Familial breast and ovarian cancer in SA:

research to diagnostic service

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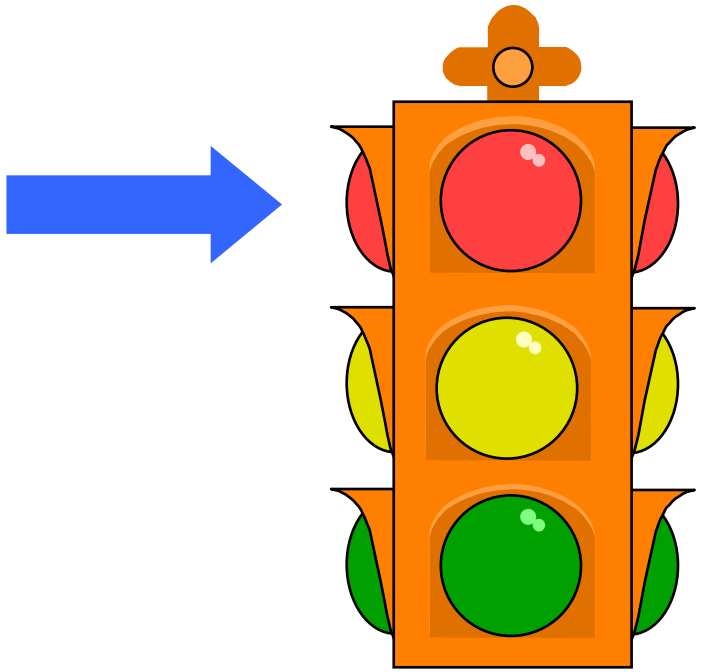
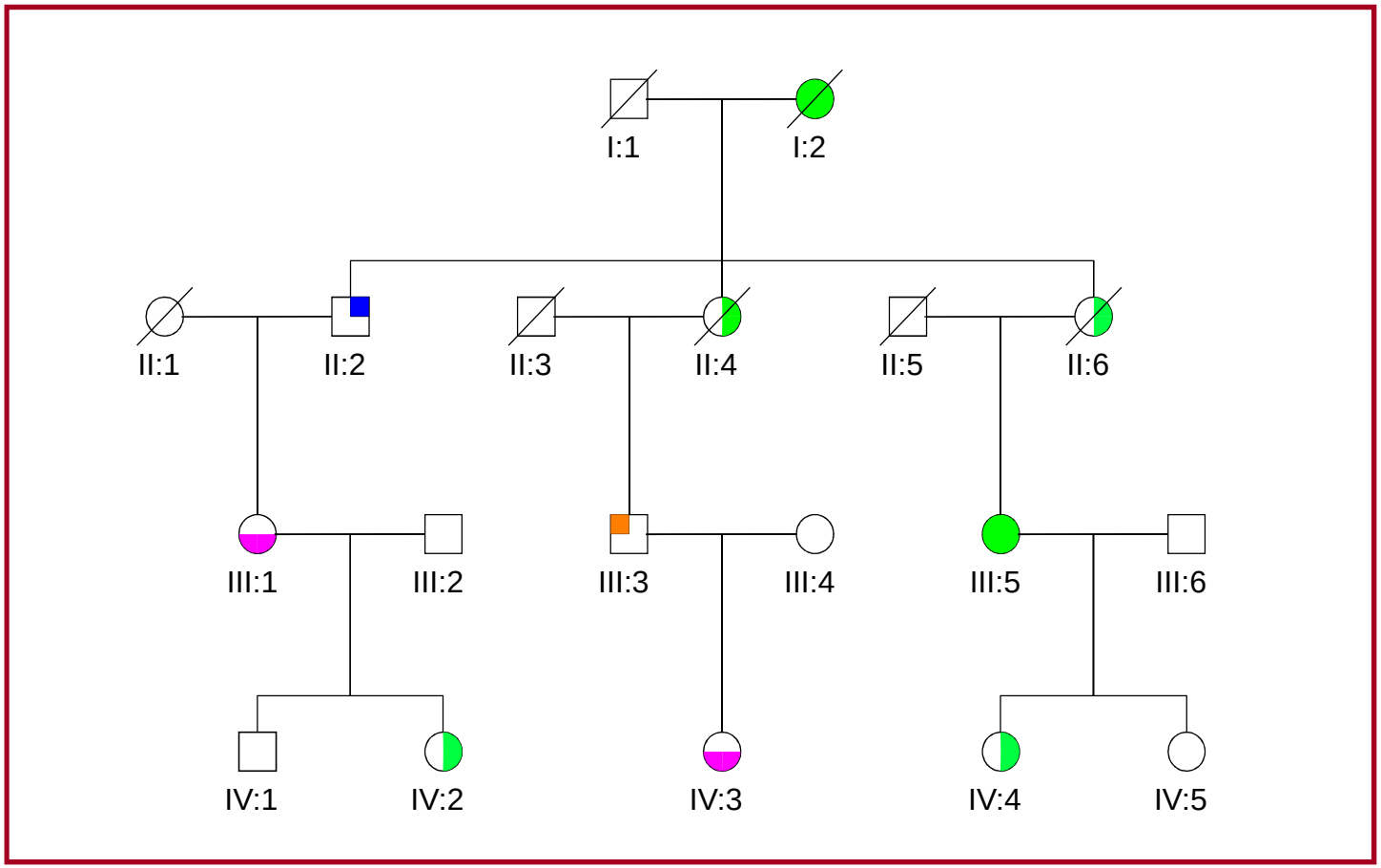
What is familial breast cancer?



