Familial breast and ovarian cancer in SA: research to diagnostic service

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What is familial breast cancer?
5 - 10% inherited susceptibility
- genetic studies - identification and localization of two familial breast cancer genes -
  - **BRCA1** on chromosome 17q (1994)
    (BR = breast; CA = cancer)
  - **BRCA2** on chromosome 13q (1995)
- genes are **tumor suppressor** genes
Chromosome 17

BRCA1 gene
AATAATAGGATTGCATGG
(tumor suppressor)

genetic mutation
no alteration

Altered BRCA1 protein unable to suppress tumor formation

BRCA1 protein suppress uncoordinated rapid cell growth
Autosomal dominant inheritance:

- 50% chance = you only need 1!
Appropriate risk at age 70

(British Journal of Surgery 2000, 87, 149-162)
Selection of families

- Families were selected based on:
  - The number of affecteds within the family
  - The age of onset of the disease
  - Bilaterality of the disease
  - Cancer types present
Methods

- EDTA or ACD blood samples
- DNA isolated, using the lymphocytes
- Four laboratory techniques:
  - linkage and haplotype analysis
  - protein truncation test (PTT)
  - single strand conformational analysis (SSCP)
  - DNA sequencing
Linkage & haplotype analysis
FAMILY 2:
BRCA1 results
DNA sequencing
=
change in genetic alphabet

manual sequencing

automated sequencing
BRCA1 founder mutations unique to South Africa: Genealogical identification of founding couples

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INTRODUCTION

- Majority of inherited BC & OV – result of mutations in BRCA1 gene
- Germline BRCA1 mutations responsible for 85% of all familial breast and/or ovarian cancers
- BRCA1 frequency varies from 1 in 833 in the general population of some countries to 1% in Ashkenazi Jewish general population
- Risk of developing by age 80 is 74% for BC and 28% for OV
- Recurrent mutations in certain populations/ethnic groups – result of founder effects
- Two recurrent BRCA1 mutations in SA (E881X & 1493delC) – preliminary genotype analyses indicated founder effects
AIM

- No information regarding genealogical origin of novel mutations was available
- We aimed to investigate the historical origin of these mutations specific to South African Afrikaner
- Since all the families representing each of the two mutations shared a common haplotype, we wanted to identify founding couples
METHODS:

Patients and families

- Study approved by appropriate ethics committees
- Informed consent obtained prior to enrolment
- For the purposes of study, Afrikaner patients were defined as individuals with maternal and paternal Afrikaans-speaking grandparents
- Of 143 Afrikaner families screened, 7 tested positive for 1493delC, whereas E881X was present in 18
Families representing mutations were genotyped to determine whether mutations were independent events or due to a common ancestor. Genotype analysis was carried out using a set of four BRCA1 markers (D17S1320, D17S855, D17S1322 & D17S1323). Forward primers were end-labelled with $^{32}$P. Denatured samples were loaded onto 6% denaturing polyacrylamide gels, together with a sequencing ladder.
METHODS:

Genealogical analysis

- Mutation positive families where genealogical evidence from at least three to four generations was available, were used.
- 10 families representing **E881X** & 5 families exhibiting **BRCA1 1493delC** met criteria.
- Information was obtained from death notices, various books on Afrikaner genealogy & personal communications with South African genealogists.
- CYRILLIC 2R computer program was used to compile family trees & to store all relevant data.
RESULTS: Genotype analysis

- The results of the haplotype analysis indicated that 10 Afrikaner families with E881X and 5 families with 1493delC each shared a common genotype (Reeves et al. 2004)
- This indicated a single mutational event for each of the two Afrikaner mutations
- Implied that the families representing each of the mutations have a common ancestor
RESULTS: Genealogical search

- Based on results of the genotype data, an attempt was made to identify the identity of founding individuals.
- Both paternal & maternal lines were historically traced, most as far back as the first individuals who arrived at the Cape of Good Hope during the late 1600’s.
- South African method of notation is used, with the first (original) settler to the Cape of Good Hope indicated as ‘a’ and all the subsequent generations after him, indicated by the following letter of the alphabet (b, c etc.).
- Children are numbered consecutively from 1 for the first born onwards to the last born.
Initially, several *interfamilial relationships* were established between pairs of families, for example BRC120 and BRC143 (1493delC) and OV1 and BRC10 (E881X) (Fig. 1). To some extent *complicated the study*, as it steered initial analysis in wrong direction.
RESULTS: Genealogy for E881X

- Genealogical & historical data indicated a distinct surname (des Prez/du Preez) common within nine of the E881X families
- A single founding couple (Hercules des Prez X Cecilia d’Athis) was identified
- They are linked to the nine families via four of their six children
- Only **one E881X family** (FS29) could not be linked to founding couple, possibly due to an incomplete branch in family tree
- Parents of V:4 (indicated in green) could not be accurately traced
For 1493delC, four of five families were initially linked to a single couple, namely Petrus Louw (III:2) married to Beatrix Olivier (Ill:1) though three of their children

After an extensive search, the fifth family (BRC27) remained unlinked to this specific couple

