Molecular pathological epidemiology of lifestyle factors and colorectal and renal cell cancer risk

Piet van den Brandt
Epidemiology
Maastricht University Medical Center
Maastricht, the Netherlands

Genetic model of CRC (Fearon, AnnRevPath 2011)

Netherlands Cohort Study (NLCS) on diet and cancer
Maastricht University & TNO

A Cohort:
- n = 120,852 (58,279 men and 62,573 women)
- Age range: 55-69 years
- Source: 204 municipal population registers

A Baseline exposure measurement (Sep 1986):
- Self-administered questionnaire (120,852)
- Toenail clippings (90,000)

A Follow-up for cancer incidence:
- National and regional cancer registries
- National database of pathology records PALGA
- Completeness of follow-up: 95%

(van den Brandt et al., JCE 1990)
Molecular subtyping of colorectal cancer in NLCS

- Tumor material (FFPE) from incident CRC patients in first 7.3 yrs FU
  - Obtained from 54 pathology labs throughout the Netherlands
  - 815 available tissue blocks
- 734 (90%) sufficient tumor material for mol. analyses (Pathology)

- Mutation analyses:
  - KRAS oncogene: mutation cluster region in codons 12-13
  - APC tumor suppressor gene: MCR in codons 1286-1520

- TP53 expression analyses:
  - IHC staining
  - Evaluated semiquantitatively by 2 independent observers

- Results overall:
  - KRAS: 33% activating mutation
  - APC: 36% truncating mutation
  - TP53: 56% overexpressed

Polyunsaturated fatty acid (PUFA) intake and K-ras mutations in colon cancer, NLCS (Brink et al, Carcinogenesis 2004)

Meat and CRC: Catalytic effects of heme on NOC formation and CRC (Bastide et al, 2011)

Heme iron intake and mutations in KRAS or APC in colorectal cancer, NLCS 7.3 yrs FU (Gisling et al, Carcinogenesis 2013)
Summary on heme iron intake and CRC

- Positive association with risk of CRC, particularly colon cancer
- Further molecular epidemiology: Heme iron intake associated with:
  - Activating mutations in KRAS oncogene
  - CRC without truncating mutations in APC
  - PI3K overexpression
  - Dose-dependent association with:
    - G>A activating transitions in KRAS
    - Overall G>A mutations in APC
  - G>A transitions characteristic of alkylating agents such as NOC
  - Endogenous NOC-formation catalyzed by heme

Prospective cohort studies applying MPE on CRC and lifestyle, with (epi)genetic tumor characteristics from FFPE tissue blocks

<table>
<thead>
<tr>
<th>Cohort study</th>
<th>Country</th>
<th>Size</th>
<th>APC</th>
<th>BRAF</th>
<th>MSI</th>
<th>CIMP</th>
<th>TP53</th>
<th>MSH2/6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health Professionals Follow-up Study (HPFS)</td>
<td>USA</td>
<td>45807</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Nurses Health Study (NHS)</td>
<td>USA</td>
<td>88397</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Israel Women's Health Study (IWHS)</td>
<td>USA</td>
<td>41836</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Netherlands Cohort Study (NLCS)</td>
<td>Netherlands</td>
<td>120852</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>EPIC Norfolk</td>
<td>UK</td>
<td>30441</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Malmö Diet and Cancer Study (MDCS)</td>
<td>Sweden</td>
<td>29038</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Melbourne Collaborative Cohort Study (MCCS)</td>
<td>Australia</td>
<td>41328</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

**Ever vs never smoking and risk of CRC, by KRAS mutation status**

(data from Hughes et al, 2017)

<table>
<thead>
<tr>
<th>Author &amp; Year</th>
<th>Cohort</th>
<th>HR (95%CI), per subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weijenberg 2008</td>
<td>NLCS</td>
<td>2.25 (1.42-3.53)</td>
</tr>
<tr>
<td>Samadder 2012</td>
<td>NLCS</td>
<td>1.80 (1.24-2.60)</td>
</tr>
<tr>
<td>Sargent 2012</td>
<td>NLCS</td>
<td>2.30 (1.60-3.30)</td>
</tr>
<tr>
<td>Weijenberg 2008</td>
<td>NLCS</td>
<td>2.25 (1.42-3.53)</td>
</tr>
<tr>
<td>Sargent 2012</td>
<td>NLCS</td>
<td>1.80 (1.24-2.60)</td>
</tr>
<tr>
<td>GAMA</td>
<td>MLHS</td>
<td>2.30 (1.60-3.30)</td>
</tr>
</tbody>
</table>