= STOP
AND THINK

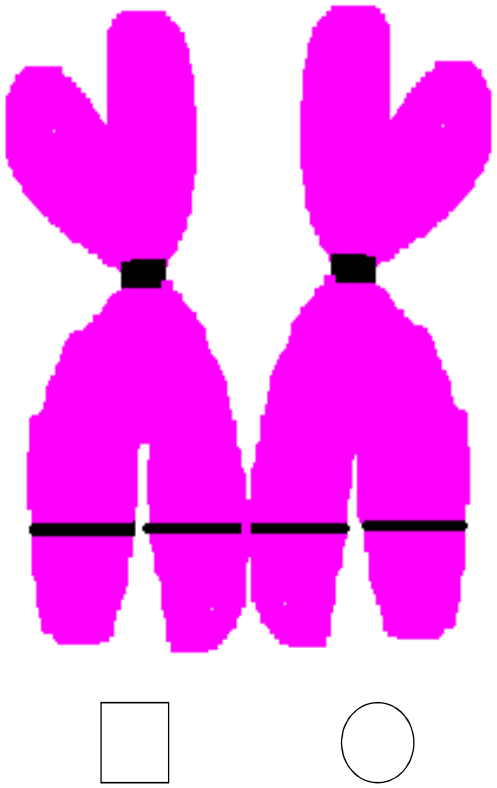


- **5 - 10% inherited susceptibility**

- 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

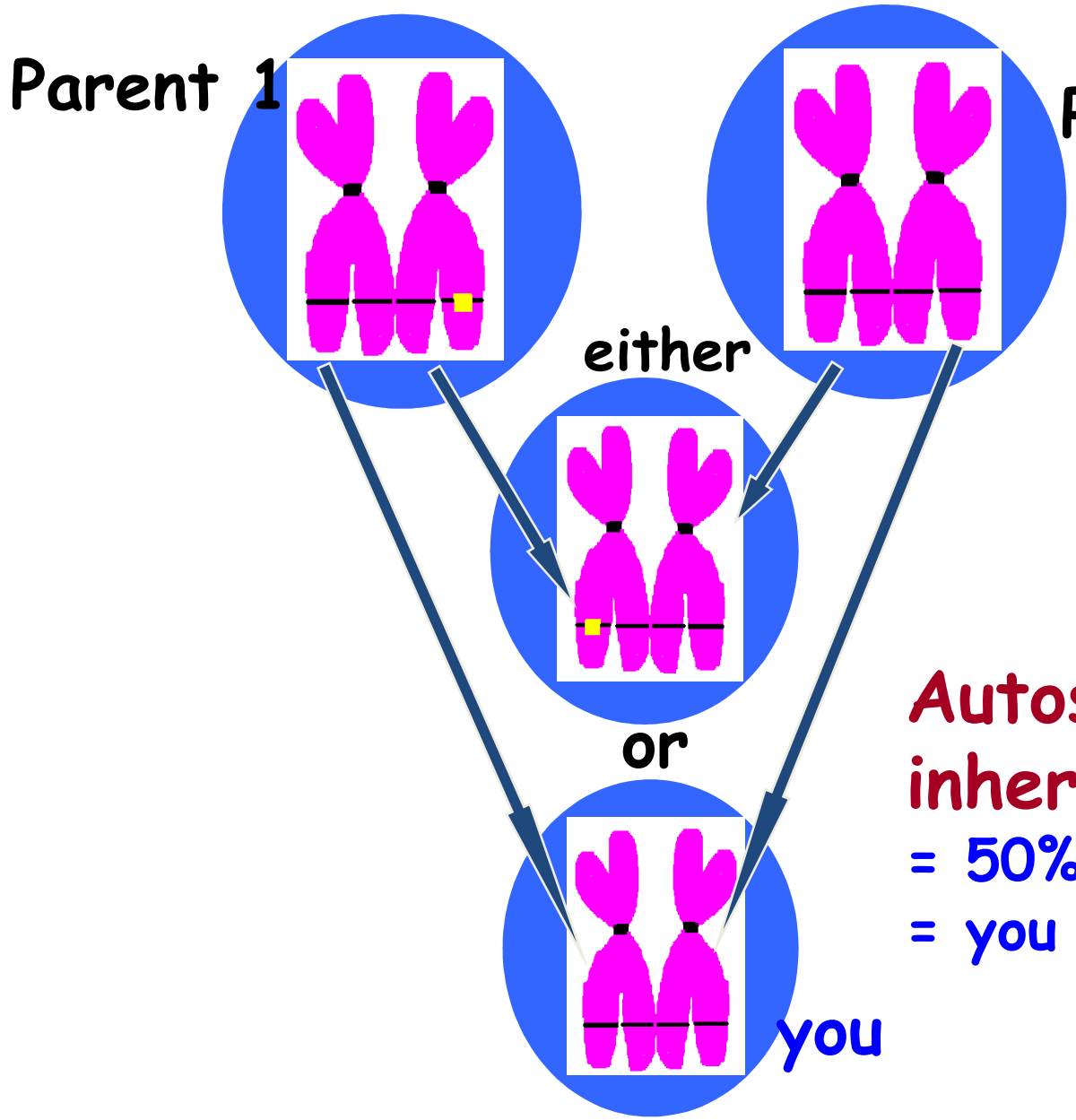
(tumor suppressor)

genetic mutation

no alteration

Altered BRCA1 protein unable to suppress tumor formation

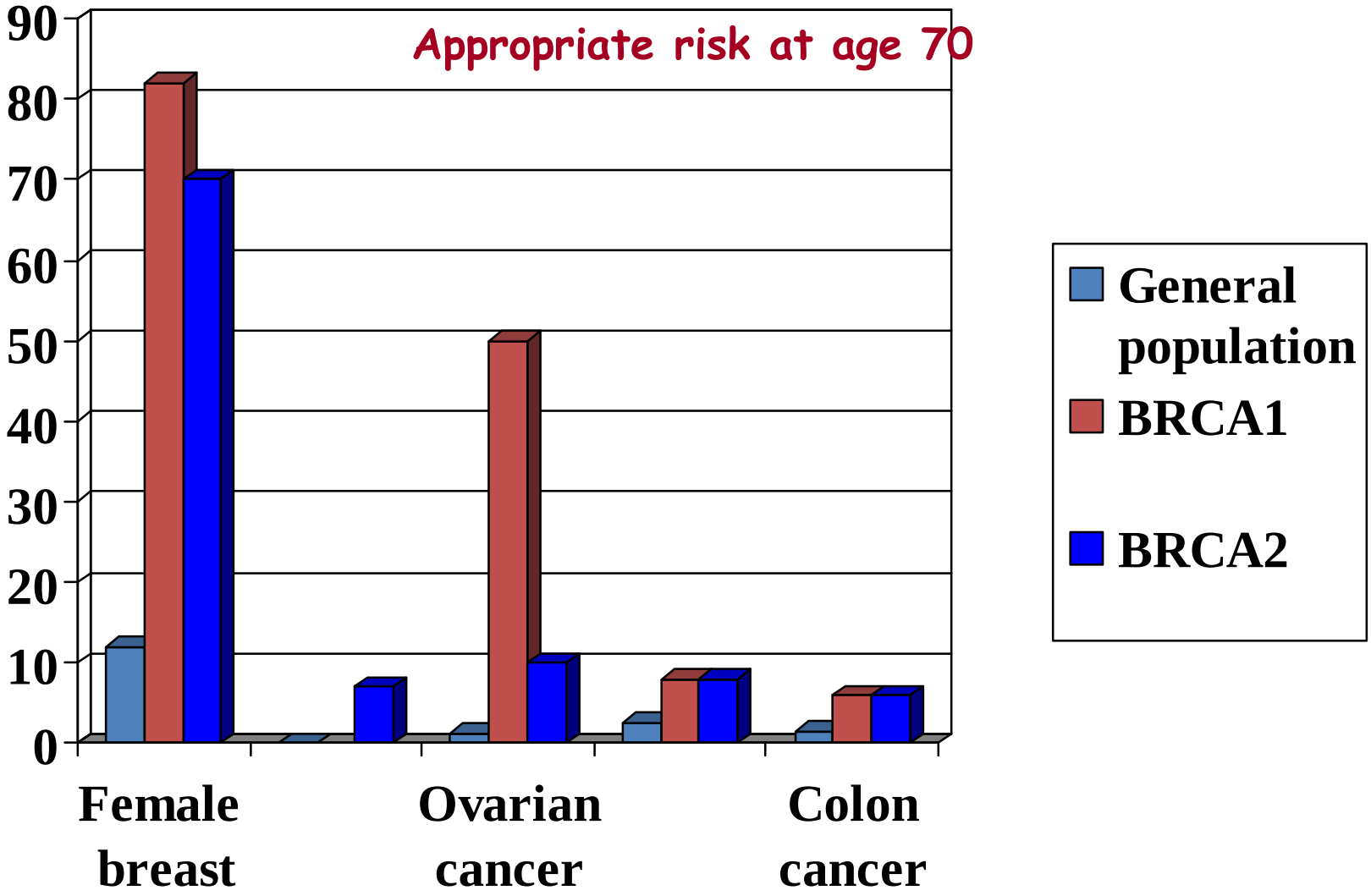
BRCA1 protein suppress unco-ordinated rapid cell growth



Autosomal dominant inheritance:

= 50% chance

= you only need 1!



