Identifying genetic syndromes – what is best practice?

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Disclosures

Relationship with companies:

AstraZeneca
Bayer
Bristol-Myers Squibb
Illumina
Janssen Pharmaceuticals
MSD
Nimagen
Roche
Informed consent for germline testing

- impact not limited to current disease
- impact not limited to individual patient
- patient has a right not to know
- explicit informed consent is good practice

**Pathogenic germline variant**

**Somatic mutation**
Roles of pathologist

- Individual patient characteristics-based role
- Reflex analysis of dMMR/MSI-high for detection of Lynch syndrome
- Test predisposition genes for combined germline and somatic mutations
- Recognize putative germline variants using gene panel testing
- Diagnostics of overgrowth syndromes
Roles of pathologist

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Hallmarks of individuals with hereditary cancer

- Relatively young age at diagnosis
- Multiple primary tumours
- Specific phenotype e.g. polyposis; developmental aberrations
- Specific tumour characteristics e.g. dMMR without *MLH1* methylation
- Germline pathogenic variant in tumour predisposition gene

>> Active role in multidisciplinary team meetings
Risk penetrance profiles

breast cancer

therapeutic options

colorectal cancer

MSI therapeutic options

Turnbull, Sud, Houlston, Nature Genetics 2018 PMID 30158684
Roles of pathologist

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• **Reflex analysis of dMMR/MSI-high for detection of Lynch syndrome**

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Lynch syndrome (LS)

- Pathogenic germline variants in mismatch repair genes *MLH1*, *MSH2*, *MSH6*, *PMS2*
- IHC loss of mismatch repair proteins (dMMR)
- Microsatellite instability (MSI-high)
- Major tumor types colorectal and endometrial cancer

>> Good practice to test these for dMMR / MSI and *MLH1* promoter methylation
Causes of microsatellite instability

1. Germline pathogenic variant inactivating a mismatch repair gene
   - Lynch syndrome (LS)
   - constitutional mismatch repair deficiency (CMMRD)

2. Hypermethylation of the $MLH1$ promoter

3. Biallelic somatic mutations inactivating a mismatch repair gene

MSI-high tumour without $MLH1$ promoter methylation $\neq$ Lynch syndrome
NGS-based MSI pan-cancer

15,045 tumours tested with MSK-IMPACT panel

2% MSI-high: mostly known Lynch syndrome associated tumour types
MSI-high as indicator for Lynch syndrome

MSS: mainly pathogenic *MSH6* and *PMS2* variants: incidental finding

Latham et al, JCO 2019 PMID: 30376427
All tumours of Lynch syndrome patients are more likely to be MSI-high: potential immunotherapeutic options.
Roles of pathologist

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Tumour test as prescreen for germline test

• Most genetic tumour tests are not designed and validated for this purpose and may miss relevant germline mutations

• Special attention is needed for:

  Coverage of all relevant regions: for most tumour suppressor genes
  • Complete coding region and flanking intronic regions
  • Detection of (multiple) exon deletions

Bio-informatic pipeline should not filter-out germline variants
New diagnosis epithelial ovarian cancer (regardless of age/family) → gynaecologist pathologist

Tumour-DNA BRCA-test

17% BRCA1/2 mutated → clinical geneticist

Counselling

chemotherapy → oncologist

Tumour-DNA BRCA-test

10% BRCA1/2 mutated

cascade testing to enable preventive measures → clinical laboratory geneticist

Germline-DNA BRCA-test

Vos et al Journal National Cancer Institute 2019 PMID: 31076742
Advantages of tumour first

- *BRCA1* and *BRCA2* mutation status available early in therapeutic process
- Efficient recognition of those at risk of hereditary mutation
- Less unnecessary tests because somatic and germline mutations are detected simultaneously
- Less patients referred to clinical geneticist:
  - Less unnecessary distress
  - More efficient

Disadvantage of tumour first

Technically more challenging than blood-based test
**Prerequisites for tumour test that replace blood test**

- Tumour test should be able to detect all germline variants & somatic mutations
  - The composition of the panel complies with clinical genetic needs
  - Test needs to be validated for this purpose
  - A minimal amount neoplastic cells to prevent performing a germline test

- Specific expertise on interpretation and reporting of variants

- Successful participation in External Quality Assessments

- Embedded in a defined care and treatment pathway:
  - Adequate pre- and post-test counselling and informed consent procedures
  - Patients with (likely) pathogenic variant in tumour should have swift access to germline testing
  - Relatives should have access to cascade testing and surveillance programs
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• Diagnostics of overgrowth syndromes
Cancer panel genes with putative germline pathogenic variants