The connection of BRC27 to the other four proved to be one generation earlier, for II:1 and II:3 were brothers (b1c1 and b1c4). Based on these results, the founding couple is proposed to be Pieter Louw (I:1), married to Elisabeth Wendels (I:2).
BRCA1 1493delC
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BRCA11493delC

I:1 Pieter Louw b1 1667-1713
I:2 Elisabeth Wendels 1671-1719
II:1 Johannes Louw b1c1 1694-1770
II:2 M Gildenhuizen Jacobus Louw b1c4 1700-1778
III:1 Beatrix Olivier
III:2 Petrus Louw
IV:1 AAVN
IV:2 MMC
IV:3 JAC
IV:4 SSDK
IV:5 JDC
IV:6 BMDT
IV:7 ECL
IV:8 ACDT
V:1 JL
V:2 JAC
V:3 MMC
V:4 SSDK
V:5 JDC
V:6 BMDT
V:7 ECL
V:8 ACDT
V:9 JL
V:10 A-MH
V:11 GMSV
V:12 JPAL
VI:1 MCPH
VI:2 PJVN
VI:3 MSDK
VI:4 JAC
VI:5 AAS
VI:6 JDC
VI:7 JEJVH
VI:8 JDC
VI:9 MEB
VI:10 JJD
VI:11 GMSV
VI:12 JPAL
VII:1 DFDT
VII:2 MMVN
VII:3 PJC
VII:4 JCB
VII:5 LDC
VII:6 EACC
VII:7 RALR
VII:8 JSDD
VII:9 AJM
VII:10 JPL
VII:11 GMSV
VII:12 JPAL
VIII:1 FS7
VIII:2 BRC143
VIII:3 BRC120
VIII:4 FS14
VIII:5 BRC27
VIII:6 BRC27
VIII:7 BRC27
VIII:8 BRC27
VIII:9 BRC27
VIII:10 BRC27
IX:1 FS7
IX:2 BRC143
IX:3 BRC120
IX:4 FS14
IX:5 BRC27
DISCUSSION

du Preez family (E881X)

- Hercules des Prez (c1645-1695) born in France - founder of du Preez family in SA
- Was married to Cecilia d'Athis (c1650-1720) – fled from France to Holland, after the Edict of Nantes was revoked in 1685
- With war looming in Europe, du Preez family departed February 1688 to Cape of Good Hope – voyage took three months
- The family arrived as paupers with very few possessions, amongst which was their valued French Bible
- Hercules died just seven years after their arrival
BRCA1 E881X
**Louw family (1493delC)**

- **Founding couple** is Pieter Louw, who was married to Elizabeth Wendels - his father was founder of Louw family in SA
- Couple had 5 children (3 sons & 2 daughters), of which only two sons married and had offspring – 1 daughter died at the age of 17, whereas the other two died in their mid twenties
- **b1 Pieter** (1667-1713) married Elizabeth Wendels (1671-1718) - for his support of Governor Willem Adriaan van der Stel, he was rewarded the farm Doornekraal - gift caused a split in the family - his younger brother was put in jail by Van der Stel during this time due to Jacobus’ friendship with his brother in law, Adam Tas (an enemy of the Governor) - Pieter (b1) & his only remaining sibling, Jacobus (b5) died in May-July 1713 due to the pox epidemic, without reconciling with each other – **had 10 children of which only 2 can be linked to this mutation**
  - **b1c1 Johannes** (1694-1770), married Margaretha Gildenhuyzen - had 7 children (link to FS7, BRC143, BRC120 & FS14)
  - **b1c4 Jacobus** (1700-1778), married Wilhelmina van Zijl - had 5 children (link to BRC27).
BRCA1 1493delC
CONCLUSION

- Genotype & genealogical data proved that two novel BRCA1 mutations, E881X and 1493delC, unique to the SA Afrikaner population, are **founder mutations that are more than 300 year old**.
- Since mutations **have not been described** for any European populations such as France, the Netherlands and Belgium, we postulate that either of the following:
  - both mutations were **de novo occurrences** in one of the founder individuals, that resulted in the mutation being carried from generation to generation in the Afrikaner population.
  - the particular mutations **are extremely rare in the European populations** where the forefathers were originally from - have not yet been detected.
  - or both these mutations **were initially present** in the European populations, but have become extinct.
Founder effects established for several heritable disorders:

- porphyria variegata
  - Gerrit Jansz from Holland, married to Adriaantje – an orphan from Rotterdam
- keratolytic winter erythema
- hypercholesterolemia
- progressive familial heart block
- Fanconi anaemia
  - Guillaume Nel married to Jeanne de la Batt
Genealogical and historical evidence was obtained with the assistance of various genealogists working on Afrikaner families. These include:

- K & M Venter
- C Jooste
- AC Fuchs (du Toit family)
- MH de Klerk (Mouton family)
- H Louw (Chair of the Louw Family Confederation of SA)
- B Cilliers and M Olivier (Cilliers family)
- PD Bosman (Verster family)
- MCH du Preez (du Preez family)
- G du Preez (du Preez family)
- J Mellville (van der Merwe family)

They are all in the process to publish their manuscripts and we would like to thank each of them for their willingness to share their data for academic purposes.