**Smoking (40+ packyr vs never) and risk of CRC molecular subtypes (serrated neoplasia pathway)**

<table>
<thead>
<tr>
<th>Author &amp; Year</th>
<th>Cohort</th>
<th>HR (95%CI), per subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCM</td>
<td>MDA90</td>
<td>2.30 (1.60-3.30)</td>
</tr>
<tr>
<td>BCM</td>
<td>MDA90</td>
<td>2.30 (1.60-3.30)</td>
</tr>
<tr>
<td>BCM</td>
<td>MDA90</td>
<td>2.30 (1.60-3.30)</td>
</tr>
<tr>
<td>BCM</td>
<td>MDA90</td>
<td>2.30 (1.60-3.30)</td>
</tr>
</tbody>
</table>
Body size and risk for colorectal cancers showing BRAF mutations or microsatellite instability: a pooled analysis

Lauret AE Hughes,1 Elizabeth J Wilkinson,1 Marcel van Engeland,1 Mark A Jenkins,2 Graham G Giles,3 John I Hopper,3 Melissa C Venturesi,3 Joanne P Young,3 Daniel D Buchman3, Michael D Wald6, Piet A van den Brandt,4 R Alexandra Goldhahn,4 Man-yi P Wurpelberg6, and Donald R English7

1Department of Epidemiology, QIMR Berghofer Medical Research Institute, Brisbane, Australia
2School of Public Health and Preventive Medicine, University of Melbourne, Melbourne, Victoria, Australia
3Department of Epidemiology, QIMR Berghofer Medical Research Institute, Brisbane, Australia
4Cancer Epidemiology Core, The Cancer Council Victoria, Carlton, Victoria, Australia
5Vanderbilt Epidemiology Laboratory, Department of Preventive Medicine, University of Medicine, Nashville, Tennessee, USA
6Department of Epidemiology, Radcliffe Institute for Infectious Diseases, University of Oxford, Oxford, UK
7Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands

*Corresponding author. Department of Epidemiology, Maastricht University, PO Box 616, 6200 MD, The Netherlands.
E-mail: ep.d.r.english@erasmusmc.nl

Int J Epidemiol 2012

BRAF V600E mutation and MSI status in CRC in NLCS and MCCS (Hughes et al, IJE 2012)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NLCS</th>
<th>MCCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>% men</td>
<td>48</td>
<td>41</td>
</tr>
<tr>
<td>No. of CRC tumors</td>
<td>734</td>
<td>717</td>
</tr>
<tr>
<td>BRAF mutation, %</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>BRAF wild type</td>
<td>84</td>
<td>86</td>
</tr>
<tr>
<td>MSI-high, %</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>MSI-stable</td>
<td>87</td>
<td>85</td>
</tr>
</tbody>
</table>

Body mass index, height and BRAF mutation status in CRC in NLCS & MCCS (Hughes et al, Int J Epi 2012)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BRAF status</th>
<th>P-value (interaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td>Mutation</td>
</tr>
<tr>
<td>BMI, per 5 kg/m²</td>
<td>1.16 (1.08-1.26)</td>
<td>1.05 (0.88-1.25)</td>
</tr>
<tr>
<td>Height, per 5 cm</td>
<td>1.08 (1.03-1.13)</td>
<td>1.23 (1.11-1.37)</td>
</tr>
</tbody>
</table>

Body height and BRAF mutation status in CRC in NLCS & MCCS (Hughes et al, Int J Epi 2012)

<table>
<thead>
<tr>
<th>Quartile of height</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4 (high)</td>
<td>2.5</td>
</tr>
</tbody>
</table>

P-trend = 0.04

P-trend = 0.01
Body height and MSI status in CRC in NLCS & MCCS (Hughes et al., Int J Epi 2012)

