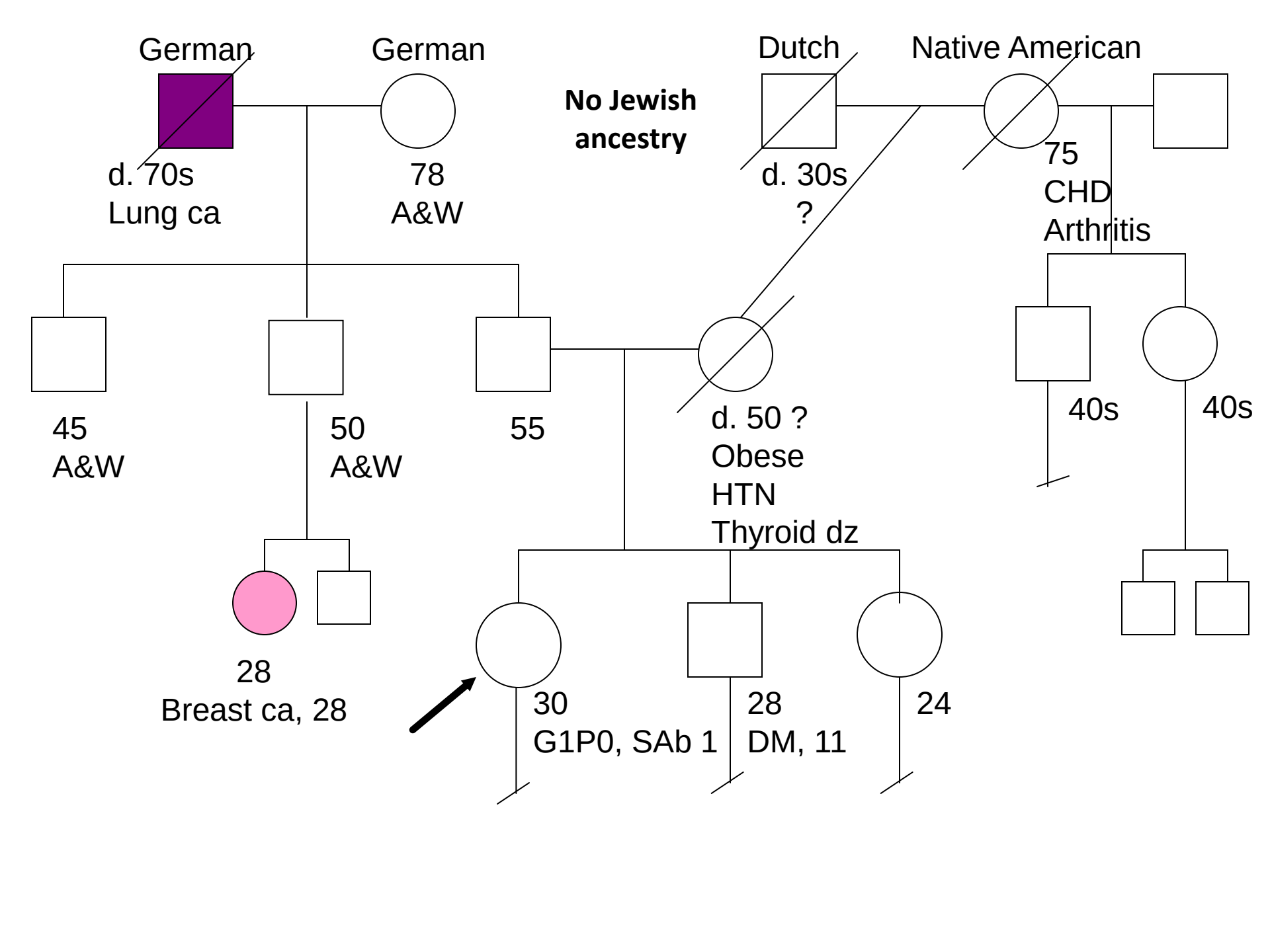
- Has maximally reduced risk for another primary breast cancer with b/l mastectomy
- Recommend b/l salpingo-oophorectomy to reduce ovarian cancer risk.
- Referral to GI to advise regarding surveillance for increased pancreatic cancer risk.
- Advise to avoid sun, use protective clothing and SPF given increased melanoma risk; annual comprehensive derm exams
- Inform at-risk family members.