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

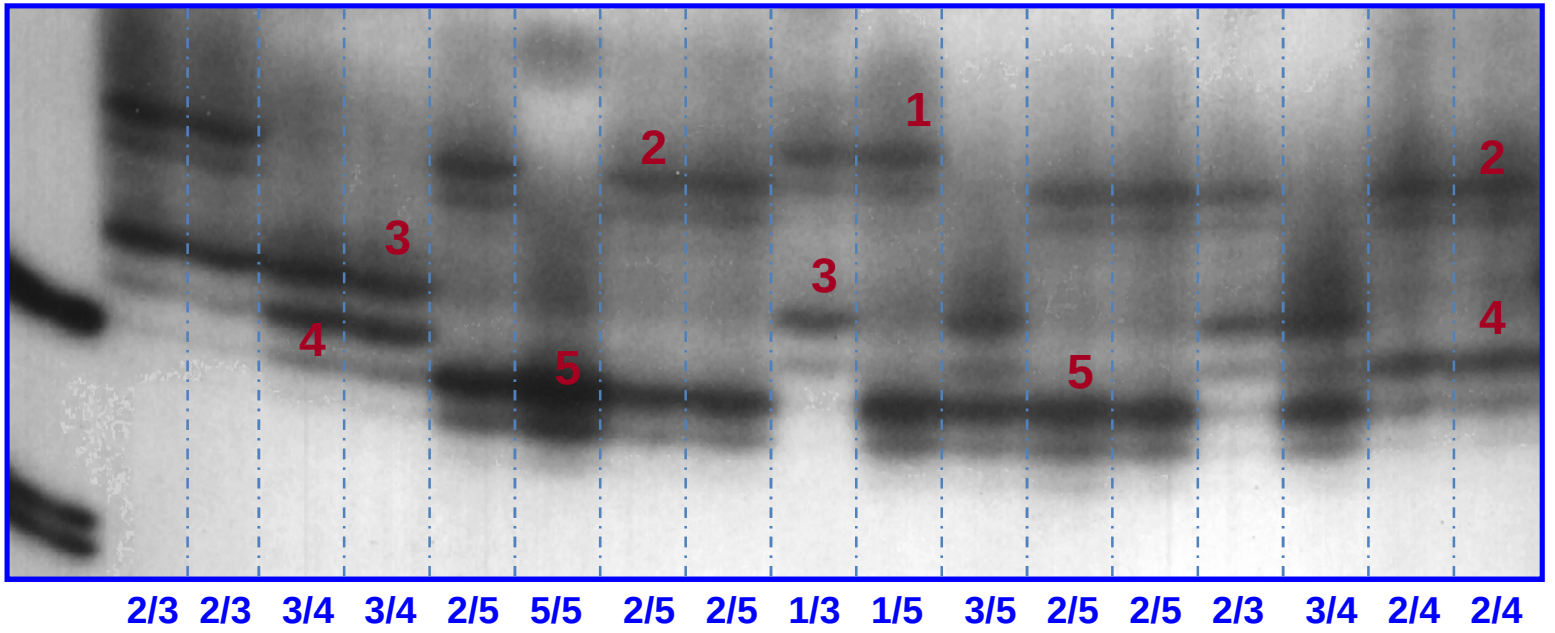
Selection of families

- 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

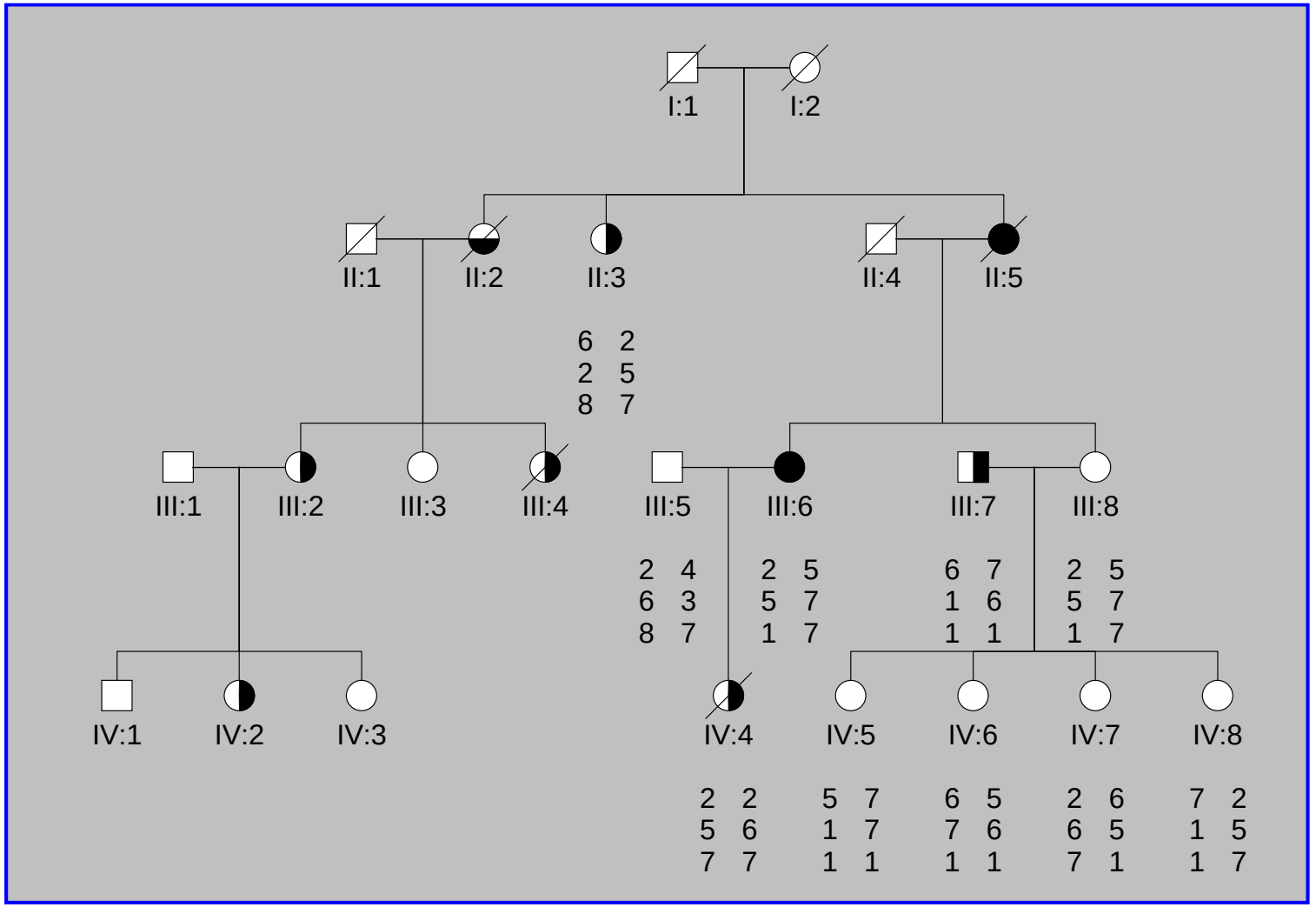
- EDTA or ACD blood samples
- DNA isolated, using the lymphocytes
- **Four laboratory techniques:**
  - linkage and haplotype analysis
  - protein truncation test (PTT)
  - single strand conformational analysis (SSCP)
  - DNA sequencing

