GENEALOGY OF A COHORT OF SOUTH AFRICAN FAMILIES AFFECTED BY FANCONI ANAEMIA, COMPLEMENTED BY CYTOGENETIC AND MOLECULAR INVESTIGATIONS

by

THOMAS PEARSON

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Department of Neurology
Faculty of Health Sciences
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Promoter: Dr S Jansen

This thesis is dedicated to my wife Marie-Ann and two children Johannes Jacobus and Joandré Pearson for their love, understanding and support during the years I spent on research in an attempt to identify the founders and authenticate the diagnosis of Fanconi anaemia

DECLARATION

I, the undersigned, hereby declare that the work contained within this thesis is my original and independent work and has not in its entirety or in part been submitted to any university for a degree.

All the sources I have made use of or quoted have been acknowledged by complete references.

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CONTENTS

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SUMMARY

Fanconi anaemia (FA) is a rare autosomal recessively inherited syndrome characterized by various phenotypic abnormalities, which inevitably, eventuates in progressive bone marrow failure. In the majority of cases a preliminary diagnosis of FA is based on the above-mentioned two criteria. The lymphocytes show an increased sensitivity to clastogenic agents such as diepoxybutane (DEB) and mytomycin C (MMC), resulting in chromosomal aberrations. This analysis is mainly used for the verification of the clinical diagnosis and screening purposes to identify family members who are possibly affected by FA.

The incidence of FA children under 16 years of age related to the white Afrikaans-speaking (Afrikaner) South African population in the Orange Free State and Northern Cape provinces is 1:22 000. Among South African Black populations and the rest of the world the approximate incidence is 1:400 000. A founder effect has been postulated as the reason for the high incidence among the white Afrikaans-speaking population.
In this study the clastogenic agent DEB was used and induced lymphocyte cultures were evaluated for the presence of chromosomal instability in inherent FA affected individuals. These patients were selected from only those families in which the FA affecteds were sensitive against DEB. Prior to the cloning of the FANCA gene, in which case if defective cause FA, a genealogical investigation was carried out on 12 FA families to substantiate the hypothesis of a founder effect. This genealogical information was then compared to the results of the molecular analysis as soon as the FANCA gene was cloned and the Afrikaner mutations became known. An additional genealogical investigation, relating to 13 supplementary FA parents known to be carriers of either the types I or II mutation, was used to verify the original genealogical investigation.

The cytogenetic results obtained in this study showed that it was not possible to differentiate between obligate carriers and the control group, however, homozygotes were clearly distinguishable from heterozygotes using only 20 metaphase spreads per person. Furthermore, when the DEB sensitivity of a patient was high, the number of unaffected cells observed in these FA patients was low.

The initial genealogical investigation pinpointed a French Huguenot couple, Guillaume Nel (or Nèel) and/or his wife Jeanne de la Batt, as possible candidates of the founder(s) of FA in South Africa. If this couple is indeed one of the founders of FA in South Africa, the same mutation (autozygosity of a gene) causing FA is suspected to occur in all their FA affected descendants. However, four mutations were present in the affected descendants, with one major mutation, a deletion stretching from exons 12 to 31 (type I), occurring on 63% of chromosomes analyzed. A hypothesis is put forward that the type I mutation is the original mutation, whereas the type II (deletion stretching from exons 11 to 17) and III (3398ΔA) mutations, with a frequency of respectively 14% and 18%, were introduced into the Afrikaner population at a later date.

The second genealogical investigation once more confirmed the French Huguenot couple Nel as possible founders of the type I Afrikaner mutation, however, the surname du Preez also featured prominently as a possible founder. As a result of numerous intermarriages, especially in the first few generations, it was not possible to distinguish between these two surnames as possible founders of the Afrikaner type I mutation. Genealogical investigations accentuated that either a Venter/Nel couple or an individual named JP du Plessis as possible founder of the type II mutation. The relationship between Nel and Venter could explain the occurrence of at least two mutations among the affected descendants from the French Huguenot couple bearing the surname 'Nel'.

KEYWORDS

Genetics, Human, Fanconi anaemia, Cytogenetic, Molecular, Genealogy, Afrikaner, South Africa

OPSOMMING

Fanconi anemia (FA) is 'n skaars outosomaal resessief oorgeërfde sindroom wat gekenmerk word deur verskeie fenotipiese abnormaliteite, met progressiewe beenmurgversaking die finale stadium van die siekte. In die meeste gevalle word bogenoemde twee kriteria gebruik vir die voorlopige diagnose van FA. Die limfosiete van geaffekteerde persone toon 'n verhoogde sensitiwiteit vir klastogeniese stowwe soos mitomisien C (MMC) of di-epoksie-butaan (DEB). Hierdie analise word dan hoofsaaklik gebruik ter bevestiging van die kliniese diagnose, asook die sifting van ander familielede met FA.

Die voorkoms van FA onder die blanke Afrikaanssprekende (Afrikaner) kinders jonger as 16 jaar in die Vrystaat en Noord-Kaap, is 1:22 000, in vergelyking met 'n voorkoms van ongeveer 1:400 000 onder swart Suid-Afrikaners en ander bevolkingsgroepe in die res van die wêreld. Daar word gepostuleer dat 'n stigterseffek die oorsaak van die hoë voorkoms van FA onder die Afrikaner is.

In hierdie ondersoek is gebruik gemaak van die klastogeen DEB, om chromosomale onstabiliteit in die limfosiete van moontlik aangetaste persone te identifiseer. Slegs families waar die aangetaste persone 'n sensitiwiteit tot DEB getoon het, is geselekteer. Aangesien die FANCA geen op daardie stadium nog nie gekloon was nie, is genealogiese data van 12 FA families gebruik om die moontlikheid van 'n stigterseffek te ondersoek. Nadat die FANCA geen gekloneer is, is die Afrikanermutasies wat in die FANCA geen identifiseer is, gebruik om die genealogiese data se akkuraatheid te toets. 'n Addisionele genealogiese ondersoek is daarna op 13 bykomende FA ouers waarby die Afrikaner tipe I of tipe II mutasie teenwoordig was, uitgevoer.

Met die sitogenetiese resultate kon geen onderskeid tussen verpligte draers en die kontrolegroep gemaak word nie. Die homosigote was duidelik onderskeibaar van heterosigote deur slegs 20 metafases per persoon te analiseer. Daar is dan ook gevind dat indien die pasiënt 'n verhoogde sensitiwiteit vir DEB toon, die aantal ongeaffekteerde selle wat voorkom, min is.

Die aanvanklike genealogies ondersoek het getoon dat 'n Franse Hugenote-egpaar by name Guillaume Nel (of Nèel) en/of sy vrou Jeanne de la Batt, kandidate is vir die stigter(s) van FA in Suid Afrika. Indien dit wel die geval is, kan verwag word dat dieselfde mutasie (outosigositeit van 'n geen) by alle FA aangetaste nasate van die egpaar sal voorkom. Daar is egter vier verskillende mutasies by die geaffekteerde afstammelinge gevind, waarvan die tipe I, 'n delesie vanaf ekson 12 tot 31, op 63% analiseerde chromosome voorkom. Die hipotese word gestel dat tipe II ('n delesie vanaf ekson 11 to 18) en tipe III (3398ΔA), wat onderskeidelik op 14% en 17% van die chromosome wat ontleed is voorkom, later tot die Afrikanerbevolking toegevoeg is.

Die resultate verkry met die tweede genealogiese ondersoek het weereens bevestig dat òf een òf beide lede van die Nel egpaar moontlik verantwoordelik was vir die voorkoms van die tipe I mutasie onder die Afrikanerbevolking. Die van du Preez het ook baie voorgekom en dit is moeilik om te bepaal watter een van die twee egpare die stigter van die Afrikaner tipe I mutasie was, aangesien ondertrouery in die vroeë generasies 'n algemene gebeurlikheid was. Die stigter van die tipe II mutasie onder die Afrikaner was moontlik òf een van die egpaar Venter/Nel òf 'n indiwidu genaamd JP du Plessis. Die verwanskap tussen Venter en Nel kan moontlik verklaar waarom ten miste twee mutasies onder die ge-affekteerde nasate van die Franse Hugenote egpaar met die van Nel voorgekom het.

CHAPTER 1
INTRODUCTION

HISTORICAL REVIEW

According to Beard et al. (1973) Ehrlich documented the first case of a refractory anaemia in childhood in 1888 and subsequently Benjam reported another case in 1911[1]. The clinical presentations of this type of anaemia include underdevelopment of the skeleton, mental retardation and hypoplasia of the genitalia. In 1927 Fanconi observed similar clinical findings in three male siblings in a family of five children who were diagnosed as anaemic with thrombocytopaenia. On examination, clinical abnormalities such as microcephaly without mental retardation, strabismus, growth retardation, testicular hypoplasia and skin pigmentation were apparent. He theorized the likelihood of a familial infantile pernicious-like anaemia[2]. The recognition of macrocytic erythrocytes, exhibiting a high colour index, were the earliest morphological features of this refractory anaemia. An autopsy report stated that the bone marrow appeared mostly pale or jelly-like and commented on the severe hypoplasia of the haemapoietic elements. The bone marrow dysfunction was regarded as one of the signs of a constitution of inferior type[2]. Incidences of patients with this syndrome were also reported from Switzerland, Holland, France, Denmark, Great Britain and the United States between 1927 and 1952[3]. By 1952 only 6 families, to have more than one child affected with Fanconi anaemia (FA), were on record. However, eight cases of sporadic occurrences were identified during this period of time. In 1952, Reinhold et al. [3] postulated that a recessive gene might be the basis of this inheritance pattern. Reasons offered were that the number of affected children born of two specific parents were higher when compared to their cousins, uncles and aunts. The only exception being a case where two brothers from one family married two sisters from another and their descendants were affected. Consanguinity, as in the case in seven of the 34 families identified to be affected by FA, constituted a factor[3].

Fanconi anaemia was first reported in South Africa during 1978 by Skikne et al.[4]. Nine years later Rosendorff et al. [5] estimated the minimum birth incidence of FA amongst white Afrikaans speaking South Africans rated 1:22 000, compared to the estimated frequency of FA homozygotes in North America at 1:348 000. Rosendorff et al. believed that the gene for FA was exceptionally common among the Afrikaans community because of random genetic

drift in the form of a founder effect. Mcdougall et al. [6] (1990) documented 25 black South African FA patients, estimating the frequency of 1 in 370 000 - 1 in 400 000 to be in accordance to the ratio quoted for the population in the rest of the world.

CLINICAL PHENOTYPE

During a study conducted by Fanconi in 1967 on 129 FA patients, it was ascertained that 77 percent of cases presented with hyperpigmentation and 66 percent with skeletal abnormalities[2]. The skeletal abnormalities were mainly limited to the hands and /or arms. These deformities included aplasia or hypoplasia of the thumb and/or radius as well as of the carpal bones[2]. Certain morphometric abnormalities, which generally involved the head and face[2], were also noted. These abnormalities included microcephaly, microphthalmia and microstomia. Urogenital malformations encompassed malformed kidneys (28%), as well as hypogenitalism and cryptorchidism in males[2]. The nervous system seemed to be unaffected, with the exception of hyperreflexia and to a lesser extent mental retardation (17%) and deafness (7%). A birth weight of less than 2 500 grams was measured in 56 % of cases[2]. In 1982 Glanz and Frazer described the phenotype of 94 FA patients, which correlated with the phenotype as expressed by Fanconi[7]. Although the weight at birth was not specified, stunted growth[7] was apparent in 77 % of patients. The description of the FA phenotype by Dos Santos et al. (1994)[8], showed strong resemblance to the phenotype as detailed by Fanconi[2], who pioneered a study 27 years prior to their study. It also fitted the description as documented by Glanz and Frazer[7]. Estern and Dameshek (1947) as well as Zaizov et al. (1969) concluded that there was considerable variation in the number and severity of congenital malformations in individuals[9, 10]. In some patients only aplastic anaemia was diagnosed, whilst others presented with all the clinical manifestations of FA disease[10].

HAEMATOLOGICAL CHARACTERISTICS

The majority of patients, usually within the first decade of life, were initially diagnosed with progressive hypoplasia of the bone marrow eventuating in aplastic anaemia. Fanconi described the anaemia as a familial infantile pernicious-like anaemia, mainly due to the presence of macrocytic red blood cells[2]. Glanz and Frazer (1982) stated that the

haematological characteristics of this invariably fatal disease encompass poikilocytosis, anisocytosis, reticulocytopaenia, thrombocytopaenia and leucopaenia[7]. The mean age of onset in probands was 7,9 ± 5,2 years, and 9.4 ± 6,4 years for non-probands[11]. In 90% of homozygotes aplastic anaemia commences during early childhood, but cases are known where individuals were asymptomatic until the third decade of their lives[12]. With reference to the International FA Registry the estimated median survival age of FA patients is 25 years yet, Alter (1991) is of the opinion that the average survival rate to be 16 years[13]. There is an increased likelihood for FA patients to develop cancers, especially acute myelogenous leukemia[14]. The incidence of cancer in FA patients is three to four times higher when compared to the normal population. Androgen therapy is the alternative treatment used for these patients however, bone marrow transplantation proved to be a better option for patients with progressive bone marrow failure. Survivors of bone marrow transplant are at increased risk of developing solid tumours.

GENETIC CHARACTERISTICS

In 1964 Schroeder et al. first demonstrated that FA is associated with abnormal susceptibility of somatic chromosomes to spontaneous aberrations. thereby validating an important cytogenetic concept and diagnostic tool[15]. Bloom et al. (1966) found an absence of chromosomal aberrations in two out of 12 patients, supporting the possibility of heterogeneity in FA[16]. Although Higurashi and Conen (1971) found that FA patients' chromosomes were radiosensitive it was Sasaki (1975) who discovered that their chromosomes were highly sensitive to cross-linking agents such as mytomycin C (MMC) and nitrogen mustard[17, 18]. This suggested a defect in one of the metabolic repair pathways, especially the mechanism for removing the interstrand cross-links from the DNA, which forms the basis of the cytogenetic technique for the diagnosis of FA[18]. Auerbach and Wolman (1976) showed that the bifunctional alkalyting agent diepoxybutane (DEB), in a non-toxic concentration for normal individuals, increases the chromosome breakage in FA homozygotes[19]. Auerbach and Wolman (1978) and Auerbach et al. (1981) were the first to claim that it was possible to distinguish between controls, heterozygotes and homozygotes using the DEB system[20, 21]. Cohen et al. (1982) demonstrated the possibility to misdiagnose non-FA patients if the DEB concentration was too high[22]. The latter group of researchers emphasized that it was impossible to distinguish between controls and heterozygotes. They found that the number of

chromosomal aberrations per cell obtained from heterozygotes overlap with that of controls. They concluded that the outcome of cytogenetic diagnosis of FA remained the same, regardless whether the DEB or MMC systems were utilised[22].