One year follow-up

- s/p hysterectomy and BSO
- Seen in GI clinic, had EUS
- Mother and both sisters tested negative for the familial *BRCA2* gene mutation.

Case 4: Ms. CJ

- 30 yo female
- Referred by primary care
- Family history of early-onset breast cancer. Genetic testing desired, *BRCA1/2* to eval for risk of breast and ovarian cancer. Pt would consider preventive surgery after childbearing.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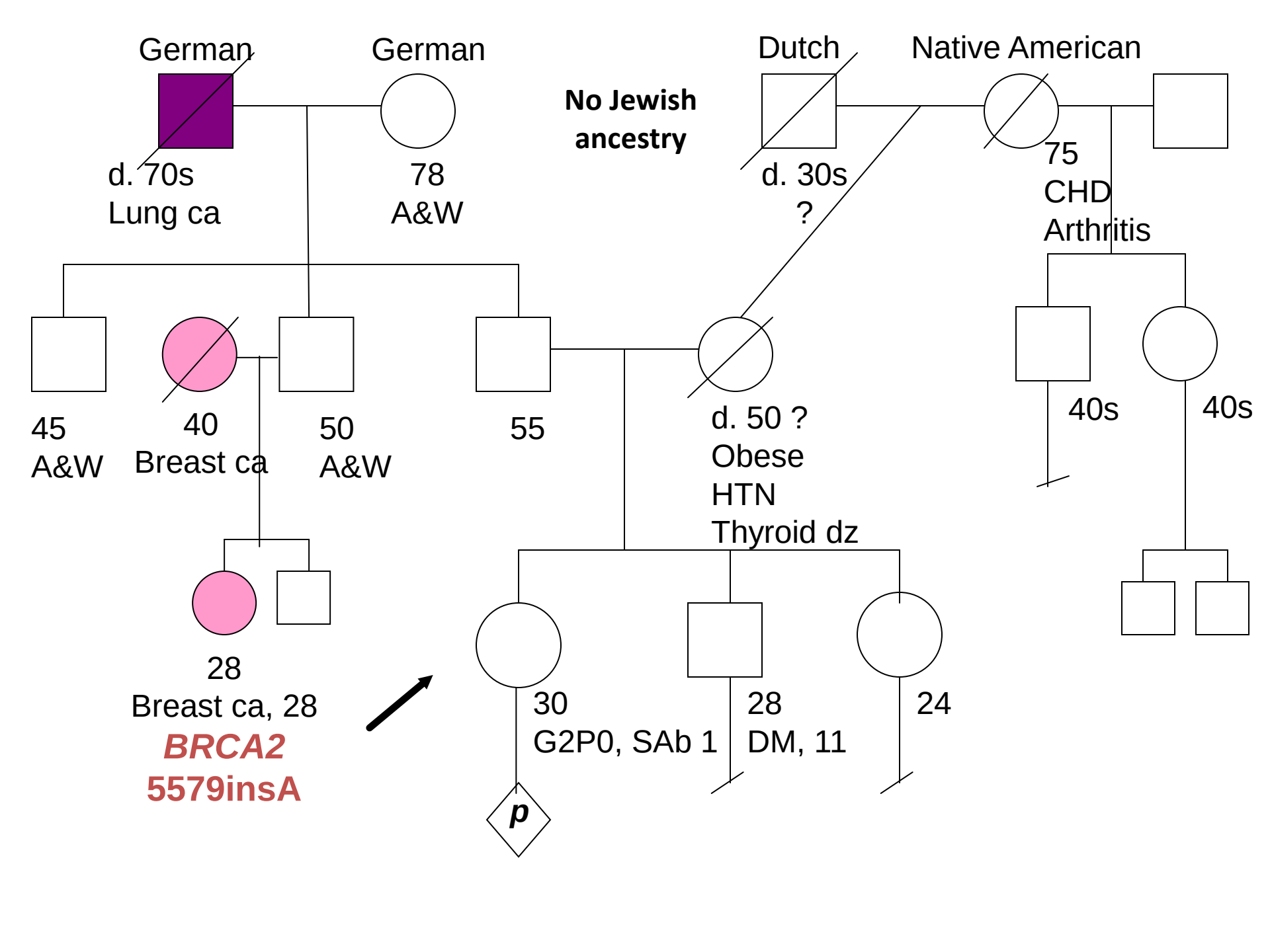
Genetic testing strategy