- RR
- Quartile of height
- MS Islable
- MSI-high

Body height and MSI status in CRC in NLCS & MCCS (Hughes et al., Int J Epi 2012)

Molecular subtyping of clear-cell renal cell carcinoma (ccRCC) in NLCS

- Tumor material (FFPE) from incident ccRCC patients in first 11.3 yrs FU
- Obtained from 54 pathology labs throughout the Netherlands
- Initial analyses focussed on: von Hippel-Lindau gene (VHL)
- Mutations in VHL distinct and early event in ccRCC development
- VHL Mutated in 61% of sporadic ccRCC in NLCS (BMC Cancer 2005)

Some results on MPE of clear-cell renal cell carcinoma in the NLCS, 1986-1997: according to mutations in the von Hippel-Lindau gene (VHL)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>VHL mut ccRCC (n=14)</th>
<th>VHL wt ccRCC (n=73)</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence VHL mut</td>
<td>61%</td>
<td></td>
<td>BMC Cancer 2005</td>
</tr>
<tr>
<td>BMl per 1 kg/m2</td>
<td>1.09 (1.02-1.16)</td>
<td>1.08 (1.03-1.15)</td>
<td>Ann Epi 2010</td>
</tr>
<tr>
<td>BMI (age 20)</td>
<td>1.09 (1.03-1.16)</td>
<td>0.95 (0.85-1.06)</td>
<td></td>
</tr>
<tr>
<td>HX of Hypertension</td>
<td>1.34 (0.87-2.07)</td>
<td>0.88 (0.51-1.53)</td>
<td>J Hypert 2005</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>1.20 (0.73-1.96)</td>
<td>1.58 (0.64-2.94)</td>
<td>Br J Ca 2006</td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.17 (0.69-1.99)</td>
<td>2.06 (1.07-3.94)</td>
<td></td>
</tr>
<tr>
<td>Alcohol, per 10 g /day</td>
<td>0.99 (0.86-1.14)</td>
<td>0.83 (0.66-1.05)</td>
<td>CEBP 2008</td>
</tr>
</tbody>
</table>

- RR2.5
  - VHL wt
  - Overall
  - VHL mut

0.5 1 1.5 2 2.5

Never Ex Current smk

Tobacco smoking

Alcohol consumption and VHL mutations in clear-cell renal cell cancer, NLCS 1986-1997 (Schouten et al, CEBP 2008)

- RR2.5
  - VHL mut
  - Overall
  - VHL wt

0 0.5 1 1.5 2

0 g/d 0.1-4.9 5-14.9 15+ g/day

Alcohol intake

Rainbow-TMA project
Rainbow-TMA project (BBMRI-NL RP7): enriching cohorts with tumor tissue microarrays and DNA

Infrastructure project, 2012-2017
- Tumors: breast, esophagus, stomach, pancreas, CRC, ovary, bladder
- Funding by BBMRI-NL
- Partners: UMCs of Maastricht, Utrecht, Nijmegen, Rotterdam, Amsterdam & PALGA
- Collection of tumor blocks from pathology labs nationwide, and TMA production
- Principal Investigator: Piet van den Brandt

**Cohorts (size, FU period, selected cancer cases):**
- Netherlands Cohort Study (NLCS) (n=120852; 8867 cancer cases) FU: 1986-
- EPIC Morgen + Prospect (n=40126, 1815 cases) FU: 1993-
- Rotterdam Study (ERGO) (n=14926; 885 cases) FU: 1989-
- Netherlands Twin Register NTR (n=9530; 49 cases) FU: 1996-
- Polygene (n=7000; 1008 cases)