The first paper describing heterogeneity in FA was that by Reinhold et al. (1952)[3]. They suggested a variation in expression of a gene to be responsible for the presence of anaemia without congenital malformations. Schroeder et al. (1976) supported this hypothesis that variability of the phenotypic characteristics and age of haematological onset of FA was additional proof of heterogeneity[11]. They proposed the use of enzyme profiles or protein structures as a tool to substantiate heterogeneity in this disease[23]. In 1982, with the introduction of alkalyting agents such as DEB and MMC for the diagnosis of FA, they found a high yield of chromosomal aberrations in patients with aplastic anaemia but without any congenital malformations[22]. Duckworth-Rysiecki et al. (1984) on the other hand, portrayed patients with congenital anomalies typical of FA, but without the spontaneous or induced chromosomal aberrations[23]. Zakrzewski and Sperling first proved genetic heterogeneity, in 1980, using complementation analysis[24]. They identified two complementation groups in FA patients and termed it types A and non-A[25]. This was in contrast to the considerable number of different biochemical lesions (at least five) attributed to the disorder. Currently the number of complementation groups increased from the at least four (FA-A to FA-D) identified in 1992 to a minimum of seven (FA-A to FA-G) [26, 27]. These different complementation groups were representative of at least seven different defective genes causing FA[27]. The distribution of the FA genes in different population groups was already delineated. In the Netherlands the majority of FA patients belong to complementation group C (FAC), whereas in Germany and Italy it is complementation group A (FAA) that predominates[28]. According to Joenje (personal communication) preliminary results suggest that the majority of French patients also belong to FAA.

DNA REPAIR IN FANCONI ANAEMIA

According to Scriver et al., mainly three different biochemical repair systems [i) photoreactivation, ii) excision repair, and iii) postreplication repair] safeguard DNA in humans from permanent damage[29]. Ultraviolet light present in sun rays resulted in pyrimidine dimers and 6-4 photoproducts whereas other chemical substances such as mitomycin C, a bifunctional alkylating agent, resulted in DNA interstrand cross-links. Some of these dimers

were reversed by photoreactivation, whereas others were eliminated by excision repair. Postreplication repair is thus responsible for the last group of dimers to be corrected. A cell with excessive DNA damage ceases to exist as a result of apoptosis. A defect in any of the enzymes needed to repair ultraviolet damage, could cause either the autosomal recessive inherited disease Xeroderma pigmentosum, or Cockayne syndrome. Vincent et al. indicated that excision repair might also be defective in Bloom syndrome, resulting in a higher than normal sister chromatid exchange and quadriradial rearangements[30]. Ataxia telangiectasia and FA present with a spontaneous chromosomal breakage, which is induced by certain chemical damaging agents such as ethyl methane sulphonate and mitomycin C, respectively. Both these inherited diseases are also sensitive to gamma irradiation, indicating that the same pathway is defective in both diseases[31, 32].

In 1978 Sasaki found that FA cells are slightly more sensitive to monofunctional agents than normal cells, but remarkably more sensitive to bifunctional agents. This indicates that FA cells lack the ability to remove the DNA interstrand cross-links formed by bifunctional alkylating agents[33]. Extensive work was done on the DNA repair mechanism defective in FA. Laquerbe et al. (1995) described that when compared to normal cells, a substantial increase in frequency of intragenic deletions occurred at the hypoxanthine phosphoribosyltransferase (HPRT) and glucophorin loci in FA-D lymphoblasts after mutagen treatment. They suggested that, concurrenly with the increased chromosomal instability, the wild-type FA gene(s) plays an important role in the maintenance of genomic integrity due to the identical 3' breakpoint of two deletions of different sizes in mutated FA-D cells, as well as a common deletion signal sequence in these mutants[34]. Therefore mutations in the FANCD gene may contribute to aberrant site-specific cleavage activity thus being the plausible reason for the chromosomal instability characteristic as found in FA patients[34]. In 1997 Escarceller et al. suggested that FANCD and FANCB gene products might play a role in end-joining fidelity of specific DNA double strand breaks after they analyzed the fate of double strand breaks with an end-joining assay[35].

Krasnoshtein and Buchwald (1996) demonstrated that FANCC polypeptides are present in all tissue types during murine embrionic development, with a high expression in prognitor cells which are downregulated in differentiating cells. They stated that the FANCC expression in rapidly dividing prognitors was consistent with a hypothesis of a protein either playing a role in DNA repair or protecting cells against oxygen toxicity[36]. However, Auerbach (1995) stated that indirectly the FANCC gene plays a role in DNA repair due to the cytoplasmic localization

of the FANCC polypeptide[37]. This was substantiated by Youssoufian (1996), who found that the FANCC activity was coupled to a cytoplasmic defense mechanism against a specific class of genotoxic agents[38]. The hypothesis that FANCC plays a role in oxygen toxicity, rather than DNA repair, was verified by Clarke et al. in a study whereby an increase in mitomycin C sensitivity was observed when compared to normal cells at an oxygen level of 20%. At an oxygen level of 5% no increase in sensitivity of FANCC cells to mitomycin C compared to normal cells were observed[40]. The function of the FANCC polypeptide was not confined to the cytoplasm but 10% of this protein was detected in nucleur fractions[42]. Escarceller et al. (1998) stated that FANC is likely to play a role in the fidelity of end-joining of specific double strand breaks, a product of DNA treatment with alkylating agents[41]. The FANCG gene has recently been cloned and is identical to the XRCC9 gene, whose product is suspected to be involved in DNA post-replication repair or cell cycle checkpoint control[39].

Brois et al. (1999) isolated a 230 kDa protein that completely inhibits the ability of the normal repair complex to incise cross-linked DNA. Their viewpoint is that the FANCA gene plays a role either in the expession or the stability of this protein[43]. Kumaresan and Lambert (2000) found that the FANCA gene product is most likely involved in the initial incision step of the excision repair pathway[44]. Speit et al. (2000) found similar results, suggesting that more than one repair pathway could be involved in the repair of DNA cross-links[44,45].

McMahon et al. (1999) indicated that in all four different FA complementation cells tested (FA-A to FA-D) the levels of human alpha spectrin II, a structural protein, were significantly reduced. This verified the fact that FA represents a disorder with a deficiency of this protein[46].

Buchwald and Moustacchi (1998) suggested that the FA proteins interact to form a complex controlling different functions, including the repair of specific DNA lesions, organized to form a web network[47]. However, the biochemical functions of the FA proteins are still abstruse.

MOLECULAR ASPECTS

The heterogeneity of FA is reflected by the existance of seven FA complementation groups, each group representing a possible defective gene[27]. Faivre et al. (2000) found in a study of 245 FA patients that 94% could be assigned to only three complementation groups, i.e. FA-A (70.2%), FA-C (13.9%) and FA-G (9.8%), whereas only 15 patients could be assigned to the

other four complementation groups[48]. The ethnic distribution of complementation groups in South Africa is such that in the Afrikaner only complementation group A has been found, whereas the black South African patients belong to group G. Joenje (1996) found that the distribution of complementation groups in Germany differed considerably from those in the Netherlands. Complementation group A patients accounted for 59% of all German FA patients, whereas complementation group C accounted for 67% patients of Dutch ancestry[49]. Savino (1997) assigned 11 of 12 Italian patients to complementation group A by means of cell-fusion studies. This demonstrates the high prevalence of complementation group A among Italian FA patients[50]. A high prevalence of FA-A patients was also found among the Japanese[51].

The hypothesis of a gene for every complementation group seems to be true due to the cloning of five out of possible seven defective genes. The first gene, FANCC, was cloned in 1992 by Strathdee et al. using functional complementation and mapped it to chromosome 9q22.3[26]. Four years later, in 1996, Lo Ten Foe et al. as well as the Fanconi Anaemia/Breast Cancer Consortium cloned the second gene, FANCA[52, 53]. A third mutated FA causing gene is localized to chromosome band 9p13, and is known as XRCC9, defective in FA-G patients[39]. The FANCD gene has been mapped to chromosome band 3p22-26, whereas the FANCE gene was mapped to chromosome 6p[54, 55].

The FANCA gene's open reading frame is 4.3 kilobases long and consists of 43 exons. The protein of this gene did not have a homology to known proteins[56]. Both Morgan et al. (1999) and Levran et al. (1998) found large intragenic deletions in this gene. The breakpoints of the deletions correspond with Alu repeat sites and suggest that Alu-mediated recombination is responsible for the majority of mutations in the FANCA gene[57, 58]. Large intragenic mutations are difficult to detect if the normal mutation screening techniques are used, which could be the reason for the low mutation detection rate among the Italian FA patients[49]. Founder mutations of the FANCA gene are rare and novel mutations are found more regularly in most population groups[50, 59]. Founder mutations are present among the Afrikaner population of South Africa, where two large intragenic deletions are responsible for disrupting the gene in respectively 65% and 17% of chromosomes analyzed, (unpublished data).

The coding sequence of the FANCC gene is 1674 base pairs long. This interrupted gene contain 14 exons which range from 53 up to 204 base pairs and the protein has no homology to known proteins[60, 25]. No founder mutation was observed in the FANCC gene except for the IVS4 + 4 A-->T (IVS4) mutation in an Ashkenazi Jewish population[61, 62].

TREAMENT OF FANCONI ANAEMIA

Fanconi anaemia evolves towards progressive bone marrow failure, and if untreated, patients die within their second decade of life[63]. Patients with FA also have an actuarial risk of 50% for developing other complications before 40 years of age, such as myelodysplasia and acute myeloid leukemia[64]. Initially FA patients are treated with blood transfusions, androgens, corticosteroids or hematopoietic growth factors[65].

Currently the only treatment to restore normal haematopoiesis is allogeneic stem cell transplantation. A 5-year survival rate of more than 65 % can be obtained if an HLA-identical sibling donor is made use of. However, whenever resorted to an alternative donor, whether HLA-non-identical sibling or HLA-matched non-sibling, the survival rate decreases to 30%[63, 66]. In eiher cases of HLA-identical and HLA-alternative donors, graft-versus-host disease is a major concern[66]. Guardiola et al. (2000) found that in FA patients where malformations of the limbs, abnormalities in the urogenital system and/or kidneys, and phenotypic abnormalities are present, there is an increased risk for graft-versus-host disease[63]. FA patients who had undergone bone marrow transplant therapy are usually completely cured of their haematological disease, but are at risk of developing secondary tumours. These squamous cell carcinomas usually involve the neck and facial areas, and pose a poor prognosis[67].

Allogeneic stem cell transplantation is limited mainly to patients with an unaffected matched sibling donor, whereas alternative donors, while successful in selected cases, are associated with a high risk of graft failure[68]. Although primitive at the moment, gene therapy seems worth persuing for the treatment of a variety of inherited defects, such as beta-thalassemia, sickle cell anaemia, FA, chronic granulomatous disease, Gaucher disease, metachromatic leukodystrophy and cystic fibrosis[69]. Gene therapy became acceptible as the treatment of FA patients after Liu et al. (1999) introduced a retrovirus with a normal FANCC gene into four FA patients. Success was evident after a demonstration of resistance against mitomycin C in peripheral blood cells, as well as haemapoietic cells[70]. The value of gene therapy as an alternative became apparent after Gush et al. (2000) demonstrated that the Fac knockout mouse became resistance against alkylating agents after mitomycin C administration and after they were tranduced with a retrovirus containing an FANCC gene[71].

The latest tool tested in the fight against FA is protein replacement therapy administered by means of receptor-mediated endocytosis[72]. A chimeric protein consisting of a full FANCC

gene and a coding portion of the interleukin-3 gene is transfected into Escherichia coli. This purified bacterial protein is then introduced into haematopoietic cells via interleukin-3 receptors[72].

THE AFRIKANER POPULATION: AN HISTORICAL REVIEW

The first known inhabitants of present-day South Africa were the Khoisan and Khoikhoi hunters and gatherers, who were followed southwards by Bantu-speaking peoples between AD 1000 and 1500. In 1488, Portuguese sailors and their commander Bartholomew Diaz were on a voyage of exploration and hoped to reach the unknown southern tip (referred to as Cape of Good Hope) of Africa and then to sail on to India and the East. The composition of endemic races was left unchanged until the 17th century, when events occurring in Europe, had a major impact on the country[73]. These events were the Reformation and the golden era of the Dutch people[74, 75].

The century before the outbreak of the Reformation was marked by increasing and widespread dismay with the venality of the bishops and their involvement in politics, the ignorance and superstition of the lower clergy, the laxity of religious orders, and the sterility of academic theology. In the early 16th century Luther, and later Calvyn, reformed the Catholic religion, a period called the Reformation[74]. The ordinary people promptly accepted these ideas and the Protestant religion was instituted. The Catholic Church established a Counter-Reformation action, and war was declared[76]. These religious wars became most relentless in France, until the Edict of Nantes was endorsed, guaranteeing liberty of conscience and equality of legal and educational rights. It allowed French Protestants to hold government office and special courts were constituted to adjudicate disputes between the different denominations. It was not only the French Protestants (Huguenots) being targeted by the Counter-Reformation of the Catholic churches, but also the Dutch and German people. Massive exodus of French Huguenots to the Cape of Good Hope and America took place during the late 17th century after the Edict of Nantes was revolted[77].

The Dutch East India Company was founded in 1602 during the golden era of the Dutch people. It was originally a trading company, receiving its charter in 1602 from the State-General to conduct all trading between the Dutch Republic and the countries on route to the Cape of Good Hope and the Strait of Magellan. Although intended primarily to conduct trade, the company conquered territories and acted as a sovereign state in order to assert itself

against competitors. From headquarters established at Batavia (Jakarta) on the island of Java in 1619, it displaced the Portuguese from most of their Asian holdings and then fought off English attempts to launch into the spice trade in the East Indies. It also gained a monopoly on trade with Japan in 1641 through the island of Dejima, off Nagasaki, after the Japanese had expelled the Portuguese. Amsterdam became the financial centre of Europe and the United Provinces, and one of the great European strongholds. The Dutch East India Company established and maintained the Dutch colonial empire in Southeast Asia through the 17th and 18th centuries[75].