- Possible inherited breast cancer in paternal female first-cousin.
- Ideally test affected family member. Ms. CJ not aware if cousin had genetic testing; lives in Germany.
- Recommended CJ contact cousin ask about genetic testing and cancer family history in her maternal family (unrelated to CJ).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Breast ca, 28
BRCA2
5579insA
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Test Results

Test Performed:

BRCA2 single site analysis 5579insA mutation

Result: No mutation detected.

Implications and Recommendations

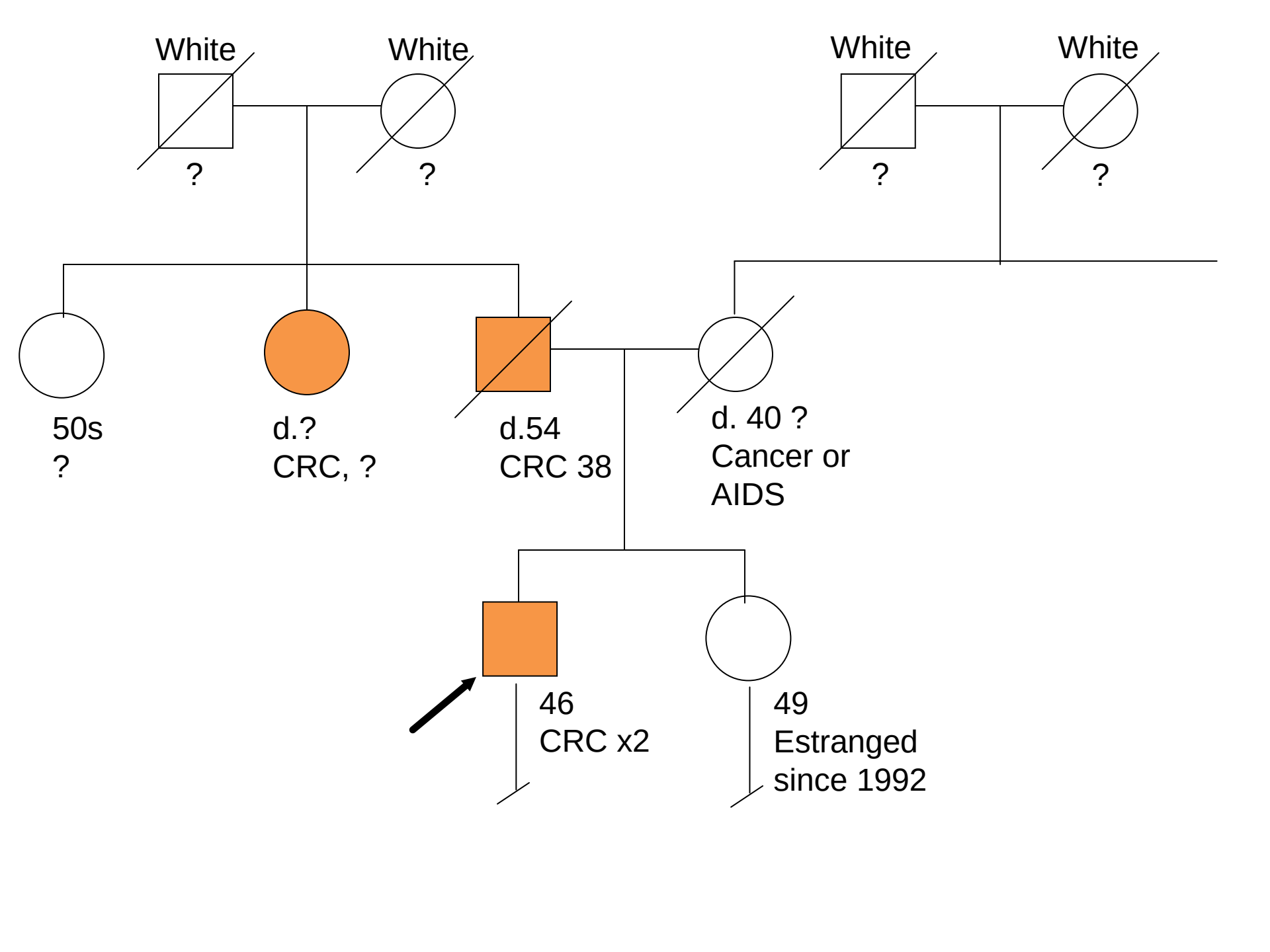
- No increased breast or ovarian cancer risk.
- No indication for enhanced surveillance or prevention.
- Cannot transmit familial risk to offspring.