Linkage & haplotype analysis



FAMILY 2:

BRCA1 results



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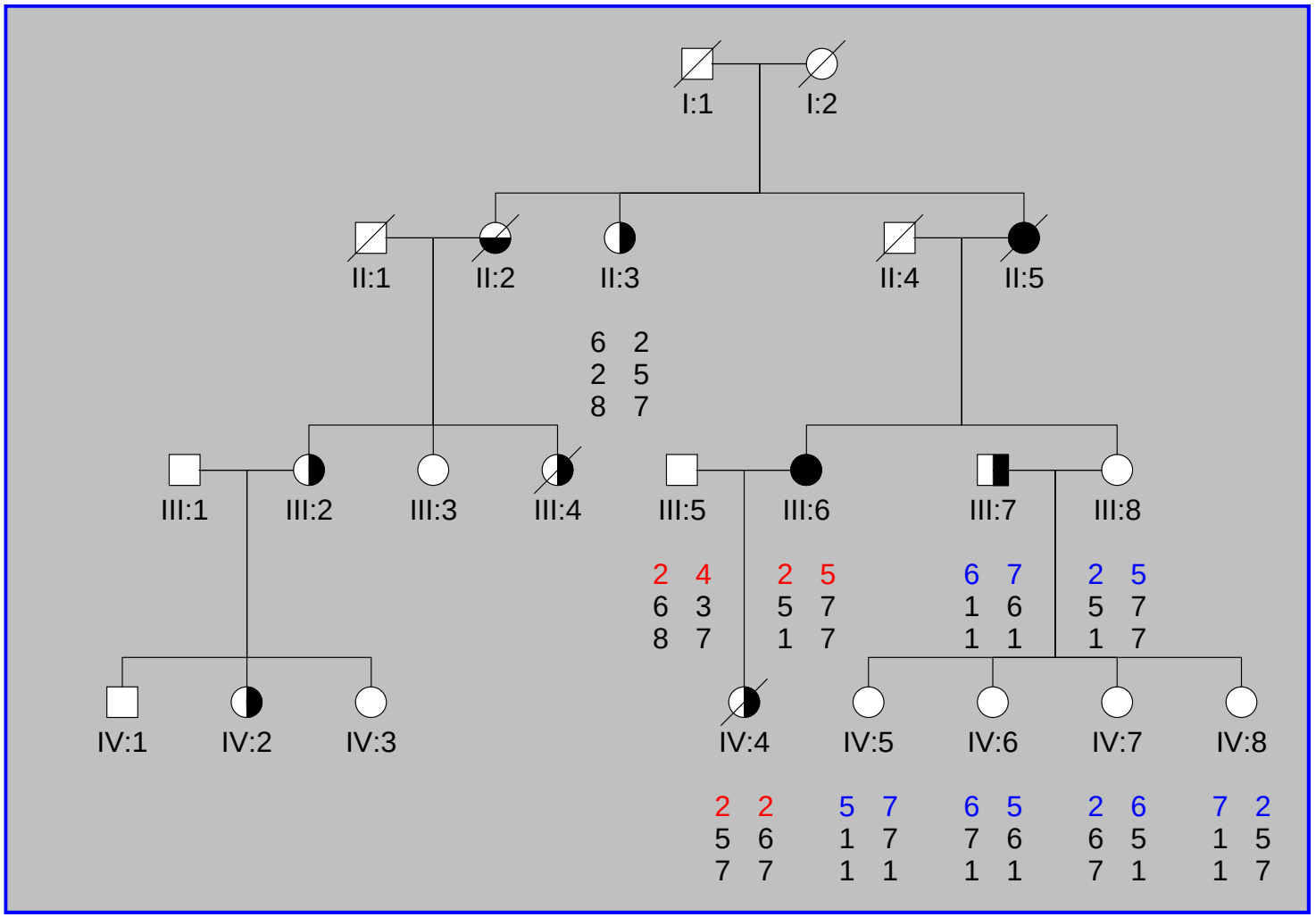
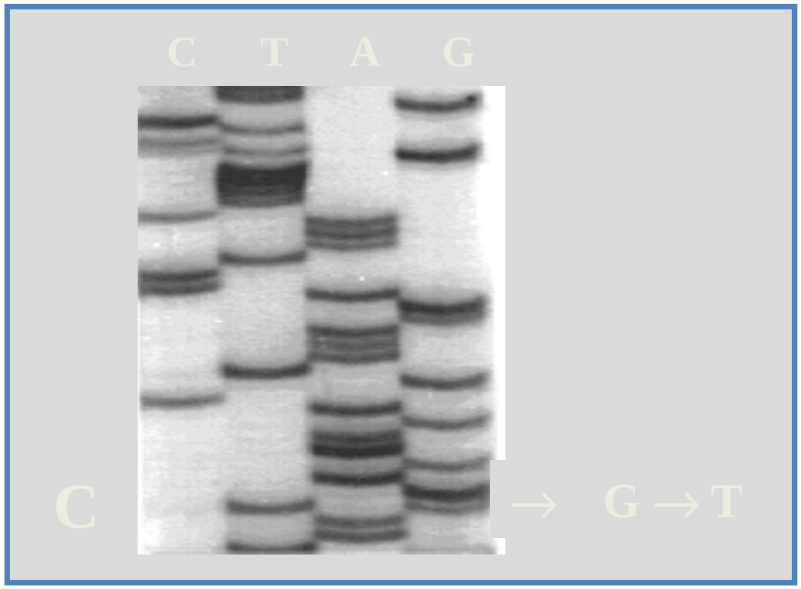
BRCA1 results



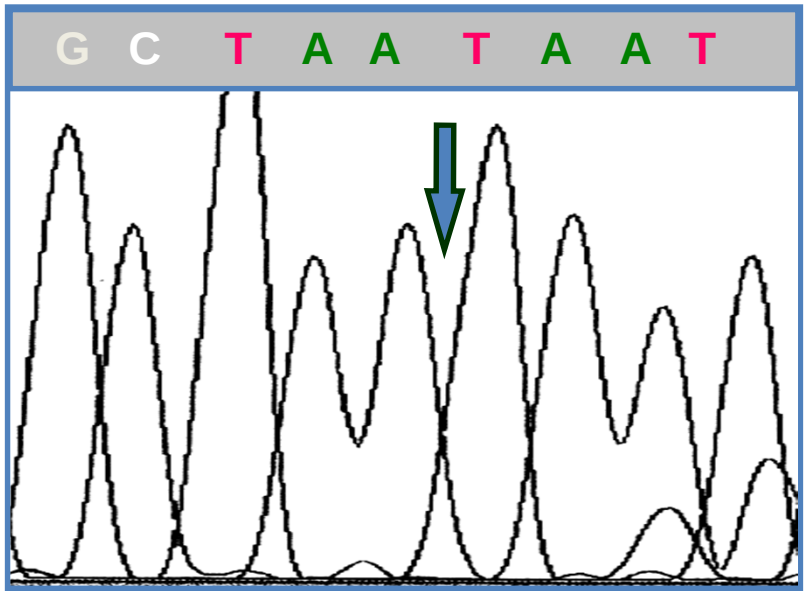
DNA sequencing

= 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

METHODS:

Haplotype analysis

- 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 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