**Rainbow-TMA: Selected records, retrieval and TMA/DNA results per tumor**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Selected (patients)</th>
<th>Retrieval tumor blocks (ptt)</th>
<th>TMA/DNA available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophagus</td>
<td>136</td>
<td>83</td>
<td>78</td>
</tr>
<tr>
<td>Stomach</td>
<td>183</td>
<td>107</td>
<td>91</td>
</tr>
<tr>
<td>CRC</td>
<td>4721</td>
<td>3579</td>
<td>3205</td>
</tr>
<tr>
<td>Pancreas</td>
<td>155</td>
<td>71</td>
<td>64</td>
</tr>
<tr>
<td>Breast</td>
<td>5335</td>
<td>3867</td>
<td>3556</td>
</tr>
<tr>
<td>Ovarium</td>
<td>765</td>
<td>611</td>
<td>581</td>
</tr>
<tr>
<td>Bladder</td>
<td>1429</td>
<td>1032</td>
<td>901 (316 TMA)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>13624</strong></td>
<td><strong>9350</strong></td>
<td><strong>8436</strong></td>
</tr>
</tbody>
</table>

**Hallmarks of Cancer** (Hanahan & Weinberg, Cell 2011)

- 1. Adapting to hypoxia
- 2. Partial resistance to apoptosis
- 3. Evading growth suppressors
- 4. Subverting of cellular senescence
- 5. Inducing inflammation
- 6. Activating oncogenes
- 7. Enabling武陵发酵
- 8. Hallmarks of Cancer: **Warburg effect**

**Warburg effect:** energy production by increased aerobic glycolysis, followed by lactate fermentation

<table>
<thead>
<tr>
<th>Glucose</th>
<th>$\rightarrow$ $\text{Pyruvate}$</th>
<th>$\rightarrow$ $\text{Lactate}$</th>
<th>$\rightarrow$ $\text{CO}_2$</th>
<th>$\rightarrow$ $\text{ATP}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\rightarrow$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Glycolysis

A. Normal cell

B. Tumor cell: shift to aerobic glycolysis driven by multiple oncogenic pathways

Lactate: a key player in tumorigenesis? (Carcinogenesis 2017)

Intrinsic and extrinsic influences on cancer cell metabolic reprogramming (Cell 2017)
Acknowledgements

Acknowledgements NLCS

Maastricht University & TNO

Sandra Goldbohm
Peter van der Vier
Ferd Sturman
Rudi Herman
Leo Volovics
Elisabeth Dorant
Leo Schouten
Jaanne van Loon
Maurice Zwijgers
Matty Wijnberg
Monique Mommers
Harry Brandt
Harry van Montfort
Sacha van der Crommert
Jolinde Nelissen
Patricia Florax
Connie de Zwart
Manja Moll
Armenie Polaters
Jacqueline Spronck
Arned Kester
Tom van Moergastel
Ron Ahls
Willy van Dijk
Gregor Fransen
Desiree de Cappellen
Rudolf Schmelz
Ralph Meijer
Linda van den Bosch
Miran Drin
Margred Lüchtenborg
Bouke van Dijk
Brenda Borgschaft
Stefan de Vogel
Laure Hughes
Colinda Simons
Jannetje Hogervorst
Kim Snels
Fret Drop
Ton de Goeij
Heike Grabsch
Manon van Engeland
Axel zur Hausen
Alex van der Saaghen
Eloan de Haan
Marc de Bruijn
Manuela Baldeweg
Kim Wouters
Peter Moerkerk
Edith van der Bossema
Jaekes van der Meier
Kim van Straten
Piet van der Brandt
and pathologists from 43 donor laboratories

Acknowledgements Rainbow-TMA

Manon van Engeland
Heike Grabsch
Leop Schouten
Jan BeckersvonderSandforth
Mayaas Bendek
Ron Ahls
Harry van Montfort
Peter Moerkerk
Edith van der Bossema
Jaekes van der Meier
Kim Wouters
Kim van Straten
Piet van der Brandt
Paul van Dijst
Petra Peeters
Bas Bueno de Mesquita
Domenico Castiglione
Petra van der Weide
S. Dubois A. Oud
Har van Krieken
Iris Nagtegaal
Bart Kromeyer
Michiel Smitt
Bert Siebers
Jaleesa van der Meer
Maailie Hermse
Dagmar Verweij
Folkert van Kemenade
Bruno Stricker
Lisa H de Vogel
Gerit Meijer
Dornit Boomstra
Charitale Strengers
Nathalie Hilmering
Arne Hofman
Lucy Ovebaek
Annette Gijbers

and pathologists from 43 donor laboratories