It was not until 1652 that the Dutchman Jan van Riebeeck established the first European settlement at Table Bay (now Cape Town), as a refreshment station for the Dutch East India Company. He was accompanied by 125 Dutch settlers. The majority of immigrants, who settled at the Cape during the late 17th and early 18th centuries, were from the Netherlands, followed by Germany and France, and of Protestant denomination.

Britain controlled the Cape infrequently during the Napoleonic Wars and took command of the territory in 1814 after agreements were made at the Congress of Vienna. Large-scale British settlement began in 1820[73]. To preserve their Calvinist way of life, hundreds of Dutch (Boer) farmers left the Cape in their ox-wagons to seek a new life in the interior. This migration of the Dutch families is known as the so-called Great Trek (1836). The Voortrekkers eventually set up independent republics, including the Orange Free State (1854) and the South African Republic (1852) later renamed as Transvaal[78].

THE CONSTITUTION OF THE AFRIKANER

According to Theal the composition of the Afrikaner people in 1795 comprised almost two thirds Dutch descent, while the French amounted to one sixth and the remaining part German and other ancestry[79]. He postulated that the prevalence of one lineage of immigrant over another was in itself not a determining aspect but more important, was the time of arrival and fertility of their marriages. Colenbrander estimated that in 1806 the Afrikaner people were a mixture of 50% Dutch, 27% German, 17% French and 5,5% other descent[80]. These estimates probably overemphasize the magnitude of the Dutch population, due to miscalculations by Colenbrander. According to the calculations by Heese the Afrikaner population is composed of an intermixture of 37% Dutch, 35% German, 13% French and 12,5% of other progeny

bloodlines[81]. The main difference between the results of Colenbrander and Heese was the number of Dutch and German immigrants. Heese found that Colenbrander had overestimated Dutch immigrants by 100 and underscored the German immigrants by approximately the same number. The difference found by these two authors may be due to the difficulty in establishing the definite origin of certain Dutch/German surnames, because the borders of these two countries changed through the 16th and 17th centuries[79]. It is therefore highly likely that a person(s) belonging to any of these three major groups introduced the FA gene to the Afrikaner population.

This new population expanded rapidly during the following century from a mere 1000 permanent immigrants in 1691 to 13 038 inhabitants in 1791. The number of tabulated founder fathers was 766 between 1691 and 1796 but Theal noted that according to church registers males with offspring in South Africa tallied to 1 526 and are the ancestors of the major part of the present-day white Afrikaans speaking-population[81].

References

1. Beard MEJ, Young DE, Bateman CJT, McCarthy GT, Smith M, Sinclair L, Franklin AW, Bodley Scott R (1973): Fanconi's anaemia. Quarterly J. Med. 166: 403-422.
2. Fanconi G (1967): Familial constitutional panmyelocytopenia, Fanconi's anemia. Sem. Hematol. 4(3):233-240.
3. Reinhold JDL, Neumark E, Lightwood R, Carter CO (1952): Familial hypoplastic anemia with congenital abnormalities (Fanconi's syndrome). Blood 7:915-926.
4. Skikne BS, Lynch SR, Bezwoda WR, Bothwell TH, Bernstein R, Katz J, Kramer S, Zucker M (1978): Fanconi's anaemia, with special reference to erythrokinetic features. S. Afr. Med. J. 53, 43:43-50.
5. Rosendorff J, Bernstein R, Macdougall LG, Jenkins T (1987): Fanconi anemia: Another disease of unusually high prevalence in the Afrikaans population of South Africa. Am. J. Med. Genet. 27:793-797.
6. Macdougall LG, Greeff MC, Rosendorf J, Bernstein R (1990): Fanconi anemia in black African children. Am. J. Med. Genet. 36:408-413.
7. Glanz A, Fraser FC (1982): Spectrum of anomalies in Fanconi anaemia. J. Med. Genet. 19:412-416.

8. Dos Santos CC, Gavih H, Buchwald M (1994): Fanconi anemia revisited: old ideas new advances. Stem Cells. 12:142-153.
9. Estern S, Dameshek W (1947): Familial hypoplastic anemia of childhood. Am. J. Dis. Child. 73:671-87.
10. Zaizov R, Matoth Y, Mamon Z (1969): Familial aplastic anaemia without congenital malformations. Acta. Paediat. Scand. 58:151-156.
11. Schroeder TM, Tilgen J, Krüger J, Vogel F (1976): Formal genetics of Fanconi's anemia. Hum. Genet. 32:257-288.
12. Lui JM, Auerbach AD, Young NS (1991): Fanconi anemia presenting unexpectedly in an adult kindred with no dysmorphic features. Am. J. Med. 91:555-557.
13. Alter BP (1992): Leukemia and preleukemia in Fanconi's anemia. Cancer Genet. Cytogenet. 58:306-208.
14. Auerbach AD, Allen RG (1991): Leukemia and preleukemia in Fanconi anemia patient a review of the literature and report of the International Fanconi Anemia Registry. Cancer Genet. Cytogenet. 51:1-12.
15. Schroeder TM, Anschütz F, Knopp A (1964): Spontane Chromosomenaberrationen bei familiärer Panmyelopathie. Hum. Genet. 1:194-196.
16. Bloom GE, Warner S, Gerald PS, Diamond LK (1966): Chromosome abnormalities in constitutional aplastic anemia. N. Eng. J. Med. 274(1):8-14.
17. Higurashi M, Conen PE (1971): In vitro chromosomal radiosensitivity in Fanconi's anemia. Blood 38(3):336-342.
18. Sasaki MS (1975): Is Fanconi's anemia defective in a process essential to repair of DNA cross links? Nature 257:501-503.
19. Auerbach AD, Wolman SR (1976): Susceptibility of Fanconi's anaemia fibroblast to chromosome damage by carcinogens. Nature 261:494-496.
20. Auerbach AD, Wolman SR (1978): Carcinogen-induced chromosome breakage in Fanconi's anaemia heterozygous cells. Nature 271:69-70.
21. Auerbach AD, Adler B, Chaganti RSK (1981): Prenatal and postnatal diagnosis and carrier detection of Fanconi anemia by a cytogenetic method. Pediatrics 67(1):128-135.
22. Cohen MM, Simpson SJ, Honig GR, Maurer HS, Nicklas JW, Martin AO (1982): The identification of Fanconi anemia genotypes by clastogenic stress. Am. J. Hum. Genet. 34:749-810.

23. Duckworth-Rysiecki G, Hulten M, Mann J, Taylor AMR (1984): Clinical and cytogenetic diversity in Fanconi's anaemia. J. Med. Genet. 21:197-203.

24. Zakrzewski S, Sperling K (1980): Genetic heterogeneity in Fanconi's anemia demonstrated by somatic cell hybrids. Hum. Genet. 56:81-84.

25. Moustacchi E, Papadopoulo D, Diatloff-Zito C, Buchwald M (1987): Two complementation groups of Fanconi's anemia differ in their phenotypic response to a DNA-crosslinking treatment. Hum. Genet. 75:45-47.

26. Strathdee CA, Duncan AMV, Buchwald M (1992): Evidence for at least four Fanconi anaemia genes including FACC on chromosome 9. Nature Genet.1:196-198.

27. Joenje H, Levitus M, Waisfisz Q, D'Andrea A, Garcia-Higuera I, Pearson T, van Berkel GM, Rooimans MA, Morgan N, Mathew CG, Arwert F (2000): Complementation analysis in Fanconi anemia: assignment of the reference FA-H patient to group A. Am. J. Hum. Genet. 67:759-762.

28. Joenje H for the European Fanconi Anaemia Research group (1996): Fanconi anaemia complementation groups in Germany and The Netherlands. Hum. Genet. 97:289-282.

29. Cleaver JE and Kraemer KH (1989): Xeroderma Pigmentosum. In: "The metabolic basis of inherited disease" 2949 – 2971, eds Scriver CR, Beaudet AL, Sly WS, Valle D, New York, McGraw-Hill.

30. Vincent RA, Hays MD(jr), Johnson RC (1978): Single-stranded breakage and repair in Bloom's syndrome cells. In: "DNA repair mechanisms", p663- 666,edt. Hanawalt PC, Friedberg EC, Fox CF, Academic Press, New York.

31. Arlett CF, Harcourt SA (1978): Cell killing and mutagenesis in repair-defective human cells. In: "DNA repair mechanisms", p633- 636,edt. Hanawalt PC, Friedberg EC, Fox CF, Academic Press, New York.

32. Paterson MC (1978): Ataxia teleangiectasia: A model inherited disease linking deficient DNA repair with radiosensitivity and cancer proneness. In: "DNA repair mechanisms", p663- 666,edt. Hanawalt PC, Friedberg EC, Fox CF, Academic Press, New York.

33. Sasaki MS (1978): Fanconi's anaemia a condition possibly associated with a defective DNA repair. In "DNA repair mechanisms", p663- 666,edt. PC Hanawalt, EC Friedberg and CF Fox, Academic Press, New York.

34. Laquerbe A, Moustacchi E, Fuscoe JC, Papadopoulo D (1995): The molecular mechanism underlying formation of deletions in Fanconi anemia cells may involve a site-specific recombination. Proc. Natl. Acad. Sci. USA. 92(3): 831-5.

35. Escarceller M, Rousset S, Moustacchi E, Papadopoulo D (1997): The fidelity of double strand breaks processing is impaired in complementation groups B and D of Fanconi anemia, a genetic instability syndrome. Somat. Cell. Mol. Genet. 23(6): 401-11.

36. Krasnoshtein F, Buchwald M (1996): Developmental expression of the Fac gene correlates with congenital defects in Fanconi anemia patients. Hum Mol Genet. 5(1): 85-93.

37. Auerbach AD (1995): Fanconi anemia. Dermatol. Clin. 13(1): 41-9.

38. Youssoufian H (1996): Cytoplasmic localization of FAC is essential for the correction of a prerepair defect in Fanconi anemia group C cells. J. Clin. Invest. 97(9): 2003-10.

39. de Winter JP, Waisfisz Q, Rooimans MA, van Berkel CG, Bosnoyan-Collins L, Alon N, Carreau M, Bender O, Demuth I, Schindler D, Pronk JC, Arwert F, Hoehn H, Digweed M, Buchwald M, Joenje H (1998): The Fanconi anaemia group G gene FANCG is identical with XRCC9. Nature Genet. 20(3): 281-3.

40. Clarke AA, Philpott NJ, Gordon-Smith EC, Rutherford TR (1997): The sensitivity of Fanconi anaemia group C cells to apoptosis induced by mitomycin C is due to oxygen radical generation, not DNA crosslinking. Br. J. Haematol. 96(2): 240-7.

41. Escarceller M, Buchwald M, Singleton BK, Jeggo PA, Jackson SP, Moustacchi E. Papadopoulo D (1998): Fanconi anemia C gene product plays a role in the fidelity of blunt DNA end-joining. J. Mol. Biol. 279(2): 375-85.

42. Hoatlin ME, Christianson TA, Keeble WW, Hammond AT, Zhi Y, Heinrich MC, Tower PA, Bagby GC(jr) (1998): The Fanconi anemia group C gene product is located in both the nucleus and cytoplasm of human cells. Blood 91(4): 1418-25.

43. Brois DW, McMahon LW, Ramos NI, Anglin LM, Walsh CE, Lambert MW (1999): A deficiency in a 230 kDa DNA repair protein in Fanconi anemia complementation group A cells is corrected by the FANCA cDNA. Carcinogenesis 20(9): 1845-53.

44. Kumaresan KR, Lambert MW (2000): Fanconi anemia, complementation group A, cells are defective in ability to produce incisions at sites of psoralen interstrand cross-links. Carcinogenesis 21(4): 741-51

45. Speit G, Schutz P, Merk O (2000): Induction and repair of formaldehyde-induced DNA-protein crosslinks in repair-deficient human cell lines. Mutagenesis. 15(1): 85-90.

46. McMahon LW, Walsh CE, Lambert MW (1999): Human alpha spectrin II and the Fanconi anemia proteins FANCA and FANCC interact to form a nuclear complex. J. Biol. Chem. 274(46): 32904-8.

47. Buchwald M, Moustacchi E (1998): Is Fanconi anemia caused by a defect in the processing of DNA damage? Mutat. Res. 408(2): 75-90.

48. Faivre L, Guardiola P, Lewis CM, Dokal I, Ebell W, Zatterale A, Altay C, Poole JE, Stones D, Kwee ML, van Weel-Sipman, Havenga C, Morgan NV, de Winter J, Digweed M, Savoia A, Pronk JC, de Ravel T, Jansen S, Joenje H, Gluckman E, Matthew CG for the European Fanconi Anemia Research Group (2000): Association of complementation group and mutation type with clinical outcome in Fanconi anemia. Blood (in press).

49. Joenje H for European Fanconi Anaemia Research group (1996): Fanconi anaemia complementation groups in Germany and The Netherlands. Hum. Genet. 97(3): 280-2.

50. Savino M, Ianzano L, Strippoli P, Ramenghi U, Arslanian A, Bagnara GP, Joenje H, Zelante L, Savoia A (1997): Mutations of the Fanconi anemia group A gene (FAA) in Italian patients. Am. J. Hum. Genet. 61(6): 1246-53.

51. Wijker M, Morgan NV, Herterich S, van Berkel CG, Tipping AJ, Gross HJ, Gille JJ, Pals G, Savino M, Altay C, Mohan S, Dokal I, Cavenagh J, Marsh J, van Weel M, Ortega JJ, Schuler D, Samochatova E, Karwacki M, Bekassy AN, Abecasis M, Ebell W, Kwee ML, de Ravel T, Mathew CG, et al. (1999): Heterogeneous spectrum of mutations in the Fanconi anaemia group A gene. Eur. J. Hum. Genet. 7(1): 52-9.

52. Lo Ten Foe JR, Rooimans MA, Bosnoyan Collins L, Alon N, Wijker M, Parker L, Lightfoot J, Carreau M, Callen DF, Savoia A, Cheng NC, van Berkel CG, Strunk MH, Gille JJ, Pals G, Kruyt FA, Pronk JC, Arwert F, Buchwald M, Joenje H (1996): Expression cloning of a cDNA for the major Fanconi anaemia gene, FAA. Nature Genet. 14(3): 320-3.