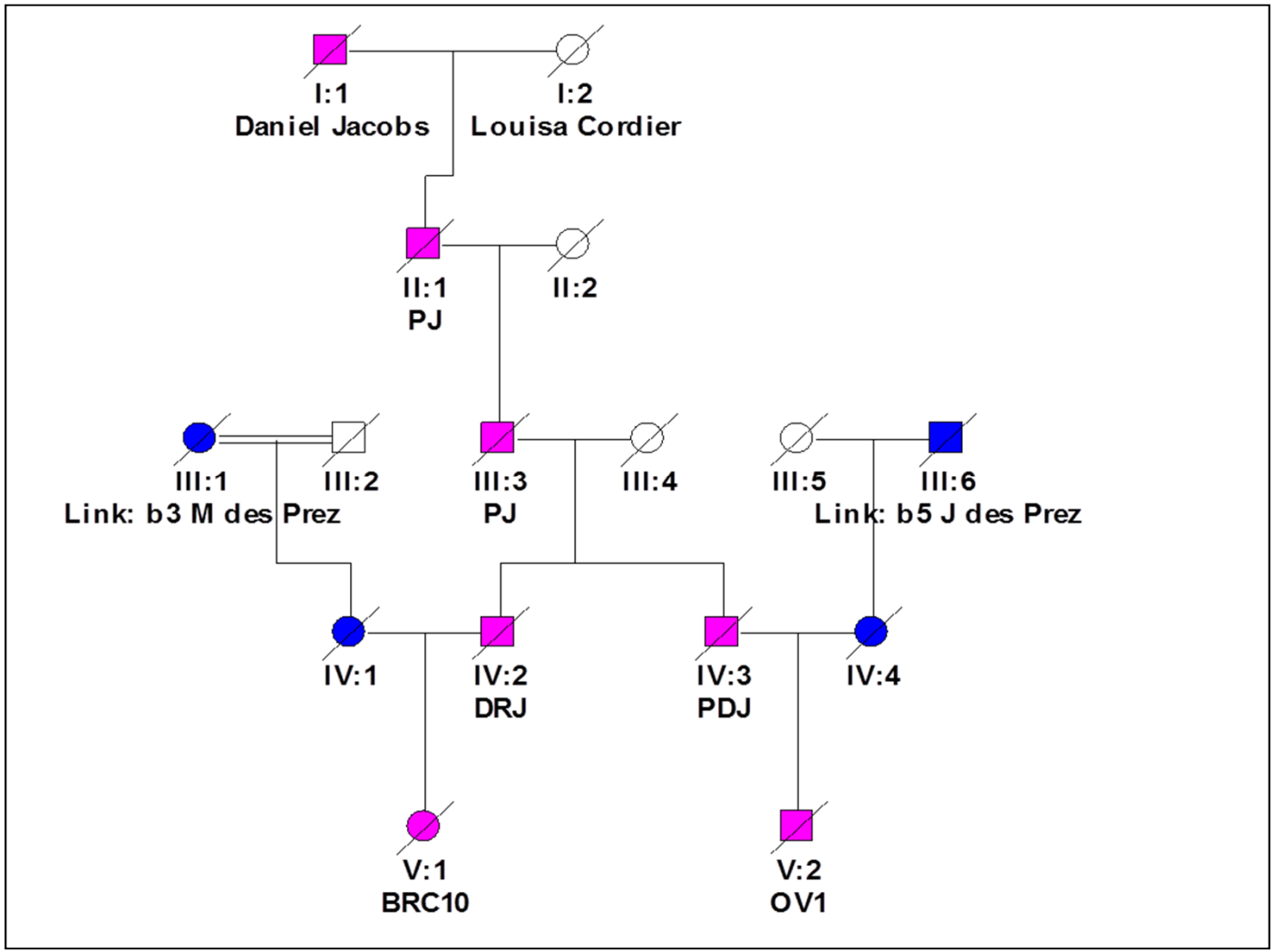
- **The results of the haplotype analysis indicated that 10 Afrikaner families with E881X and 5 families with 1493delC each shared a common genotype (Reeves *et al.* 2004)**
- **This indicated a single mutational event for each of the two Afrikaner mutations**
- **Implied that the families representing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

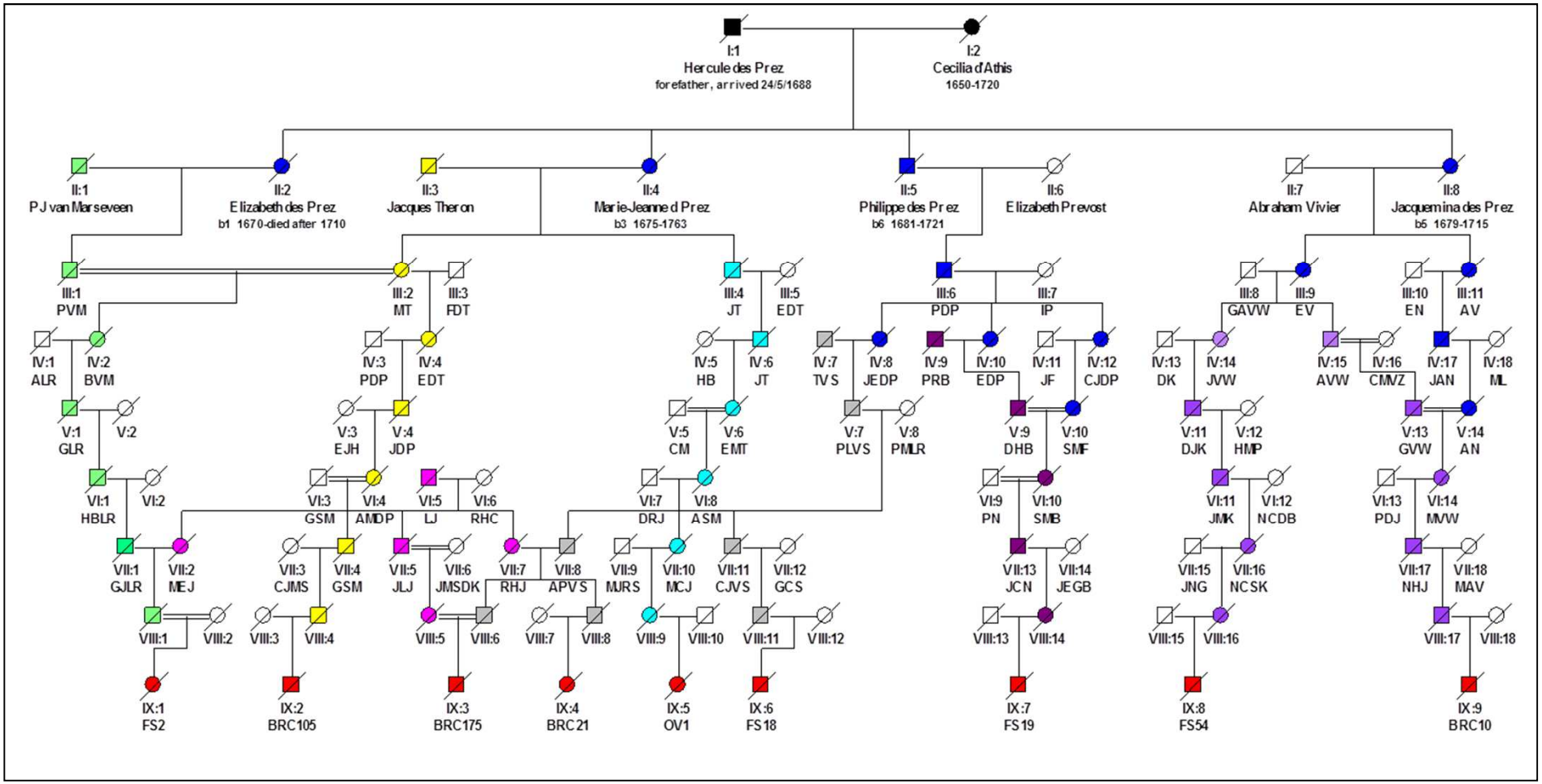


RESULTS:

