Germline POLE and POLD1 variation in persons with colorectal cancer from the Colon Cancer Family Registry Cohort

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InSiGHT, 2019
Germline POLE and POLD1 in Colorectal Cancers (CRCs)

• Germline mutations in POLE and POLD1 are associated with CRCs

• Recurring germline exonuclease domain mutations (EDMs):
  • POLE: p.Leu424Val
  • POLD1: p.Leu474Pro and p.Ser478Asn

• EDMs result in defective proofreading function
  • structural proximity to DNA binding/active sites likely perturbs function
  • resulting in characteristic hypermutator phenotype
  • also shown by functional assays in yeast and T4 bacteriophage

Palles et.al. (2013), Valle et.al. (2014) and Bellido et.al. (2016), Kokoska RJ et.al. (2000)
Aims

• Identify new germline mutations in POLE and POLD1 using a large cohort of persons with CRC

• Incorporate clinico-pathological and tumour molecular features to classify new POLE and POLD1 pathogenic variants
### Study cohort: Colon Cancer Family Registry (CCFR)

<table>
<thead>
<tr>
<th>Australian cohort</th>
<th>Ontario cohort</th>
<th>Seattle cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases: recruited as population based probands diagnosed with CRC</td>
<td></td>
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</tr>
<tr>
<td>Incident CRC dx between 18-59 yrs (independent of FHx)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incident CRC dx between 20-74 yrs (weighted to FHx)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incident CRC dx between 18-74 yrs (independent of FHx)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls: recruited by random sampling from population without prior CRC</td>
<td></td>
<td></td>
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<tr>
<td>Cases = 861 Controls = 247</td>
<td></td>
<td></td>
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<tr>
<td>Cases = 633 Controls = 575</td>
<td></td>
<td></td>
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<tr>
<td>Cases = 459 Controls = 385</td>
<td></td>
<td></td>
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<tr>
<td>Cases = 1953 Controls = 1207</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results: germline POLE predicted pathogenic variants

<table>
<thead>
<tr>
<th>Cases (n=1953)</th>
<th>POLE</th>
<th>POLE_exo</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 (1.09%)</td>
<td>4 (0.21%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Controls (n=1207)</th>
<th>POLE</th>
<th>POLE_exo</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (0.84%)</td>
<td>0 (0%) OR 1.3 [0.61-2.75] P=5.8E-01</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 (0%) OR 2.27 [0.84-6.16] p=1.1E-01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>gnomAD (n=123134)</th>
<th>POLE</th>
<th>POLE_exo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1168 (0.96%)</td>
<td>111 (0.1%) OR 2.27 [0.84-6.16] p=1.1E-01</td>
<td></td>
</tr>
</tbody>
</table>
Results: germline POLD1 predicted pathogenic variants

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=1953)</th>
<th>Controls (n=1207)</th>
<th>gnomAD (n=123134)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19 (0.98%)</td>
<td>5 (0.42%)</td>
<td>438 (0.36%)</td>
</tr>
<tr>
<td>OR 2.35 [0.88-6.27]</td>
<td></td>
<td>OR 2.47 [0.53-11.62]</td>
<td>OR 2.73 [1.73-4.32]</td>
</tr>
<tr>
<td>P=9.3E-02</td>
<td></td>
<td>p=3.4E-01</td>
<td>P=1.4E-04</td>
</tr>
<tr>
<td></td>
<td>8 (0.41%)</td>
<td>2 (0.17%)</td>
<td>84 (0.07%)</td>
</tr>
<tr>
<td>OR 2.47 [0.53-11.62]</td>
<td></td>
<td>OR 6.0 [2.91-12.38]</td>
<td>OR 6.0 [2.91-12.38]</td>
</tr>
<tr>
<td>p=3.4E-01</td>
<td></td>
<td>p=7.6E-05</td>
<td>p=7.6E-05</td>
</tr>
</tbody>
</table>
Characterising POLE exonuclease domain mutations (EDMs)

Use matching tumour molecular features to characterise POLE EDMs

POLE:p.Lys280Asn
POLE:p.Asp301Gly
POLE:p.Lys398Arg
Results: Somatic mutational signature analysis

[Somatic mutational signatures per sample]

POLE:p.Asp301Gly - Sig.10 POLE mutations 67%

POLE:p.Lys280Asn - Sig.1 age related 65%

POLE:p.Lys398Arg - Sig.4 tobacco mutagens 18%

MMR_d_1 - Sig.1 age related 20%

MMR_d_2 - Sig.1 age related 25%

MMR_p_1 - Sig.1 age related 65%

Sig.15 18%

Sig.18 10%

Sig.18 66%

Sig.18 5%

Sig.15 12%

Sig.15 21%

Sig.17 15%

Sig.6 defective MMR 52%

Sig.6 defective MMR 65%

[TCG→T] and [TC→A] mutations are enriched in tumours with POLE EDMs
Results: Tumour mutation burden analysis

Mutation burden (per Mb)

- POLE:p.Asp301Gly: 206.36
- POLE:p.Lys280Asn: 213.41
- MMRd: 70.26

**Ultra-hypermutators**
- >100 mut/Mb*

**Hypermutator**
- >10 mut/Mb*

*Campbell BB. et.al. Cell (2017)
<table>
<thead>
<tr>
<th>POLE EDMs</th>
<th>In silico prediction (CADD, REVEL)</th>
<th>gnomAD</th>
<th>Predicted structure stability (DynaMut)* yeast POL2 structure 4m8o</th>
<th>Mutational Signature.10</th>
<th>Mutation burden (mut/Mb)</th>
<th>ClinVar classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.Asp301Gly</td>
<td>31.0, 0.7</td>
<td>8.12E-06 (1 in 100k)</td>
<td>destabilising</td>
<td>High</td>
<td>Ultra-hypermutator</td>
<td>VUS</td>
</tr>
<tr>
<td>p.Lys280Asn</td>
<td>27.7, 0.28</td>
<td>ultra-rare</td>
<td>destabilising</td>
<td>Low</td>
<td>Low mutation burden</td>
<td>VUS</td>
</tr>
<tr>
<td>p.Lys398Arg</td>
<td>17.8, 0.15</td>
<td>ultra-rare</td>
<td>stabilising</td>
<td>Low</td>
<td>Ultra-hypermutator</td>
<td>VUS</td>
</tr>
</tbody>
</table>

*DynaMut, Rodrigues CHM et.al. NAR (2018)
Conclusions and Future directions

• Analysing matching tumour molecular features can help characterise EDMs

• Future directions for EDM characterisation
  • incorporate structural and functional data
  • perform segregation analysis for variant characterisation

predicted pathogenic variants
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Prof. Laney Lindor

Australasian Colorectal Cancer Family Registry
Colon Cancer Family Registry Cohort
Genetics of Colonic Polyposis Study
Structural analysis

Lys280Asn
Lys398Arg
Asp301Gly
Leu424Val

predicted pathogenic variants
Somatic POLE and POLD1 mutations (COSMIC)