53. The Fanconi anaemia/Breast cancer consortium (1996): Positional cloning of the Fanconi anaemia group A gene. Nature Genet. 14(3): 324-8.

54. Diatloff-Zito C, Duchaud E, Viegas-Pequignot E, Fraser D, Moustacchi E (1994): Identification and chromosomal localization of a DNA fragment implicated in the partial correction of the Fanconi anemia group D cellular defect. Mutat. Res. 307: 33-42.

55. Waisfisz Q, Saar K, Morgan NV, Altay C, Leegwater PA, de Winter JP, Komatsu K, Evans GR, Wegner RD, Reis A, Joenje H, Arwert F, Mathew CG, Pronk JC, Digweed M (1999): The Fanconi anemia group E gene, FANCE, maps to chromosome 6p. Am. J. Hum. Genet. 64: 1400-1405.

56. Ianzano L, D'Apolito M, Centra M, Savino M, Levran O, Auerbach AD, Cleton-Jansen AM, Doggett NA, Pronk JC, Tipping AJ, Gibson RA, Mathew CG, Whitmore SA,

Apostolou S, Callen DF, Zelante L, Savoia A (1997): The genomic organization of the Fanconi anemia group A (FAA) gene. Genomics 41(3): 309-14.

57. Morgan NV, Tipping AJ, Joenje H, Mathew CG (1999): High frequency of large intragenic deletions in the Fanconi anemia group A gene. Am. J. Hum. Genet. 65(5): 1330-41.
58. Levran O, Doggett NA, Auerbach AD (1998): Identification of Alu-mediated deletions in the Fanconi anemia gene FAA. Hum. Mutat. 12(3): 145-152.
59. Tachibana A, Kato T, Ejima Y, Yamada T, Shimizu T, Yang L, Tsunematsu Y, Sasaki MS (1999): The FANCA gene in Japanese Fanconi anemia: reports of eight novel mutations and analysis of sequence variability. Hum. Mutat. 13(3): 237-44.
60. Gibson RA, Buchwald M, Roberts RG, Mathew CG (1993): Characterisation of the exon structure of the Fanconi anaemia group C gene by vectorette PCR. Hum. Mol. Genet. 2(1): 35-38.
61. Gibson RA, Morgan NV, Goldstein LH, Pearson IC, Kesterton IP, Foot NJ, Jansen S, Havenga C, Pearson T, de Ravel TJ, Cohn RJ, Marques IM, Dokal I, Roberts I, Marsh J, Ball S, Milner RD, Llerena JC(jr), Samochatova E, Mohan SP, Vasudevan P, Birjandi F, Hajianpour A, Murer-Orlando M, Mathew CG (1996): Novel mutations and polymorphisms in the Fanconi anemia group C gene. Hum. Mutat. 8(2): 140-8.
62. Verlander PC, Kaporis A, Liu Q, Zhang Q, Seligsohn U, Auerbach AD (1995): Carrier frequency of the IVS4 + 4 A→T mutation of the Fanconi anemia gene FAC in the Ashkenazi Jewish population. Blood 86(11): 4034-8.
63. Guardiola P, Pasquini R, Dokal I, Ortega JJ, van Weel-Sipman M, Marsh JC, Ball SE, Locatelli F, Vermylen C, Skinner R, Ljungman P, Miniero R, Shaw PJ, Souillet G, Michallet M, Bekassy AN, Krivan G, Di Bartolomeo P, Heilmann C, Zanesco L, Cahn JY, Arcese W, Bacigalupo A, Gluckman E (2000): Outcome of 69 allogeneic stem cell transplantations for Fanconi anemia using HLA-matched unrelated donors: a study on behalf of the European Group for Blood and Marrow Transplantation. Blood 95(2): 422-9.
64. Butturini A, Gale RP, Verlander PC, Adler-Brecher B, Gillio AP, Auerbach AD (1994): Hematologic abnormalities in Fanconi anemia: an international Fanconi Anemia Registry study. Blood 84: 1650-1655.
65. Guinan EC, Lopez KD, Huhn RD, Felser JM, Nathan DG (1994): Evaluation of granulocyte-macrophage colony-stimulating factor for treatment of pancytopenia in children with Fanconi anemia. J. Pediatr. 124: 144-150.

66. Gluckman E, Auerbach AD, Horowitz MM, Sobocinski KA, Ash RC, Bortin MM, Butturini A, Camitta BM, Champlin RE, Friedrich W, Good RA, Gordon-Smith EC, Harris RE, Klein JP, Ortega JJ, Pasquini R, Ramsay NKC, Speck B, Vowels MR, Zhang MJ, Gale RP (1995): Bone marrow transplantation for Fanconi anemia. Blood 86(7): 2856-62.

67. Guardiola P, Socie G, Pasquini R, Dokal I, Ortega JJ, van Weel-Sipman M, Marsh J, Locatelli F, Souillet G, Cahn JY, Ljungman P, Miniero R, Shaw J, Vermylen C, Archimbaud E, Bekassy AN, Krivan G, Di Bartolomeo P, Bacigalupo A, Gluckman E (1998): Allogeneic stem cell transplantation for Fanconi Anaemia. Severe Aplastic Anaemia Working Party of the EBMT and EUFAR. European Group for Blood and Marrow Transplantation. Bone Marrow Transplant. 21 Suppl 2: S24-7.

68. Liu JM (1998): Gene transfer for the eventual treatment of Fanconi's anemia. Semin. Hematol. 35(2): 168-79.

69. Hallek M, Wendtner CM (1996): Recombinant adeno-associated virus (rAAV) vectors for somatic gene therapy: recent advances and potential clinical applications. Cytokines Mol Ther. 2(2): 69-79.

70. Liu JM, Kim S, Read EJ, Futaki M, Dokal I, Carter CS, Leitman SF, Pensiero M, Young NS, Walsh CE (1999): Engraftment of hematopoietic progenitor cells transduced with the Fanconi anemia group C gene (FANCC). Hum. Gene Ther. 10(14): 2337-46.

71. Gush KA, Fu KL, Grompe M, Walsh CE (2000): Phenotypic correction of Fanconi anemia group C knockout mice. Blood 95(2): 700-4.

72. Youssoufian H, Kruyt FA, Li X (1999): Protein replacement by receptor-mediated endocytosis corrects the sensitivity of Fanconi anemia group C cells to mitomycin C. Blood 93(1): 363-9.

73. Hockly HE (1957): "The story of the British settlers of 1820 in South Africa" Juta & Co, Johannesburg.

74. Boucher M (1981): "French speakers at the Cape: The European background". NHW-Pers, Pretoria. pp6-25.

75. Sleigh, D (1980): Jan Compagnie : the world of the Dutch East India Company. Tafelberg, Cape Town.

76. Coertzen P (1988): Die opkoms van die Gereformeerde Kerk in Frankryk tot 1559. In "Die Hugenote van Suid-Afrika 1688-1988". Tafelberg uitgewers, Cape Town. pp1-16.

77. Reaman GE (1963): The trail of the Huguenots in Europe, the United States, South Africa and Canada. Frederich Muller Limited, London.

78. Walker EA (1934); The Great Trek. A&C Black Ltd, London.

79. Pama C (1981): "The composition of the Afrikaner people." In: de Villiers CC, Pama C (eds). "Genealogies of old South African families." 2nd ed. Cape Town/Rotterdam: AA Balkema, pp.xviii-xxv.

80. Colenbrander HT (1902): "De afkomst der Boeren." Dordrecht: Algemeen Nederlandsch Verbond.

81. Botha MC, Pritchard J (1972): Blood group, gene frequencies an indication of the genetic constitution of population samples in Cape Town. S. Afr. Med. J. Suppl 1 April: 1-27.

CHAPTER 2

OBJECTIVES OF THE STUDY

1. To determine the aberration yield of diepoxybutane-induced chromosome damage in cultured lymphocytes, and to consolidate/refine the technique of distinguishing between FA homozygotes, -heterozygotes and relevant controls by means of statistical analysis of the aberration yields obtained.

In the majority of cases a preliminary diagnosis of FA is made using certain haematological and phenotypical abnormalities. The induced chromosomal breakage studies are applied to verify this diagnosis. Auerbach et al.[1] and Marx et al.[2], using the same alkylating agent, and in the latter case some of the same patients and family members as ourselves, were able to distinguish between FA homozygotes, obligate heterozygotes and controls. Our aim was to determine the accuracy of differentiating between these groups.

2. To obtain and analyse genealogical information on Afrikaner FA patients and their families, and establish the possible founder(s) of the disease.

Rosendorff et al. described a possible founder effect of FA among the Afrikaner, based on prevalence, which we wanted to verify by using genealogical information[3]. This could also lead to the identification of possible founder cases and the establishment of a database of high risk Afrikaner family members who are at risk of being FA heterozygotes.

3. To determine the mutations present in the parents of FA children, limit the genealogy to the carriers of one or two mutation types, and compare these findings with those of the first genealogical investigation.

The first genealogical investigation identified a single founder couple. Since then it was found that all investigated Afrikaner FA patients belong to complementation group A, and that in ~90% of cases one of three mutations was involved. This immediately raised the possibility of more diversity in the origins of the disease. The second genealogical investigation was carried out to explore this possibility by tracing the ancestors of the types I and II FANCA mutation carrier parents.

References

1. Auerbach AD, Adler B, Changanti RSK (1981): Pre- and Postnatal diagnosis and carrier detection of Fanconi anaemia by a Cytogenetic method. Pediatrics 67 (1): 128 - 135.
2. Marx MP, Smith S, Heyns AduP, Van Tonder IZ (1983): Fanconi's Anemia: A Cytogenetic Study on Lymphocyte and Bone Marrow Cultures Utilizing 1,2:3,4-Diepoxybutane. Cancer Genet. Cytogenet. 9: 51-60.
3. Rosendorff J, Bernstein R, Macdougall LG, Jenkins T (1987): Fanconi anemia: Another disease of unusually high prevalence in the Afrikaans population of South Africa. Am. J. Med. Genet. 27:793-797.

CHAPTER 3

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FANCONI ANAEMIA: A STATISTICAL EVALUATION OF CYTOGENETIC RESULTS OBTAINED FROM SOUTH AFRICAN FAMILIES

T Pearson[1], S Jansen[1], C Havenga[2], D.K. Stones[2], G Joubert[3].

Depts. of Human Genetics[1], Paediatrics[2] and Biostatistics[3], UOFS, Bloemfontein.

ABSTRACT

Fanconi Anaemia (FA) is a rare autosomal recessive genetic disorder characterized by various phenotypic abnormalities and inevitably resulting in progressive bone marrow failure. The lymphocytes exhibit an increased sensitivity to the clastogenic agents diepoxybutane (DEB) or mytomycin C (MMC), measured as chromosomal aberrations.

Statistical analysis of chromosome aberration yield showed that: (i) differentiation between obligate carriers and the control group was not possible; (ii) homozygotes were clearly distinguishable from heterozygotes as well as controls by analyzing only 20 metaphase spreads per person; (iii) most of the FA patients had only one cell line present as measured by distribution of chromosomal damage among cells analysed; (iv) when the DEB sensitivity of a patient was high, the number of unaffected cells were low.

INTRODUCTION

Fanconi anaemia (FA) is a rare autosomal recessive genetic disorder actuating progressive bone marrow failure, various phenotypic abnormalities, and an increased risk of developing malignant disease, particularly acute myelomonocytic leukemia[1]. This genetically

heterogeneous disorder, in which 7 complementation groups have already been described[2], has an estimated gene frequency of 1 in 300[3].

Schroeder et al. (1964)[4] first demonstrated that FA is associated with abnormal susceptibility of the somatic chromosomes to spontaneous aberrations. Bloom et al. (1966)[5] found no spontaneous chromosomal aberrations in two out of 12 patients, supporting the concept of heterogeneity in Fanconi anaemia. Although Higurashi et al. (1971)[6] found that Fanconi anaemia patients' chromosomes were radiosensitive it was Sasaki (1973)[7] who demonstrated that their chromosomes were highly sensitive to cross-linking agents such as mytomycin C (MMC) and nitrogen mustard. They suggested that Fanconi anaemia patients were defective in one of the repair metabolic pathways, especially the mechanism for removing the interstrand cross links from the DNA. This principle forms the basis of the cytogenetic technique for the diagnosis of Fanconi anaemia. Auerbach and Wolman (1976)[8] determined that the bifunctional alkalyting agent Diepoxybutane (DEB), in a non-toxic concentration for the lymphocytes of normal individuals, increased the chromosome breakage in those of Fanconi anaemia homozygotes. Auerbach et al. (1979)[9] were the first to claim that by utilizing the DEB system, it was possible to distinguish between controls, heterozygotes and homozygotes. Cohen et al. (1982)[10] demonstrated the possibility of misdiagnosing non-Fanconi anaemia patients when the DEB concentration was too high and claimed that it was impossible to distinguish between controls and heterozygotes. They noticed that the amount of chromosomal aberrations per cell obtained from heterozygotes overlap with those of controls. They also affirmed the usage of DEB or MMC to be equally reliable in the cytogenetic diagnosis of Fanconi anaemia. The only study pioneered by Marx et al (1983)[11] detected an increase in DEB sensitivity of lymphocytes of obligate carriers sufficient enough to differentiate them from controls. Kwee et al. (1983)[12] described a case of a FA patient who showed hypersensitivity to bifunctional alkalyting agents in only a minority of this cultured lymphocytes, the majority being as sensitive as those from healthy controls. They postulated that the clastogen resistant cells had arisen de novo. Auerbach et al. [13] reported a similar phenomenon in 1981, raising the question whether the occurrence of clastogen non-responsive cells, as documented, may be more common among FA patients.

The aim of this study was to determine by means of a statistical evaluation of the yield of chromosomal aberrations obtained in lymphocyte cultures to which DEB was added, the accuracy of differentiating between FA homozygotes, obligate carriers and controls.

MATERIAL AND METHODS

Individuals Studied

Included in this study were individuals whose clinical profiles suggested FA and these patients were referred to this laboratory on the diagnosis of a progressive pancytopaenia, though not evident at birth, as the main haematological criterion. Certain phenotypical characteristics such as growth retardation, assessed on low birth weight or small stature and other demonstrable dysmorphic features, were used as additional criteria. A total of 25 patients, from 22 South African families, were selected and comprised of 18 white, 5 black and 2 of mixed origin. The 44 parents of these patients were classified as obligate heterozygotes. Ten phenotypically normal individuals were randomly selected regardless of sex, race or age to serve as the control group.