<table>
<thead>
<tr>
<th>GERMLINE OR SOMATIC MUTATION</th>
<th>RARE GERMLINE-ASSOCIATED SYNDROME</th>
<th>MAIN CANCER APPLICABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>Li-Fraumeni</td>
<td>Sarcomas, and cancers of the breast and brain</td>
</tr>
<tr>
<td>MSH2, MLH1, MSH6, PMS2, EPCAM</td>
<td>Lynch</td>
<td>Cancers of the GI tract (particularly colorectal), endometrium, ovary, brain, breast, and renal pelvis</td>
</tr>
<tr>
<td>BRCA1, BRCA2</td>
<td>Hereditary breast, ovarian, prostate, and pancreatic cancers</td>
<td>Cancers of the breast, ovary, prostate, and pancreas</td>
</tr>
<tr>
<td>PTEN</td>
<td>Cowden</td>
<td>Cancers of the breast, endometrium, and thyroid gland</td>
</tr>
<tr>
<td>APC, MUTYH</td>
<td>Familial adenomatous polyposis</td>
<td>Cancers of the colon and rectum, small intestine, stomach, brain, bone, and skin</td>
</tr>
<tr>
<td>CDH1</td>
<td>Hereditary diffuse gastric cancer</td>
<td>Cancers of the stomach and breast</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>Familial atypical multiple mole melanoma</td>
<td>Melanoma, pancreatic adenocarcinoma, and cerebral astrocytoma</td>
</tr>
<tr>
<td>MEN1</td>
<td>Werner</td>
<td>Pancreatic endocrine cancer and pituitary gland tumors</td>
</tr>
<tr>
<td>RB1</td>
<td>Retinoblastoma</td>
<td>Eye cancer, pinealoma, osteosarcoma, melanomas, and soft-tissue sarcomas</td>
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<tr>
<td>RET</td>
<td>Multiple endocrine neoplasia type 2</td>
<td>Medullary thyroid cancer, and pheochromocytoma</td>
</tr>
<tr>
<td>VHL</td>
<td>Von Hippel-Lindau</td>
<td>Kidney cancers and multiple noncancerous tumors</td>
</tr>
<tr>
<td>STK11</td>
<td>Peutz-Jeghers</td>
<td>Cancers of the breast, colon and rectum, pancreas, and stomach and hamartomas</td>
</tr>
<tr>
<td>SDHD, SDHB, SDHC</td>
<td>Familial paraganglioma</td>
<td>Paragangliomas and pheochromocytomas</td>
</tr>
<tr>
<td>FLCN</td>
<td>Birt-Hoge-Dube</td>
<td>Chromophobe renal cell cancers</td>
</tr>
<tr>
<td>TSC1, TSC2</td>
<td>Tuberosis sclerosis</td>
<td>Angiofibromas, angiomylipomas, giant cell astrocytomas</td>
</tr>
<tr>
<td>NF1</td>
<td>Neurofibromatosis type 1</td>
<td>Optic gliomas and neurofibromas</td>
</tr>
<tr>
<td>NF2</td>
<td>Neurofibromatosis type 2</td>
<td>Schwannomas, meningiomas, gliomas, neurofibromas</td>
</tr>
<tr>
<td>PTCH1</td>
<td>Gorlin</td>
<td>Childhood primitive neuroectodermal tumors, skin basal cell carcinomas</td>
</tr>
<tr>
<td>BMPR1A, SMAD4</td>
<td>Juvenile polyposis</td>
<td>Multiple noncancerous growths in the colon</td>
</tr>
</tbody>
</table>
Prostate cancer example 1

- Metastatic castrate resistant prostate cancer at age 62
- Externally tested for inclusion in clinical trial
- **BRCA2** pathogenic variant c.5101C>T; p.Gln1701*

- Clinical genetic counselling >> germline test for **BRCA2**
- Germline pathogenic variant
- Family history negative for breast, ovarian and prostate cancer
- Cascade testing is indicated

Treatment options and consequences for his relatives
Prostate cancer example 2

- Metastatic castrate resistant prostate cancer (mCRPC) age 76
- Predictive NGS test: (hotspot) regions in 33 genes and MSI
- MSI-High (33 of 55 markers unstable); IHC MSH2-deficient
- PTEN likely pathogenic variant c.333G>T; p.Trp111Cys VAF 53%; 50% tumour cell

- Clinical genetic counselling >> no germline variants in MSH2, MSH6, PTEN
- MSH2-deficiency in mCRPC is often due to bi-allelic somatic mutations (not tested)
- Tumour with copy number neutral LOH of somatically mutated PTEN

Treatment options but no consequences for his relatives
Genetic susceptibility based on a tumour test?