Case 5: Mr. MC

- 46 yo male
- Referred by gastroenterology
- Possible Lynch syndrome

More history

- Diagnosed with synchronous CRC at 2 and 18 cm on first colonoscopy at age 46.
- Both tumors screened for Lynch syndrome with IHC of MMR proteins and MSI. Nl IHC, MSI-High and no *MLH1* promoter methylation.
- HTN
- Current smoker
- No alcohol, no other drugs. Walks nightly.
- Family history of CRC in father and pat aunt.


White
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CRC, ?
d.54
CRC 38
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Cancer or
AIDS
46
CRC x2
49
Estranged
since 1992

Genetic testing strategy

- Personal and family history consistent with Lynch syndrome; no polyposis, meets Amsterdam criteria and MSI-High tumors.
- May have other hereditary CRC cancer syndrome; however, currently no utility in trying to confirm Lynch syndrome diagnosis.
  - Normal test results cannot exclude LS diagnosis
  - No impact on cancer surveillance and prevention

Recommendations

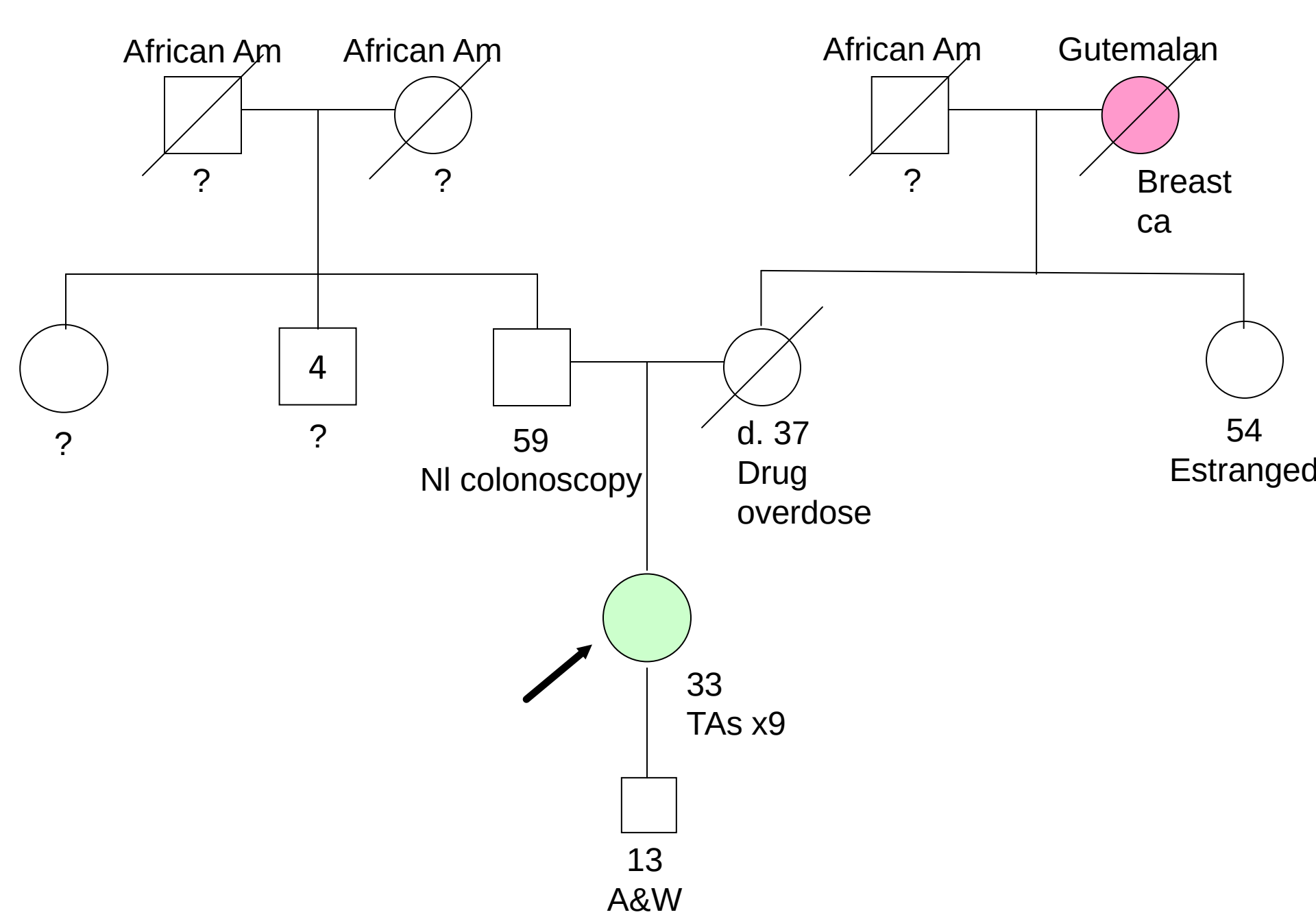
- Colonoscopy every 1-2 years
- Upper endoscopy every 3-5 years
- Urine for blood and cytology every year
- Inform relatives (sister – but estranged)
- Quit smoking, start daily aspirin