Peripheral Blood Lymphocyte Cultures

Phytohemagglutinin (PHA)-stimulated peripheral lymphocytes were used to obtain chromosome spreads for cytogenetic studies, cultured according to conventional procedures[14]. Two sets of cultures were set up containing 0.5 ml heparinized whole blood and 9 ml medium TC199, supplemented with 10% fetal calf serum. Cultures were incubated at 37 ^{0}C for 69 hours[11,13].

Drug treatment

The bifunctional alkalyting agent DEB was used to induce chromosomal aberrations. DEB was diluted with sterile commercially available saline solution to a final concentration of 0.1 μg/ml. The DEB solution was added to only 1 of 2 cultures, 24 hours after initiation. The other culture served as control and was treated identically to the DEB-supplemented cultures in all other aspects.

Scoring Technique

Slides were prepared from both cultures, stained with Giemsa and chromosomal aberrations were scored according to Auerbach et al.[13] and Marx et al. [11] on a maximum of 40 or as many as possible consecutive metaphases containing well-defined chromosome spreads. Structural rearrangements such as chromatid exchange configurations, dicentrics, rings and obvious translocations were scored as 2 breakage events. Chromatid and chromosome breaks were scored as 1 breakage event. Aberrations per cell were calculated as follows:

$$\text{Aberrations per cell} = \frac{\text{Total amount of aberrations detected}}{\text{Total amount of metaphases analysed}}$$

The amounts of normal and aberrant metaphases were also scored for each individual.

Statistical Methods

The aberrations per cell were calculated after analysing 10, 20, 30 and 40 or as many as possible consecutive well spread metaphases in all 25 patients. The mean of the aberrations per cell was calculated for 10, 20, 30 and 40 metaphase spreads of each patient. The difference between the mean aberrations per cell of 10 and 20, 10 and 30, 10 and 40, 20 and 30, 20 and 40, and 30 and 40 metaphase spreads were calculated and summarized by means, standard deviation and 95% limits of agreement. Pearson correlations were calculated between percentage aberrant metaphases, aberrations per aberrant cell and breakage index.

RESULTS

The analysis of Giemsa-stained metaphases did not reveal any stable, constitutional, or acquired clonal chromosome abnormalities. The spontaneous and clastogen-induced chromosome breakage results (aberrations per cell) are shown in Table 1. Unequivocal differentiation between FA homozygotes and the remaining individuals was possible. Although the values obtained, both spontaneous and induced were slightly higher for obligate carriers than for controls, this difference was statistically not significant enough allow accurate distinction between the two groups.

Table 2 represents the sensitivity to DEB of the 25 FA patients, as measured by chromosome aberrations present. The lowest sensitivity to DEB, measured as 0.61 aberrations per cell in 39% of metaphase spreads containing aberrations, was observed in the cells of patient 1.1, being of mixed origin. However, in another patient (21.1) of the same ethnic origin, the second highest DEB-induced value of 12.9 aberrations per cell was recorded. On average the lymphocytes of black patients were more sensitive to DEB than those of their white counterparts, but overlap of values ranged from a low 1.3 in patient 3.1 (Black) to a high of 15.68 in patient 15.1 (White). A sizable measure of difference was also encountered within each ethnic group. A notable correlation was obtained between the DEB-sensitivity of the

patient, measured as the breakage index and aberrations per aberrant cell (r = 1.00). The difference between these two measurements is that breakage index represents number of the average aberrations occurring in all metaphases analysed, whereas aberrations per aberrant cell only takes damage to affected cells into account. The correlation between % aberrant metaphases and aberrations per aberrant cell was 0.67 and with breakage index 0.72. Therefore, greater sensitivity to DEB will be reflected by an increase in breakage index, and vice versa.

Individuals 5.1, 5.2, 10.1, 10.2, 13.1 and 13.2 (Table 2) represent 3 sets of siblings affected by FA of 3 different families. Lymphocyte cultures of all 6 patients were set up at the same time and treated in identical fashion. The difference in breakage index between sibs exceeds the standard deviation for FA homozygotes (4.19, Table 1) in families 5 (6.51) and 13 (8.43).

The minimum amount of DEB-stressed metaphases needed for analysis to obtain reliable as well as repeatable results is reflected in Table 3. Unfortunately it was possible to analyse 40 metaphases in only 9 FA patients, with breakage indices ranging from 1.00 to 7.75 aberrations per cell. The DEB-stressed lymphocyte cultures of another 9 patients each produced at least 30 well defined metaphases. The narrow 95% limits of agreement and the small standard deviation of 20 metaphase spreads compared to 30 and 40, indicate that the analysis of 20 metaphase spreads are enough to obtain an accurate diagnosis of FA homozygotes (Table 4).

DISCUSSION

The composition of FA homozygotes in this study is 18 white, 5 black and 2 of mixed origin. The majority of the white FA homozygotes are Afrikaans-speaking, known in South Africa as Afrikaners, which is similar to the observation of Rosendorff et al.(1987)[15].

A considerable interpatient variation was observed in the chromosome breakage results obtained spontaneously and with clastogenic stressing (Tables 1 and 2). The aberrations however, both in number and type, seen in the clastogen-stressed lymphocyte cultures of FA homozygotes are distinctive for these patients and differ remarkably from that found in the lymphocyte cultures of obligate carriers and controls. A small increase in mean aberrations per cell, both stressed and unstressed, of obligate carriers was detected, but was insufficient to enable differentiation between obligate carriers and controls. Although most of the FA patients and obligate carriers included in this investigation, were the same individuals as

reported by Marx et al.[11], it was not possible to verify their results. It is important to emphasize that the volume of DEB in the culture medium is minimal and the slightest variation in technique may have a substantial influence on results obtained. Since higher concentrations of clastogen in the medium are known to prompt the misdiagnosis of unaffected persons as FA homozygotes, it seems improbable that increase in DEB concentrations can be used to distinguish between carriers and non-carriers.

The interracial differences of sensitivity to DEB as seen in Table 2, (e.g. 4 of the 5 black patients showed higher values for mean aberrations per cell as their white counterparts) are not significant and do not differ in extent to interracial, inter- or intrafamilial differences.

Kwee et al. (1983)[12] reported an FA case where 60% of cells failed to show any damage, even at the highest concentration of clastogen. Patient 1.1, Table 2, shows great resemblance to this case. Waisfisz et al. (1999)[16] found that a proportion of T-lymphocytes (80%, 58% and 30%) were refractory to cross-linker induced breakage, suggesting mosaicism. We suspect a correlation between sensitivity of cells to alkylating agents, measured as breakage index, and the amount of cells containing aberrations, measured as the percentage aberrant cells.

We conclude that induced chromosome breakage supports the diagnosis of FA homozygotes and still remains an important diagnostic tool, since it is the only general screening strategy to detect FA homozygotes of known and as yet unknown mutations. The variety of mutations and possible founder effects will determine future screening strategies based on mutation screening.

The breakage index for each patient did not differ significantly whether 10, 20, 30 or 40 spreads were analysed, especially if the main purpose of the investigation is either to rule out or to confirm a cytogenetic diagnosis of FA. In particular, the critical 95% limits of agreement and the negligible standard deviation between the results of 20 metaphase spreads compared to 30 and 40, indicate that it is ineffective to analyze more than 20 metaphase spreads. The authors therefore propose that analysis of a minimum of 10 to 20 cells is mandatory for accurate cytogenetic diagnosis of FA.

References

1. Duckworth-Rysiecki G, Hulten M, Mann J, Taylor AMR (1984): Clinical and Cytogenetic diversity in Fanconi's anaemia. J. Med. Genet. 21: 197-203.
2. Joenje H, Levitus M, D'Andrea A, Garcia-Higuera I, Pearson T, van Berkel CGM, Rooimans MA, Morgan N, Mathew CG, Arwert F (2000): Complementation Analysis in Fanconi Anemia: Re-assignment of the Reference FA-H patient to group A. Am. J. Hum. Genet. 99:1529.
3. Swift M (1971): Fanconi's Anaemia in the Genetics of Neoplasia. Nature 230:370-373.
4. Schroeder TM, Tilgen D, Kruger J, Vogel F (1976): Spontane Chromosomenaberrationen bei Familiärer Panmyelopathie. Hum. Genet. 32: 257-288.
5. Bloom GE, Warner S, Gerald PS, Diamond LK (1966): Chromosome Abnormalities in Constitutional Aplastic Anemia. N. Engl. J. Med. 6: 8-14.
6. Higurashi M, Conen PE (1971): In vitro Chromosomal Radiosensitivity in Fanconi's Anemia. Blood 38: 336-342.
7. Sasaki MS, Tonomura A (1973): A High Susceptibility of Fanconi's Anemia to Chromosome Breakage by DNA Cross-linking Agents. Cancer Res. 33: 1829-1836.
8. Auerbach AD, Wolman SR (1976): Susceptibility of Fanconi's Anaemia Fibroblasts to Chromosome Damage by Carcinogens: Nature 261: 494-496.
9. Auerbach AD, Warburton D, Bloom AD, Chaganti RSK (1979): Prenatal Detection of the Fanconi Anemia Gene by Cytogenetic Methods. Am. J. Hum. Genet. 31: 77-81.
10. Cohen MM, Simpson SJ, Honig GR, Maurer HS, Nicklas JW, Martin AO (1982): The Identification of Fanconi Anemia Genotypes by Clastogen Stress. Am. J. Hum. Genet. 34: 794-810.
11. Marx MP, Smith S, Heyns AduP, Van Tonder IZ (1983): Fanconi's Anemia: A Cytogenetic Study on Lymphocyte and Bone Marrow Cultures Utilizing 1,2:3,4-Diepoxybutane. Cancer Genet. Cytogenet. 9: 51-60.
12. Kwee ML, Poll EHA, van de Kemp JJP, de Koning H, Eriksson AW, Joenje H (1983): Unusual response to bifunctional alkalyting agents in a case of Fanconi anaemia. Hum. Genet. 64:348-387.

13. Auerbach AD, Adler B, Changanti RSK (1981): Pre- and Postnatal diagnosis and carrier detection of Fanconi anaemia by a Cytogenetic method. Pediatrics 67 (1): 128 - 135.

14. Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA (1960): Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell Res. 20:613-616.

15. Rosendorff J, Bernstein R, Macdougall L, Jenkins T (1987): Fanconi Anemia; Another Disease of Unussually High Prevalence in the Afrikaans Population of South Africa. Am. J. Med. Genet. 27:793-797.

16. Waisfisz Q, Morgan NV, Savino M, de Winter JP, van Berkel CG, Hoatlin ME, Ianzano L, Gibson RA, Arwert F, Savoia A, Mathew CG, Pronk JC, Joenje H (1999): Spontaneous functional correction of homozygous Fanconi anaemia alleles reveals novel mechanistic basis for reverse mosaicism. Nature Genet. 22(4): 379-83.

Table 1 The mean index of chromosomal breakage with standard deviation at a 95% confidence interval.

	Spontaneous aberrations	Induced aberrations
FA homozygotes	0,29 ± 0,17	5,83 ± 4,19
Obligate heterozygotes	0,08 ± 0,06	0,13 ± 0,06
Controls	0,02 ± 0,02	0,07 ± 0,09

Table 2 Chromosome breakage of DEB stressed FA homozygote lymphocytes

Patient	Race	Metaphases analysed	Aberrant metaphases	% Aberrant metaphases	Aberrations per aberrant cell	Breakage Index
1.1	c	28	11	39	1.55	0.61
2.1	w	40	17	42	2.35	1.00
3.1	b	20	17	85	1.53	1.30
4.1	w	40	29	72	2.69	1.95
5.1	w	14	14	100	8.57	8.57
5.2	w	32	23	72	2.87	2.06
6.1	w	40	37	92	4.08	3.78
7.1	w	40	36	90	4.47	4.03
8.1	w	40	37	92	4.97	4.60
9.1	w	19	18	95	5.33	5.05
10.1	w	10	9	90	8.33	7.50
10.2	w	40	37	92	5.57	5.15
11.1	w	40	37	92	6.35	5.88
12.1	w	20	20	100	5.95	5.95
13.1	w	22	22	100	15.68	15.68
13.2	w	24	24	100	7.25	7.25
14.1	w	20	18	90	8.22	7.40
15.1	w	40	40	100	7.75	7.75
16.1	w	40	38	95	8.24	7.83
17.1	w	20	19	95	9.32	8.85
18.1	b	27	27	100	9.26	9.26
19.1	b	15	15	100	9.80	9.80
20.1	b	30	30	100	10.43	10.43
21.1	c	21	21	100	12.90	12.90
22.1	b	10	20	100	9.80	9.80

c = patients of mixed races, w = Afrikaner patients and b = black patients

Tables 3 Mean number of aberrations per cell in DEB-stressed lymphocytes of FA homozygotes if 10, 20, 30 or 40 consecutive metaphase spreads were analysed.

Patient	Race	Metaphase spreads analysed			
		10[a]	20[b]	30[c]	40[d]
2.1	w	0.60	0.80	1.07	1.00
4.1	w	1.90	1.80	1.83	1.95
8.1	w	1.80	3.30	4.67	4.60
11.1	w	6.10	5.70	6.10	5.88
16.1	w	7.40	7.80	6.70	7.84
10.2	w	4.30	4.95	4.83	5.15
6.1	w	3.70	3.80	3.63	3.78
7.1	w	4.30	4.20	4.17	4.03
15.1	w	8.40	8.60	7.67	7.75
5.2	w	1.40	1.90	1.93	
20.1	b	7.90	9.80	10.43	
1.1	c	0.40	0.50	0.61	
18.1	b	11.40	9.55	9.26	
13.2	w	7.50	7.55	7.25	
13.1	w	14.50	16.35	15.68	
21.1	c	14.70	12.90	12.90	
17.1	w	9.60	8.85	8.85	
3.1	c	1.30	1.30		
14.1	w	7.80	7.40		
12.1	w	5.70	5.95		
9.1	w	4.80	5.05		
19.1	b	8.90	9.80		
5.1	w	7.80	8.57		
10.1	w	7.50			
22.1	b	9.80			

c = patients of mixed races, w = Afrikaner patients and b = black patients

Table 4 Comparison of mean number of aberrations per cell obtained from 10,20.30 and 40 metaphase spreads.