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

BRCA1 E881X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forefather, arrived 24/5/1688
I:2
Cecilia d'Athis
1650-1720
II:1
PJ van Marseveen
II:2
Elizabeth des Prez
b1 1670-died after 1710
II:3
Jacques Theron
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Marie-Jeanne d Prez
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III:1 PVM
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III:6 PDP
III:7 IP
III:8 GAVW
III:9 EV
III:10 EN
III:11 AV
IV:1 ALR
IV:2 BVM
IV:3 PDP
IV:4 EDT
IV:5 HB
IV:6 JT
IV:7 TVS
IV:8 JEDP
IV:9 PRB
IV:10 EDP
IV:11 JF
IV:12 CJDP
IV:13 DK
IV:14 JVW
IV:15 AVW
IV:16 CMVZ
IV:17 JAN
IV:18 ML
V:1 GLR
V:2
V:3 EJH
V:4 JDP
V:5 CM
V:6 EMT
V:7 PLVS
V:8 PMLR
V:9 DHB
V:10 SMF
V:11 DJK
V:12 HMP
V:13 GVW
V:14 AN
VI:1 HBLR
VI:2
VI:3 GSM
VI:4 AMDP
VI:5 LJ
VI:6 RHC
VI:7 DRJ
VI:8 ASM
VI:9 PN
VI:10 SMB
VI:11 JMK
VI:12 NCDB
VI:13 PDJ
VI:14 MVW
VII:1 GJLR
VII:2 MEJ
VII:3 CJMS
VII:4 GSM
VII:5 JLJ
VII:6 JMSDK
VII:7 RHJ
VII:8 APVS
VII:9 MJRS
VII:10 MCJ
VII:11 CJVS
VII:12 GCS
VII:13 JCN
VII:14 JEGB
VII:15 JNG
VII:16 NCSK
VII:17 NHJ
VII:18 MAV
VIII:1
VIII:2
VIII:3
VIII:4
VIII:5
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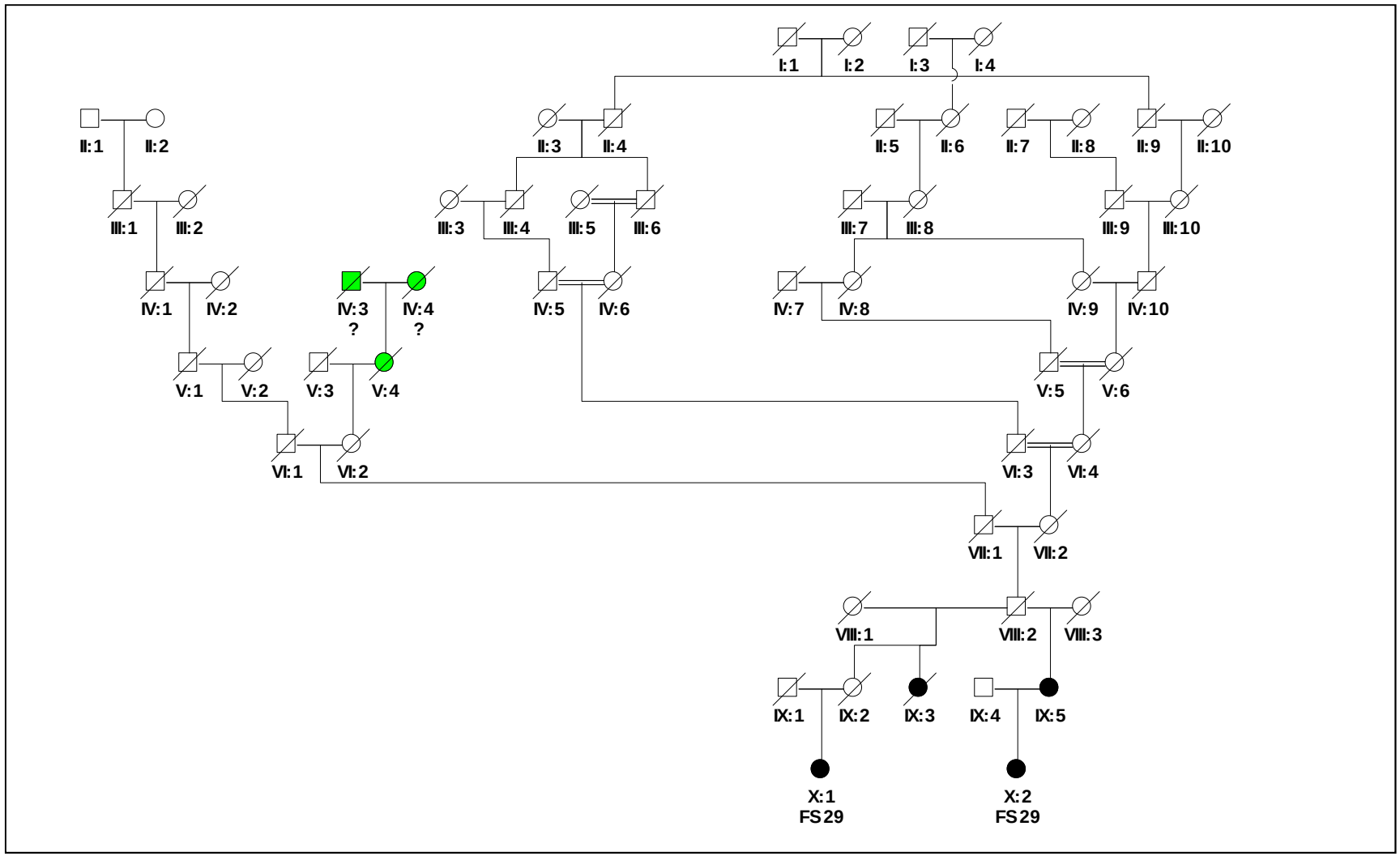
IX:2 BRC105
IX:3 BRC175
IX:4 BRC21
IX:5 OV1
IX:6 FS18
IX:7 FS19
IX:8 FS54
IX:9 BRC10