• Frequency of somatic mutations – genetic syndrome
  • E.g. TP53, PTEN, CDKN2A mutations are often somatic but rarely germline

• Tumour type – gene combination
  • STK11 mutations common in lung cancer; Peutz Jegher syndrome is rare
  • POLE mutations common in endometrial; PPAP is rare

• Tumour type and clinical context
  • APC is commonly mutated in CRC:
    • APC associated with Familial Adenomatous Polyposis
    • Only consider genetic susceptibility in case of polyposis

• Mutational burden of a tumour
  • High mutational burden > high a priori risk of mutation being somatic
Extended gene panels in molecular pathology

- Introduction of 523 gene panel for single nucleotide variants (SNV), copy number variants (CNV); tumour mutational burden (TMB) and microsatellite instability (MSI)

- Definition of subpanels dependent on clinical request
  e.g.
  - Lung cancer: 14 genes
  - Lymphoma: 26 genes
  - Prostate cancer: 46 genes
  - Actionable target panel: 54 genes

Focus on genes that are relevant for the clinical problem
Reduce unnecessary interpretations of variants
Reduce secondary findings
Optimization of care pathway

- Pre-test information: test may give indication for genetic predisposition
- Define which (likely) pathogenic variants need follow up test in germline
  - Clinical relevance for patient and/or relatives
  - *A priori* risk of germline variant
- Define who initiates germline testing
- Priority level at clinical genetics

Provisional list for prostate cancer panel:

Always:  
- BRCA1; BRCA2; BRIP1; RAD51C; RAD51D; PALB2

Discuss in MTB:  
- ATM; CHECK2; MLH1; PMS2; MSH2; MSH6

No action:  
- TP53; PTEN and other genes
Molecular tumour board

- metastatic prostate cancer
- pathogenic mutation in $BRCA1$
- clinical trial options
- genetic counselling

medical oncologists; pulmonologists; pathologists; clinical geneticists; clinical molecular biologist/geneticist
Roles of pathologist

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Diagnostics of mosaic mutations

A 7 year old boy with Proteus syndrome
Somatic AKT1 p.E17K variant.

Essential to test affected tissue at high sensitivity
Involved genes also frequently mutated in tumours

https://en.wikipedia.org/wiki/Proteus_syndrome
<table>
<thead>
<tr>
<th>gene</th>
<th>Clinical manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AKT1</strong></td>
<td>Proteus syndroom</td>
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<tr>
<td><strong>AKT2</strong></td>
<td>Hypoinsulinemic hypoglycemia and hemihypertrophy</td>
</tr>
<tr>
<td><strong>AKT3</strong></td>
<td></td>
</tr>
<tr>
<td><strong>PIK3R2</strong></td>
<td>Megalencephalyepolymicrogyria-polydactyly-hydrocephalus syndrome</td>
</tr>
<tr>
<td><strong>MTOR</strong></td>
<td></td>
</tr>
<tr>
<td><strong>GNA11</strong></td>
<td>Diverse: e.g. hemangioma, Phacomatosis pigmentovascular</td>
</tr>
<tr>
<td><strong>GNA14</strong></td>
<td>Congenital en vascular tumours</td>
</tr>
<tr>
<td><strong>GNAQ</strong></td>
<td>Sturge Weber, congenital hemangioma</td>
</tr>
<tr>
<td><strong>IDH1</strong></td>
<td>Mafucci syndrome, haemangioma</td>
</tr>
<tr>
<td><strong>IDH2</strong></td>
<td>Mafucci syndrome, haemangioma</td>
</tr>
<tr>
<td><strong>KRAS</strong></td>
<td>Vascular tumours (granuloma pyogenicum)</td>
</tr>
<tr>
<td><strong>PIK3CA</strong></td>
<td>PIK3CA related overgrowth syndrome (PROS)</td>
</tr>
<tr>
<td><strong>PTEN</strong></td>
<td>PTEN Hamartooom Tumor syndroom (PHTS)</td>
</tr>
<tr>
<td><strong>TEK/TIE2</strong></td>
<td>veneuze malformaties en Blue Rubber Bleb Nevus (BRBN) Syndrome</td>
</tr>
<tr>
<td><strong>RASA1</strong></td>
<td>Parkes Weber, capillary malformation/arteriovenous malformation</td>
</tr>
</tbody>
</table>
Somatic mutations in vascular malformations

Ten Broek, Genes Chromosomes Cancer. 2019; PMID: 30677207
Conclusions

• Good practice to consider genetic predisposition based on individual criteria

• Most common tumour predisposition genes (or their signatures) are more widely tested in molecular pathology due to new treatment options

• Explicit informed consent is needed to test for germline mutations

• Workflow for follow-up on possible germline variants need to be defined in collaboration with clinicians and clinical geneticists

• Genetic predisposition enables surveillance options for patient and relatives
Thank you
Support clinical geneticists

Genetic variant classification

- segregation analysis in deceased relatives
- study of tumours for signatures and/or loss of heterozygosity

Differential diagnostics

- Identification of somatic mutations in tumours in specific genes may exclude a genetic syndrome
- Detection of genetic mosaics in presumed somatic overgrowth syndrome