	N	Mean difference	Standard deviation	95% limits of agreement
10 - 20	23	-0.18	0.918	-2.02; 1.65
10 - 30	17	-0.10	1.279	-2.66; 2.46
10 - 40	9	-0.39	1.008	-2.40; 1.62
20 - 30	17	0.05	0.572	-1.10; 1.19
20 - 40	9	-0.11	0.555	-1.22; 0.99
30 - 40	9	-0.15	0.408	-0.96; 0.67

CHAPTER 4

GENEALOGICAL AND MOLECULAR EVIDENCE FOR A FOUNDER EFFECT IN FANCONI ANAEMIA FAMILIES OF THE AFRIKANER POPULATION IN SOUTH AFRICA

Thomas Pearson[1], Neil V. Morgan[2], Alex J. Tipping[2], Lambertus P. Kuyt[3], Christopher G. Mathew[2] and Stander Jansen[1]

1 Division of Human Genetics, Department of Neurology, Faculty of Health Sciences, University of the Orange Free State, P.O. Box 339 Bloemfontein, 9300 South Africa

2 Division of Medical and Molecular Genetics, GKT School of Medicine, 8th Floor Guy's Tower, Guy's Hospital, London Bridge, London SE1 9RT, UK

3 Department of Human Genetics, Free University of Amsterdam, Van der Boechorststraat 7,1081-BT Amsterdam, The Netherlands

ABSTRACT

Fanconi anaemia (FA) is a rare autosomal recessive syndrome characterized by various phenotypic abnormalities and inevitably results in progressive bone marrow failure. The lymphocytes show an increased sensitivity to the clastogenic agents diepoxybutane (DEB) and mytomycin C (MMC), resulting in chromosomal aberrations. The incidence of white South African Afrikaans-speaking FA children, under 16 years of age, living in the Free State and Northern Cape provinces averages 1:22 000 as compared to the ratio of 1:400 000 among South African Black populations and the rest of the world. A founder effect has been postulated as the reason for the high incidence among the white Afrikaans-speaking population. Outcome of the genealogical analyses on 12 families affected by FA confirmed the founder effect as a pivotal factor. A common ancestor was found for

at least one parent of an FA child in all 12 families, and in 7 of these families both parents shared this familiar ancestor lineage. Three common mutations within the Fanconi anaemia A gene were detected in 11 patients tested, which supports the hypothesis of a founder effect. We propose that the type I mutation, present in at least one chromosome of all 11 patients tested, is the original mutation, and that the type II and III mutations were introduced into the Afrikaner population at a later date.

INTRODUCTION

Fanconi anaemia (FA) is an autosomal recessive syndrome that clinically manifests as a progressive aplastic anaemia. In addition to history of low birth weight, variable congenital malformations, growth retardation and abnormal skin pigmentation[1-4] are evident on physical examination. Cells from FA patients show spontaneous chromosome instability and hypersensitivity to DNA cross-linking agents such as mitomycin C (MMC) and diepoxybutane (DEB)[5, 6]. Bone marrow failure may lead to death within a few months or years. In affected individuals there is an increased incidence of malignancies such as acute leukaemias, hepatomas, and squamous cell carcinomas[7].

FA is phenotypically and genetically heterogeneous[8, 9], as indicated by the fact that at least 7 complementation groups (FA-A to FA-G) have been described, representing 7 different genes (*FANCA* - *FANCG*)[9, 10]. The *FANCD* and *FANCE* genes have been mapped to chromosomes 3p22-26 and 6p, respectively[11, 12], while the *FANCA* (16q24.3), *FANCC* (9q22.3), *FANCF* (11p15) and *FANCG* (9p13) genes have been cloned[13-16]. Complementation group A (FA-A) accounts for 60-65% of FA patients[10]. The *FANCA* gene has an open reading frame of 4365 bp encoding a protein of 1455 amino acids, with its coding sequence interrupted by 43 exons[17]. It has a very heterogeneous mutational spectrum, with more than 100 different *FANCA* mutations having been described, but at low mutation detection rates of ~30% - ~ 70%[18-22]. This was probably caused by a high frequency of intragenic deletions, which would not be detected by the majority of screening protocols if they occurred in compound heterozygotes whose one mutant allele was due to a point mutation[20]. Morgan et al. [1999] found a significant correlation between the breakpoints of the large intragenic deletions and the amount of *Alu* repeats in that specific intron, suggesting that this may be a common method for the generation of *FANCA* deletions[20].

This may also indicate a higher mutation rate of *FANCA* as compared to the other FA genes, which could explain why FA-A is by far the most common complementation group in this disorder.

The incidence of FA in children under the age of 16 years in the white Afrikaans-speaking South African population (commonly referred to as Afrikaner) of the Free State and Northern Cape regions rates 1:22 000. A ratio of 1:400 000 is being quoted among South African Black populations and the rest of the world[23,24]. A founder effect has been postulated as the reason for this high occurrence of FA among the Afrikaner population[23]. This hypothesis is supported by the detection of strong linkage disequilibrium between polymorphic markers at the *FANCA* gene locus and the FA mutation-bearing chromosome in South African Afrikaner families'[25]. Mutation screening of the *FANCA* gene in this population revealed the presence of three mutations, which accounted for the majority of cases[26]. A deletion of exons 12-31 (type I), as well as a deletion of exons 11-17 (type II), and a single base pair deletion (3398delA) the type III were noted. An additional mutation, 795-808del (type IV) was detected in only one of the families included in this study.

The Afrikaner population stemmed from Jan van Riebeeck and his party of Dutch settlers who were ordained by the Dutch East India Company to establish a settlement at the Cape of Good Hope (now known as Cape Town) in 1652. According to Theal, almost two thirds of the Afrikaner population in 1795 were of Dutch descent, while one sixth were of French descent, and the remainder of German or other ancestry[27]. He reiterated that the predominance of one group of immigrants over another was in itself not a cardinal factor, but that the time of arrival and the fertility of their marriages were more important. Colenbrander [1902] estimated that by 1806 the Afrikaner people bloodline constituted of 50% Dutch, 27% German, 17% French, and 5.5% of other ancestry because of intermarriage[28]. It is therefore eminent that a descendant(s) of one of these three major ethnic groups introduced the FA gene to the Afrikaner population. FA patients in the Netherlands mainly belong to complementation group C, whereas in Germany and Italy the majority of patients are classified as FA-A[16, 29]. Preliminary results indicate that most French FA patients also belong to complementation group A [Joenje - personal communication]. This information divulges that the countries Germany and France are the most likely candidates of origin of the founder(s). Further support for a founder effect among Afrikaans-speaking South

African FA patients, using genealogical data complemented by molecular results, is presented in this paper.

MATERIALS AND METHODS

Genealogy

Afrikaner families with one or more children affected by FA were identified. Only families whose members showed sensitivity to DEB, and where genealogical evidence of at least 4 to 5 generations was available, were selected and included in this project. Twelve families met these criteria.

Genealogical evidence on the 4 to 5 generations preceding the index case, dating back to 1880 - 1910, was obtained primarily from the families themselves, while information prior to this period was obtained from death notices and books on Afrikaner genealogy. Death notices are retained by the Master of the Supreme Court of South Africa in Pretoria, as part of the documentation on the estates of deceased persons. Records of individuals whom at the time of their death lived in the Free State province and died during or after 1960 are kept in Bloemfontein, while Pretoria retains records of all individuals in the old Transvaal Province whom have died since 1975. Records of individuals who died prior to these dates are stored at the State Archives of the above-mentioned cities. The information from these records was used to complement and to find a correlation with information recorded in the literature on Afrikaner genealogy. The Cyrillic 2® computer program was used to store all relevant data and for drawing family trees.

Molecular analysis

Blood samples were obtained with informed consent. DNA was extracted from the lymphocytes of 11 patients with FA, and 18 parents, according to standard molecular techniques. Different polymerase chain reactions were set up as described in table 1. The 11 FA patients were first screened for the deletion of exons 12 - 31 (type I mutation). The deletion was confirmed in homozygotes by testing for the presence of exons 11, 18 and 31. Patients where exons 10, 18 and 31 were present as a single copy and exons 11 and 17 were absent, were designated compound heterozygotes for the type I and type II mutations. Those patients showing a heterozygous pattern for the type I mutation were confirmed as compound heterozygotes for the type I and type III

mutations, or for the type I and type IV mutations, by DNA sequencing. All 18 parents were first screened for the type I mutation. In families where the affected child was a compound heterozygote for the type I and II mutations, the presence of the type II mutation was verified in the parent who was not a carrier of the type I mutation, using dosage analysis[20].

RESULTS

The first two FA patients found to be related were SD ($\overline{\underline{X}}$II-2) and PS ($\overline{\underline{X}}$I-3) (Fig. 1). They share the common ancestors JFS ($\overline{\underline{VIII}}$-3) and MSS ($\overline{\underline{VIII}}$-4), 4 generations back from SD on the maternal side, and 3 generations back from PS on the paternal side. Another inter-familial relationship was found between CCWS ($\overline{\underline{X}}$-1) and JG ($\overline{\underline{XI}}$-1) (Fig. 2). They share paternal ancestors MECS ($\overline{\underline{VII}}$-1) and HAS ($\overline{\underline{VII}}$-2), 3 and 4 generations back, respectively. A third familial relationship established was between SO ($\overline{\underline{XII}}$-5) and ZMM ($\overline{\underline{X}}$-12) (Fig. 3). In this case the common ancestors were DDM ($\overline{\underline{V}}$-9) and ACMM ($\overline{\underline{V}}$-10), 6 generations back from SO and 5 generations back from ZMM, both on the paternal side. The surnames Botha, Swanepoel and van der Merwe occurred more frequently in these 6 families than the other common Afrikaner surnames, suggesting that they may be candidate surnames for the founder(s). However, no subsidiary family ties could be established between these 3 groups, or between 4 additional families, over a period 10 to 13 generations.

Genealogical information became available on another two families where the surname Nel appeared frequently in the line of descent. Upon considering the surname Nel as that of a possible common ancestor, it soon became evident that this surname was commonly referred to in the ancestry of the other 10 families (Table 2). This led to the deduction that there is a linkage between the first 6 families investigated and the other families (Figs. 1–4). All 12 families shared common ancestors, in the persons of Guillaume Nel and his wife Jeanne de la Batt (Fig. 5), who disembarked on South African soil on 5th June 1688 from France, via the Netherlands. Guillaume and Jeanne had 10 children, 4 of whom (PWN, HV, JN and JP) were the ancestors of 19 of the 24 parents in the 12 FA families (Table 3). Genealogical information was incomplete for four of the remaining five parents, and was unobtainable for one individual, who as a child, was put up for adoption.

Four mutations, comprising a deletion of exons 12 to 31 (type I), a deletion of exons 11 to 17 (type II), a single base pair deletion in exon 34 (type III), and a 14 base pair deletion in exon 9 (type IV) were detected in the 11 FA patients (Table 4). The type I mutation was detected in at least one chromosome of all 11 patients. The type I mutation was present in 63% (14/22) of the 22 chromosomes analysed, type II in 14% (3/22), type III in 18% (4/22), and type IV in 4% (1/22). The distribution of mutations in the FA parents descended from PWN, HV, JN and JP is given in Table 5. This shows that 9 of the 19 FA parents traced back to the Nel family were carriers of the type I mutation, whereas 2 were type II carriers and 3 were carriers of type III and 1 a carrier of type IV.

DISCUSSION

We suggest that two French Huguenots who arrived in South Africa in 1688 from France via the Netherlands, introduced a mutated FANCA gene into the Afrikaner population. Guillaume Nel and Jeanne de la Batt were found to be the common ancestors of 12 families belonging to this ethnic group, each with one or more children in the present generation affected by FA. Our genealogical investigations, extending over 13 generations, have shown that at least one parent of an FA patient(s) in each of these 12 families can trace their ancestry back to one of four children of the Guillaume Nel and Jeanne de la Batt couple.

When a founder effect is present in an autosomal recessive genetic disease, it can be expected that in most cases both parents of an affected individual, homozygous for a specific mutation, will be related to the founder(s). We have shown that in at least 7 (58%) of the 12 families investigated in this study, both parents are offspring of one of 4 Nel sibs, while the remaining 5 families all have one parent who is descended from one of these four. Although the ancestry of 5 parents cannot be traced back to Guillaume and Jeanne Nel (Table 3), the possibility that they descend from the Nel couple cannot be excluded.

The proposal that the founder for FA in the Afrikaner population of South Africa was either Guillaume Nel or his wife Jeanne de la Batt is substantiated by the fact that 4 of their 10 children (40%) are ancestors of the 12 FA families investigated. This assumption is close to the parameters of the anticipated 50% carrier rate for an autosomal recessive disease when one of the parents is a carrier.

Two of the major events in 17th and 18th century French history were the Revocation of the Edict of Nantes, in October 1685, and the French Revolution, in 1789. The Revocation of the Edict of Nantes led to an exodus of the French Huguenots to Germany (especially to Brandenburg), and also to South Africa. Emigration to certain areas of Italy only started after the French Revolution. According to Sagarra [1977], most merchants and industrialists in the Ruhr area of Germany were Calvinists who descended from the French Huguenots[30].

The high incidence of a disease thought to be as result of a founder effect, is usually characterized by the predominance of one or a few mutations, known as founder mutations. Several examples of these founder mutations are documented in the Afrikaner population, for instance: familial hypercholesterolemia (FH), where 3 mutations account for more than 90% of all clinically affected cases, and variegate porphyria (VP), where the R59W mutation accounts for >90% of cases[31, 32]. However, by means of genealogical references, pinpointing the probable founders of an hereditary disease in South Africa has only been accomplished for a few diseases, e.g. variegate porphyria[33]. Besides this being one of the major achievements of the present investigation, a database of possible carriers of the FANCA gene has also been created. The molecular results specify the mutations involved, and greatly simplify the molecular diagnosis of FA in the Afrikaner population. The mutational status of Afrikaner FA patients is similar to that in FH and VP with type I, II and III mutations accounting for 21 of the 22 mutant alleles tested (95%) in the samples submitted for investigation.