Case 6: Ms. AW

- 33 yo female
- Referred by gastroenterology
- Underwent colonoscopy today for rectal bleeding, multiple polyps removed, two >1cm. Grandmother had breast cancer. Question of whether polyp(s) should be tested. Histology pending.

More history

- Abdom pain, passing clots.
- Colonoscopy – 9 polyps found throughout colon
- Cyst removed from back
- Migraine, acne, lumbago
- Meds: sumatriptan succinate, daily MVI; not taking aspirin
- No smoking, alcohol or drug use
- Walks daily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DDX and Genetic testing strategy

- Young age and 9 adenomas suggests possible inherited predisposition to polyps/CRC.
- DDX: Lynch syndrome, attenuated FAP due to APC gene, MUTYH-associated polyposis, other polyposis syndromes known/unknown.
- Testing of adenoma low yield for Lynch syndrome.
- Gene panel testing to inform diagnosis, cancer spectrum and risk, and surveillance and prevention options.

Genetic test results

- CRC gene panel: *APC, BMPR1A, CDH1, CHEK2, EPCAM, GREM1, MLH1, MSH2, MSH6, MUTYH, PMS2, POLD1, POLE, PTEN, SMAD4, STK11,* and *TP53.*
- APC gene, heterozygous pathogenic mutation, c.266C>G (p.S89*), consistent with attenuated FAP
- RAD50 heterozygous variant of uncertain significance, c.610A>C

Implications and Recommendations

- Colonoscopy in one year, then every 1-3 years, no more than 5 year interval. If polyps become innumerable, consider colectomy.
- Aspirin or NSAID (sulindac), though not known if effective in preventing polyps/cancer in AFAP, as in classic FAP.
- Upper GI polyp/cancer risk: EGD every 2-3 years.
- Thyroid ca risk: exam every year with low threshold to image and biopsy
- Relatives at risk, including son and mat/pat rels

Selecting the right test: Clinical context is key

- Patient characteristics: age, gender, past history, family history, motivations for testing, acceptance of available interventions.
- Characteristics of the genetic disorder: inheritance, prevalence, penetrance, variable expressivity, clinical heterogeneity and genetic heterogeneity.
- Genetic test characteristics: test availability, targeted vs. comprehensive testing, one gene at a time or gene panels, methodology.
- Provider characteristics: expertise/familiarity with genetic disorder, with genetics and genetic testing.

Summary

- Genetic diagnosis can…
  - End a diagnostic oddysey
  - Guide management and surveillance recommendations
  - Inform reproductive and life-planning decisions
  - Have health and reproductive implications for family
- Genetic diagnosis can be a complex process, and relies heavily on genetic testing.
- Selecting the right genetic test relies on accurate differential diagnosis.
- There are multiple genetic testing methods and each has limitations.
- Genetic counseling is important, including discussion of benefits, risks and limitations of genetic testing options.

Thank You!

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