- 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

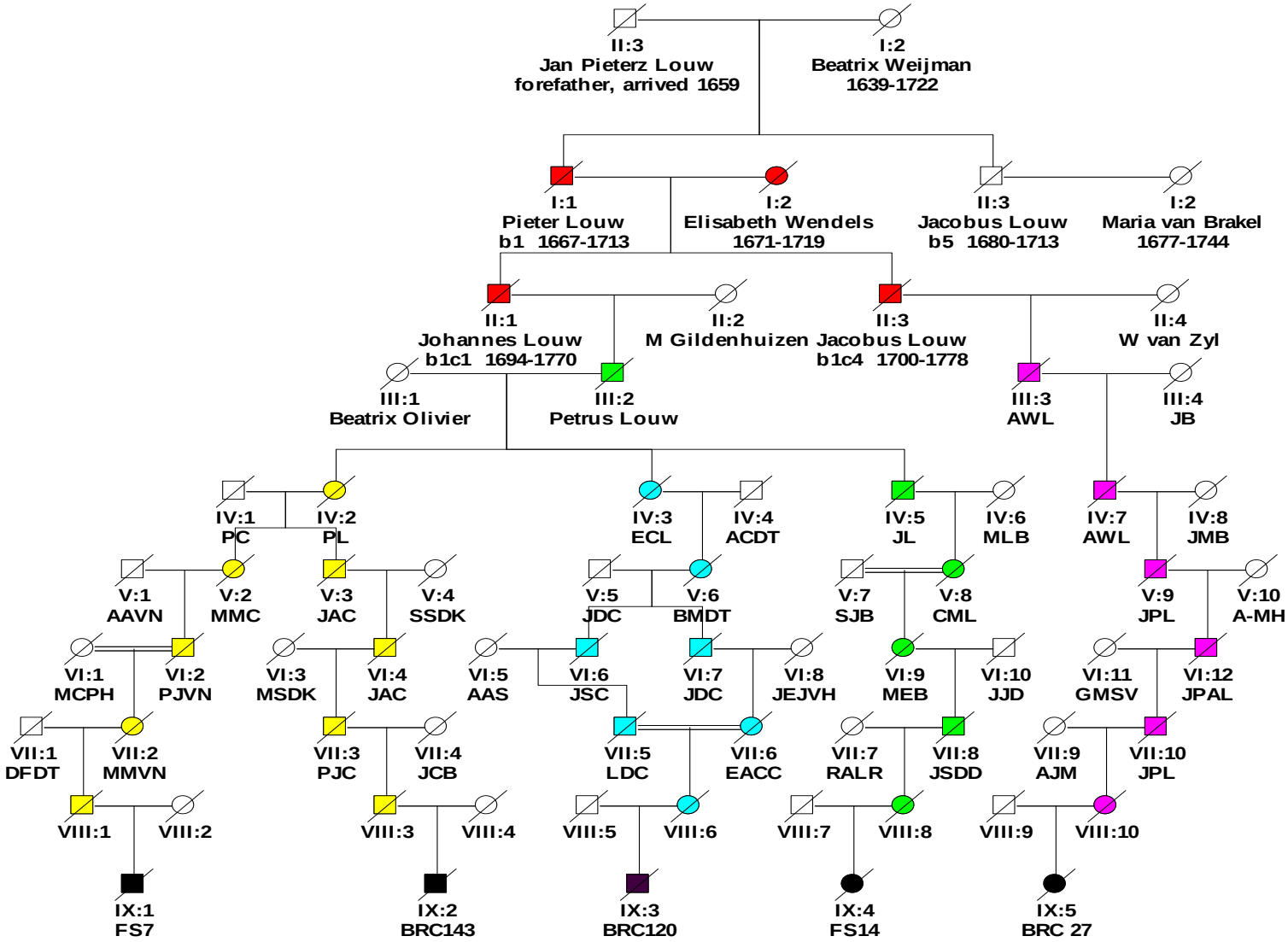


RESULTS:

Genealogy for 1493delC

- For 1493delC, four of five families were initially linked to a single couple, namely Petrus Louw (III:2) married to Beatrix Olivier (III: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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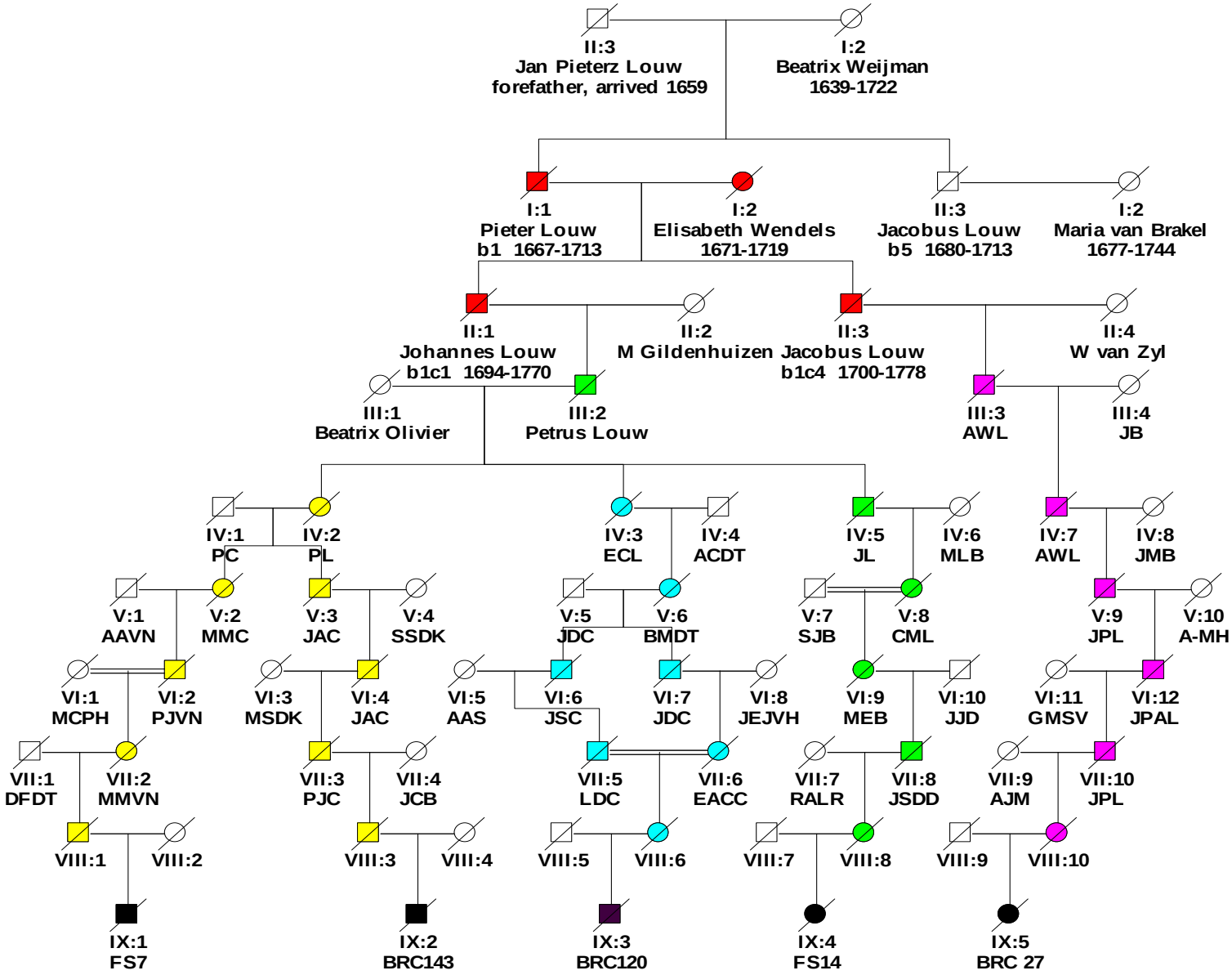
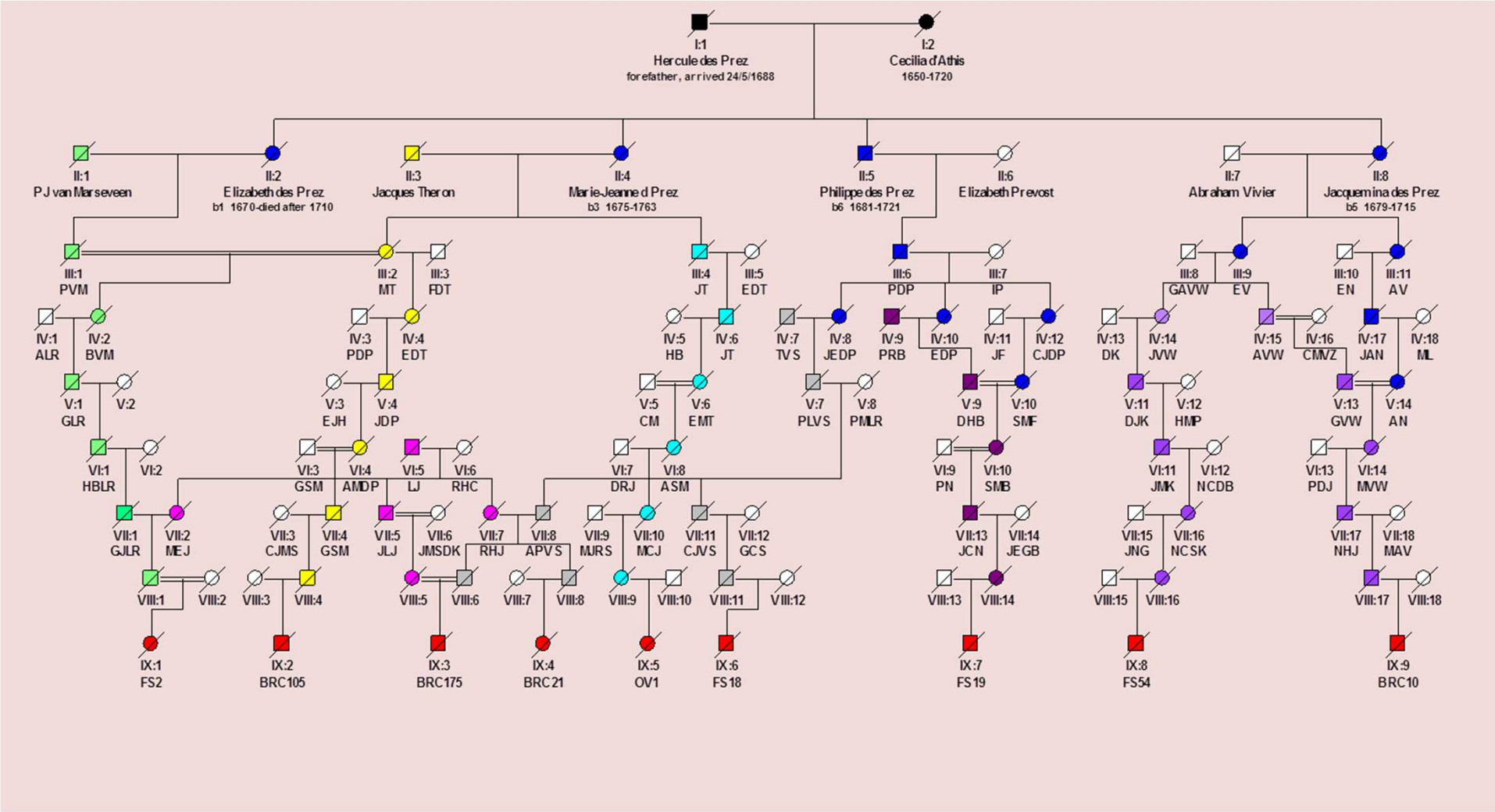