We postulate that the type I mutation, present in at least one chromosome of all 11 FA patients, represents the founder mutation, and that the other 3 mutations were introduced to the Afrikaner population at a later date, hence their lower prevalence. The fact that some of the carriers of these other mutations also trace their ancestry to Guillaume and Jeanne Nel may be explained by later inter-marriage. The genealogical data collected during this investigation suggest the strong possibility that the French Huguenots Guillaume Nel and Jeanne de la Batt were the founders who introduced the mutated FANCA gene into the Afrikaner population of South Africa. We will continue this genealogical investigation. The geographical distribution of FA in South Africa is variable, characterized by areas with higher prevalence, which in itself merits further investigation and will probably also shed more light on the origins of the disease in this country.

The molecular findings confirm the presence of at least 4 mutations among the offspring of the proposed founders, a situation similar to that for other founder-related hereditary diseases in the Afrikaner population. As more information becomes available, especially regarding the ancestry of parents with mutation types II and III, it will be interesting to determine whether this leads to other possible founders. Pinpointing the founders of an autosomal recessive disease will always be impeded by the fact that carriers are usually without clinically recognisable symptoms. Possible links between the FANCA gene in the South African White population and its European counterparts merit further investigation.

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References

1. Gordon-Smith, E.C., & Rutherford, T.R. (1991) *Hematology* **28**, 104-112.
2. Auerbach, A.D., Ghosh, R., Pollio, P.C., & Zhang, M. (1989) In: *Fanconi anemia: clinical, cytogenetic and experimental aspects*, eds. Schroeder-Kurth T.M., Auerbach, A.D., & Obe, G. (Springer-Verlag, Berlin), pp.71-82.
3. Auerbach, A.D., Rogatko, A., & Schroeder-Kurth, T.M. (1989) *Blood* **73**, 391-396.
4. Glanz, A., & Frazer, F.C. (1982) *J. Med. Genet.* **19**, 412-416.
5. Schroeder, T.M., Anschuts, F., & Knopp, A. (1964) *Hum. Genet.* **1**, 194-196.
6. Sasaki, M.S., & Tonomura, A. (1973) *Cancer Res.* **33**, 1829-1833.
7. Schroeder, T.M., Tilgen, D., Kruger, J., & Vogel, F. (1976) *Hum. Genet.* **32**, 257-288.
8. Strathdee, C.A., Duncan, A.M.V., & Buchwald, M. (1992) *Nat. Genet.* **1**, 196-198.
9. Strathdee, C.A., Gavish, H., Shannon, W.R., & Buchwald, M. (1992) *Nature* **356**, 763-767.

10. Joenje, H., Levitus, M., Waisfisz, Q., D'Andrea, A., Garcia-Higuera, I., Pearson, T., van Berkel, C.G.M., Rooimans, M.A., Morgan N.V., Mathew, C.G., & Arwert F. (2000) *Am. J. Hum. Genet.* **67**, 759-762.

11. Whitney, M., Thayer, M., Reifsteck, C., Olson, S., Smith, L., Jakobs, P.M., Leach, R., Naylor, H., Joenje, H., & Grompe M. (1995) *Nat. Genet.* **11**:341-343.

12. Waisfisz, Q., Saar, K., Morgan, N.V., Altay, C., Leegwater, P.A., de Winter, J.P., Komatsu, K., Evans, G.R., Wegner, R., Reis, A., *et al.* (1999) *Am. J. Hum. Genet.* **64**, 1400-1405.

13. The Fanconi anaemia/Breast Cancer Consortium (1996) *Nat. Genet.* **14**, 324-328.

14. Lo Ten Foe, J.R., Rooimans, M.A., Bosnoyan-Collins, L., Alon, N., Wijker, M., Parker, L., Lightfoot, J., Carreau, M., Callen, D.F., Savoia, A., *et al.* (1996) *Nat. Genet.* **14**, 320-323.

15. de Winter, J.P., Waisfisz, Q., Rooimans, M.A., van Berkel, C.G.M., Bosnoyan-Collins, L., Alon, N., Carreau, M., Bender, O., Demuth, I., Schindler, D., *et al.* (1998) *Nat. Genet.* **20**, 281-283.

16. de Winter, J.P., Rooimans, M.A., van der Weel, L., van Berkel, C.G.M., Alon, N., Bosnoyan-Collins, L., de Groot, J., Zhi, Y., Waisfisz, Q., Pronk, J.C., *et al.* (2000) *Nat. Genet.* **24**, 15-16.

17. Ianzano, L., D'Apolito, M., Centra, M., Savino, M., Levran, O., Auerbach, A.D., Cleton-Jansen, A., Doggett, N.A., Pronk, J.C., Tipping, A.J., *et al.* (1997) *Genomics* **41**, 309-314.

18. Levran, O., Erlich, T., Madgalena, N., Gregory, J.J., Batish, S.D., Verlander, P.C., & Auerbach, A.D. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 13051-13056.

19. Savino, M., Ianzano, L., Strippoli, P., Ramenghi, U., Arslanian, A., Bagnara, G.P., Joenje, H., Zelante, L., & Savoia, A. (1997) *Am. J. Hum. Genet.* **61**, 1246-1253.

20. Morgan, N.V., Tipping, A.J., Joenje, H., & Mathew, C.G. (1999) *Am. J. Hum. Genet.* **65**, 1330-1341.

21. Tachibana, A., Kato, T., Ejima, Y., Yosuke, T., Shimizu, T., Yang, L., Tsunematsu, Y., Sasaki, M.S. (1999) *Hum. Mutat.* **13**, 237-244.

22. Wijker, M., Morgan, N.V., Herterich, S., van Berkel, C.G.M., Tipping, A.J., Gross, H.J., Gille, J.J.P., Pals, G., Savino, M., Altay, C., *et al.* (1999) *Eur. J. Hum. Genet.* **7**, 52-59.

23. Rosendorff, J., Bernstein, R., Macdougall, L., & Jenkins, T. (1987) *Am. J. Med. Genet.* **27**: 793-797.

24. Smith, S., Marx, M.P., Jordaan, C.J., & van Niekerk, C.H. (1989) In: *Fanconi Anemia: Clinical, Cytogenetic and Experimental Aspects*, eds. Schroeder-Kurth, T.M., Auerbach, A.D. & Obe, G. (Springer, Heidelberg), pp. 34-46.

25. Pronk, J.C., Gibson, R.A., Savoia, A., Wijker, M., Morgan, N.V., Melchionda, S., Ford, D., Temtamy, S., Ortega, J.J., Jansen, S., *et al.* (1995) *Nat. Genet.* **11**, 338-340.

26. Tipping, A.J., Morgan, N.V., Gibson, R., Pearson, T., Havenga, C., Gluckman, E., Joenje, H., de Ravel, T., Jansen, S., & Mathew, C.G. (2000) *Proc. Natl. Acad. Sci. USA* (submitted)

27. Pama, C. (1981) in *Genealogies of old South African families*, eds. de Villiers C.C., & Pama C., 2nd ed. (AA Balkema, Cape Town, Rotterdam), pp.xviii-xxv.

28. Colenbrander, H.T. (1902) *De afkomst der Boeren* (Algemeen Nederlandsch Verbond, Dordrecht).

29. Joenje, H, for EUFAR (1996) *Hum. Genet.* **97**, 280-282.

30. Sagarra, E. (1977) *A social history of Germany 1648-1914.* (Methuen, London).

31. Kotze, M.J., Langenhoven, E., Warnich, L., du Plessis, L., & Retief, A.E. (1991) *Ann. Hum. Genet.* **55**, 115-121.

32. Hift, R.J. (1999) *S. Afr. Med. J.* **89**, 10-14.

33. Dean, G. (1971) *The Porphyrias: a story of inheritance and environment.* 2nd ed. (Pitman Medical, London).

Table 1 Primers and PCR conditions of biplex PCR reactions used to detect the major Afrikaner mutations.

Reac-tion	PCR Primers 5' → 3'	Observation	Pro-duct	PCR conditions	
			Size (bp)	Annealing (°C)	$[Mg^{2+}]$ (nM)
1	Forward: AAG AAA ATT CAG AAT TAT GAG TGG Reverse: CAC ATC ATT TTT GCC TCA CAA G	Deletion of exons 12-31	720	58	1.5
	Forward: CCC ACA ACT TTT TGA TCT CTG Reverse: GCT GAC AGC AAG GTT GCT CAC	Presence of exons 12-14	1300		
2	Forward: GAT TGT AGA AGT CTT GAT GGA TGT G Reverse: ATT TGG CAG ACA CCT CCC TGC TGC	Presence of exon 10	259	58	1.5
	Forward: GAT GAG CCT GAG CCA CAG TTT GTG Reverse: AGA ATT CCT GGC ATC TCC AGT CAG	Presence of exon 11	301		
3	Forward: GAT GAG CCT GAG CCA CAG TTT GTG Reverse: AGA ATT CCT GGC ATC TCC AGT CAG	Presence of exon 11	301	58	1.5
	Forward: CCA CAA CTT TTT GAT CTC TGA CTTT G Reverse: GTG CCG TCC ACG GCA GGC AGC ATG	Presence of exon 12	224		
4	Forward: CCA TGC CCA CTC CTC ACA CC Reverse: AAA AGA AAC TGG ACC TTT GCA T	Presence of exon 17	203	58	1.0
	Forward: CGC ACA GCA TGT GGG CCT TTA CC Reverse: GCA CAC CCT GCA GGC ATC AG	Presence of exon 18	333		
5	Forward: CAC ACT GTC AGA GAA GCA CAG CCA Reverse: CAC GCG GCT TAA ARG AAG TGA ATG C	Presence of exon 31	205	58	1.5
	Forward: CTT GCC CTG TCC ACT GTG GAG TCC Reverse: CTC ACT ACA AAG AAC CTC TAG GAC	Presence of exon 32	369		

Table 2 Ancestors of FA patients sharing the surname Nel

FIGURE 1		FIGURE 2	
Pedigree number	Generations separating patient and a Nel ancestor	Pedigree number	Generations separating patient and a Nel ancestor
V-3	6 & 7	VI-2	4 & 5
VI-5	3	V-3	6
		VII-5	4
		III-7	7
		VI-9	4
		VII-12	3

Table 3 Mutual relationship between patients.

PATIENT	PATERNAL ANCESTOR	MATERNAL ANCESTOR
BS	Genealogy incomplete (4)*	Pieter Willem Nel (PWN)
CCWS	Pieter Willem Nel (PWN)	Jean Nel (JN)
DMK	Johanna Pieterse(n) (née Nel) (JP)	Pieter Willem Nel (PWN)
JG	Pieter Willem Nel (PWN)	Unknown (Adoption)
KPVDW	Hester Venter (née Nel) (HV)	Genealogy incomplete (4)*
OS	Genealogy incomplete (6)*	Pieter Willem Nel (PWN)
PS	Jean Nel (JN)	Hester Venter (née Nel) (HV)
RB	Pieter Willem Nel (PWN)	Hester Venter (née Nel) (HV)
RP	Genealogy incomplete (5)*	Pieter Willem Nel (PWN)
SD	Johanna Pieterse(n) (née Nel) (JP)	Jean Nel (JN)
SO	Hester Venter (née Nel) (HV)	Hester Venter (née Nel) (HV)
ZMM	Hester Venter (née Nel) (HV)	Jean Nel (JN)

* Number of generations on which genealogy is available

Table 4 Mutation status of the Fanconi anaemia patients and their parents.

PATIENT	MUTATION STATUS	FATHER		MOTHER	
		ANCESTOR	MUTATION DETECTED	ANCESTOR	MUTATION DETECTED
BS	Type I/I	NF	Type I	Pieter W	Type I
CCWS	Type I/III	Pieter W	Type I	Jean	Type III
DMK	Type I/II	Johanna	Type I	Pieter W	Type II
JG	Type I/III	Pieter W	unknown	adoption	unknown
KPVDW	Type I/II	Hester	Type II	NF	Type I
OS	Type I/I	NF	Type I	Pieter W	Type I
PS	Type I/II	Jean	unknown	Hester	unknown
RB	Type I/I	Pieter W	Type I	Hester	Type I
RP	unknown	NF	unknown	Pieter W	unknown
SD	Type I/IV	Johanna	Type IV	Jean	Type I
SO	Type I/III	Hester	Type I	Hester	Type III
ZMM	Type I/III	Hester	Type III	Jean	Type I

NF = Ancestors not found due to insufficient genealogical data

Table 5 Distribution of the Afrikaner mutations among the four proposed ancestors.

NEL ANCESTOR	NUMBER AND TYPE OF MUTATIONS PRESENT				
	TYPE I	TYPE II	TYPE III	TYPE IV	UNKNOWN
PIETER WILLEM (PWN)	4	1			2
HESTER (HV)	2	1	2		1
JEAN (JN)	1		1		1
JOHANNA (JP)	2			1	
NOT FOUND	3				2

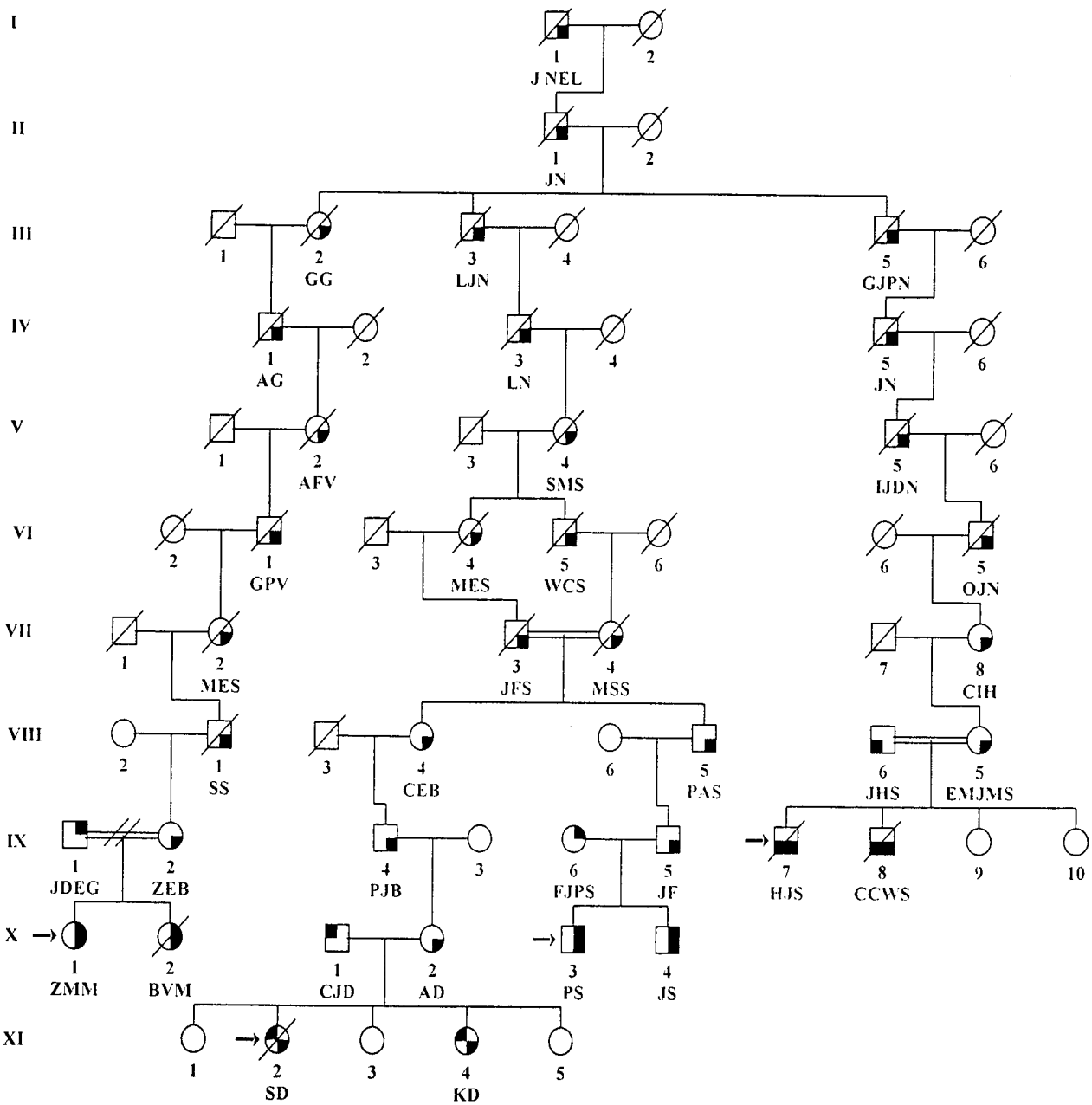

Figure 1. Possible segregation of the FANCA gene via Jean Nel's descendants.