DISCUSSION

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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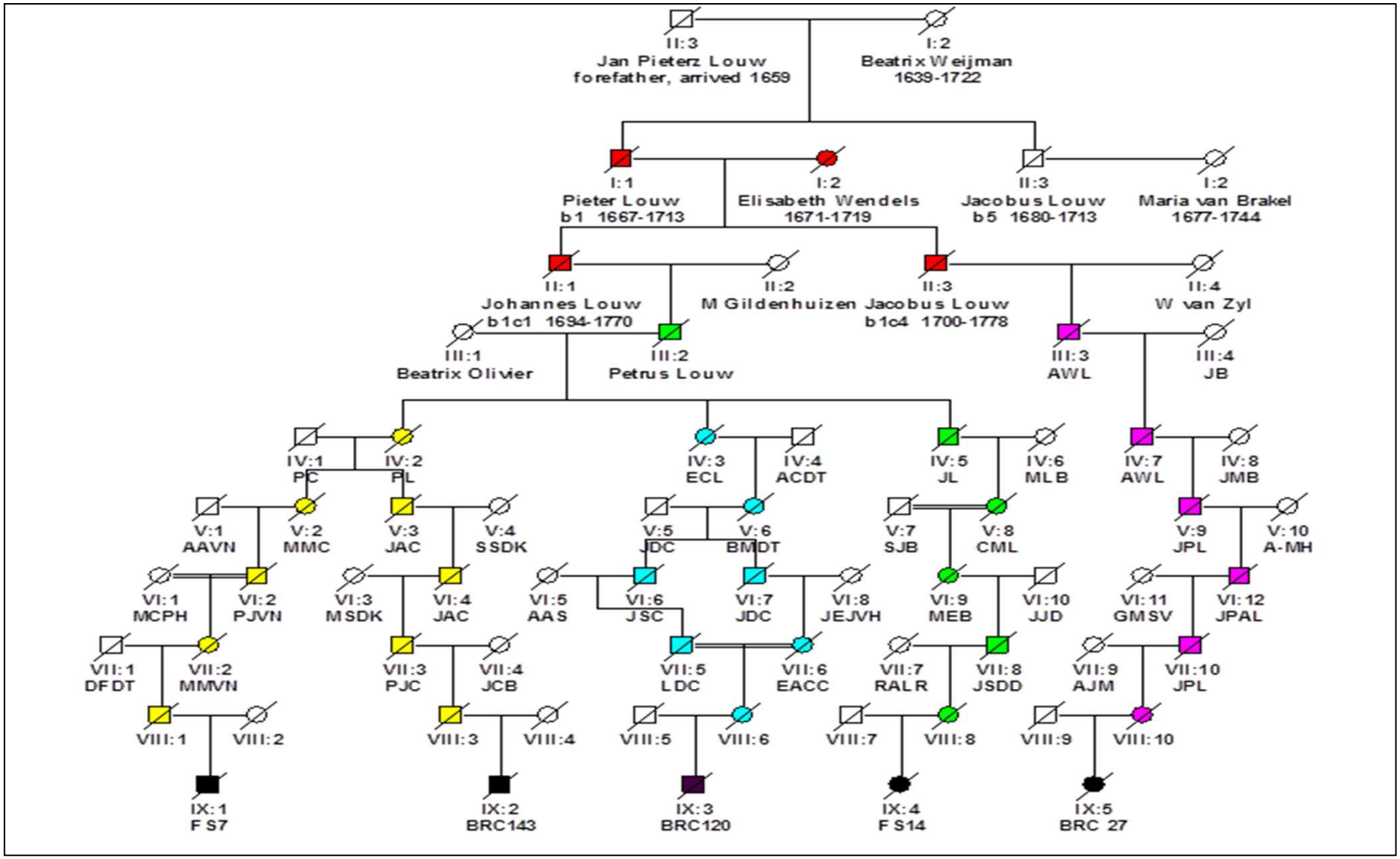
Louw family (1493delC)

- **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

OTHER DISEASES

- **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**

ACKNOWLEDGEMENTS

- 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