□ Male; ○ Female; ◧◔ Descendants of J Pieterse(n)'s (née Nel); ◪◔ Descendants of J Nel; ◪◔ Descendants of PW Nel; ◧◔ Descendants of H Venter(née Nel); ◧◐ Descendants of PW Nel and Pieterse(n); ◪◔ Descendants of J Nel and J Pieterse(n); ◨◑ Descendants of J Nel and H Venter; ◪◒ Descendants of J Nel and PW Nel.

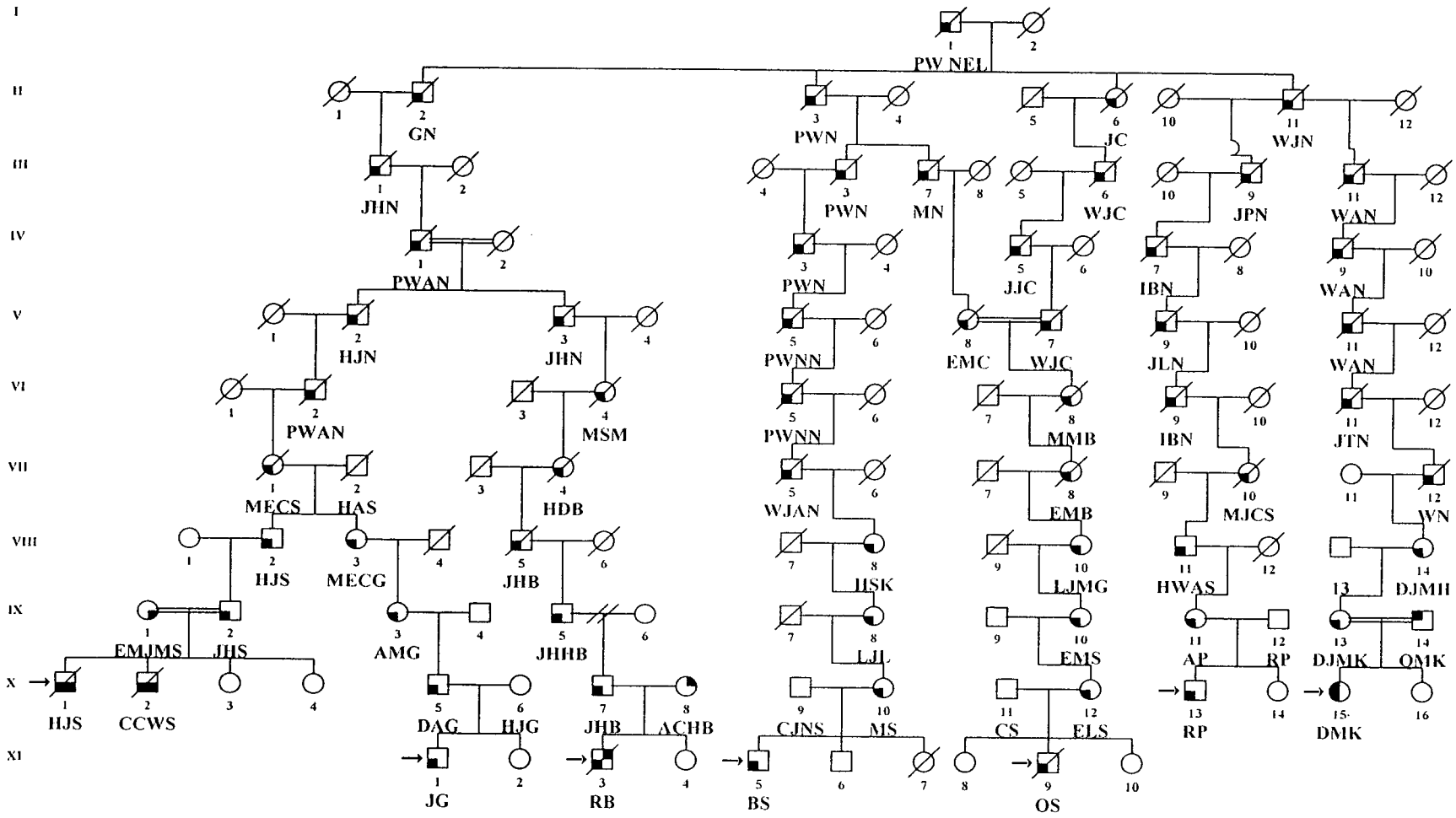

Figure 2. Possible segregation of the FANCA gene via Pieter Willem Nel's descendants.

□ Male; ○ Female; Descendants of J Pieterse(n)'s (née Nel); Descendants of J Nel; Descendants of PW Nel; Descendants of H Venter(née Nel); Descendants of PW Nel and J Pieterse(n); Descendants of PW Nel and H Venter; Descendants of J Nel and H Venter; Descendants of J Nel and PW Nel.

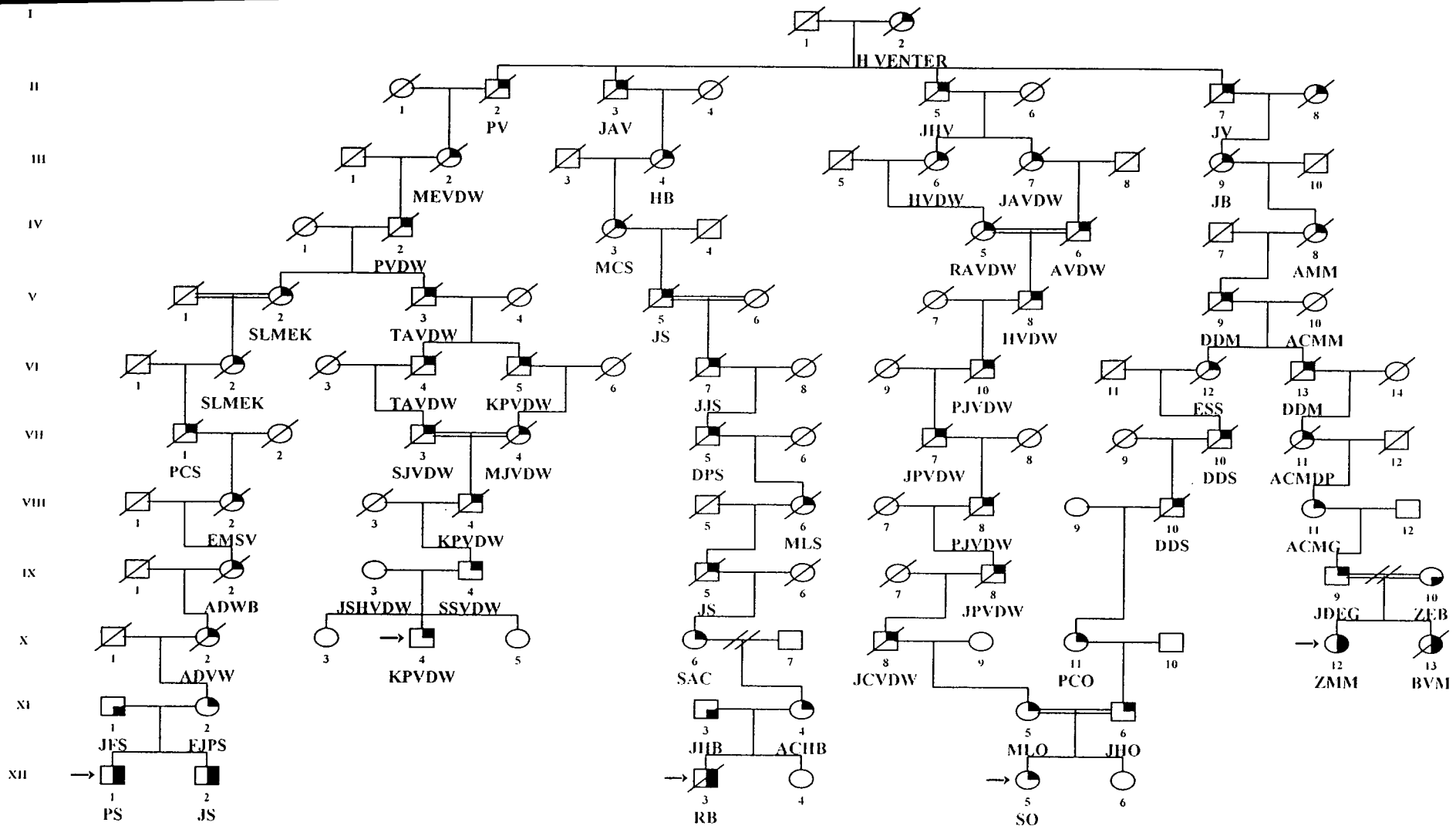

Figure 3. Possible segregation of the FANCA gene via Hester Venter's (née Nel) descendants.

□Male; ○ Female; ◔ Descendants of H Venter(née Nel); ◔ Descendants of J Nel; ◗ Descendants of J Nel and H Venter.

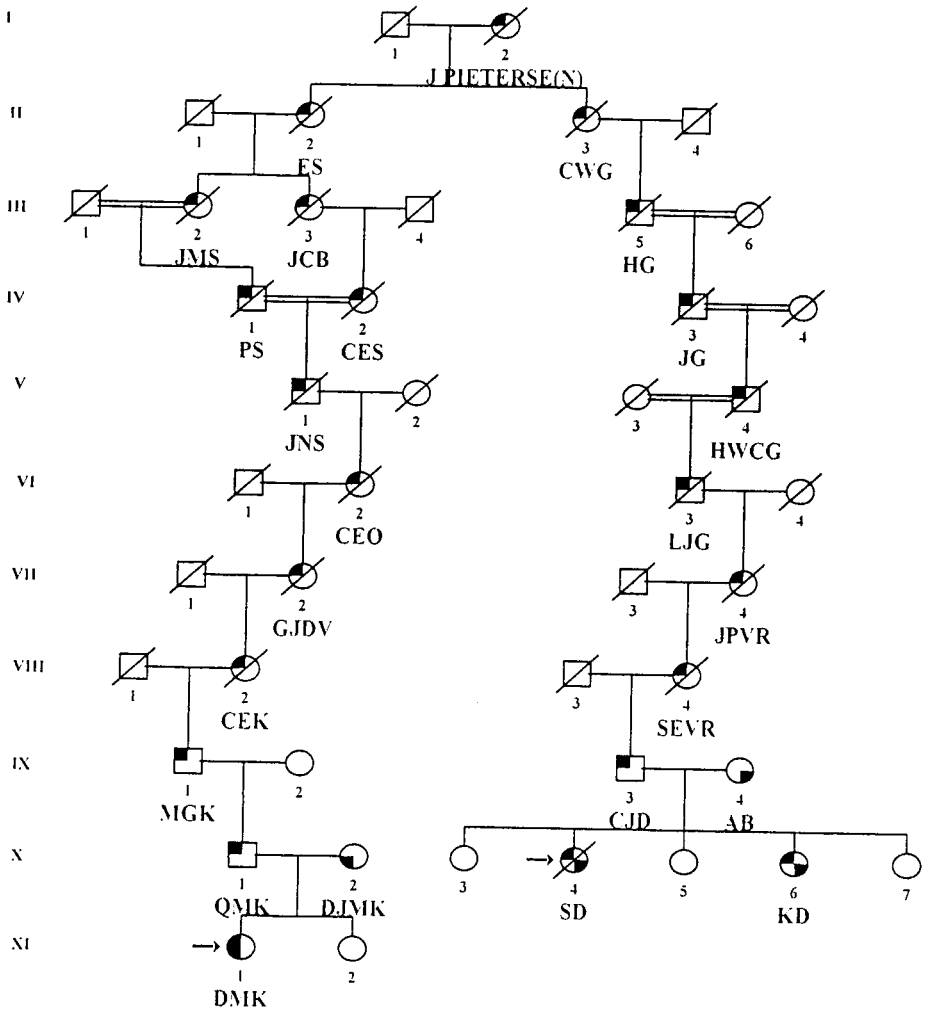

Figure 4. Possible segregation of the FANCA gene via Johanna Pieterse(n)'s (née Nel) descendants.

□ Male; ○ Female; ◪◴ Descendants of J Pieterse(n)'s (née Nel); ◪◶ Descendants of J Nel; ◪◵ Descendants of PW Nel; ◧◐ Descendants of PW Nel and J Pieterse(n); ◪◔ Descendants of J Nel and J Pieterse(n),

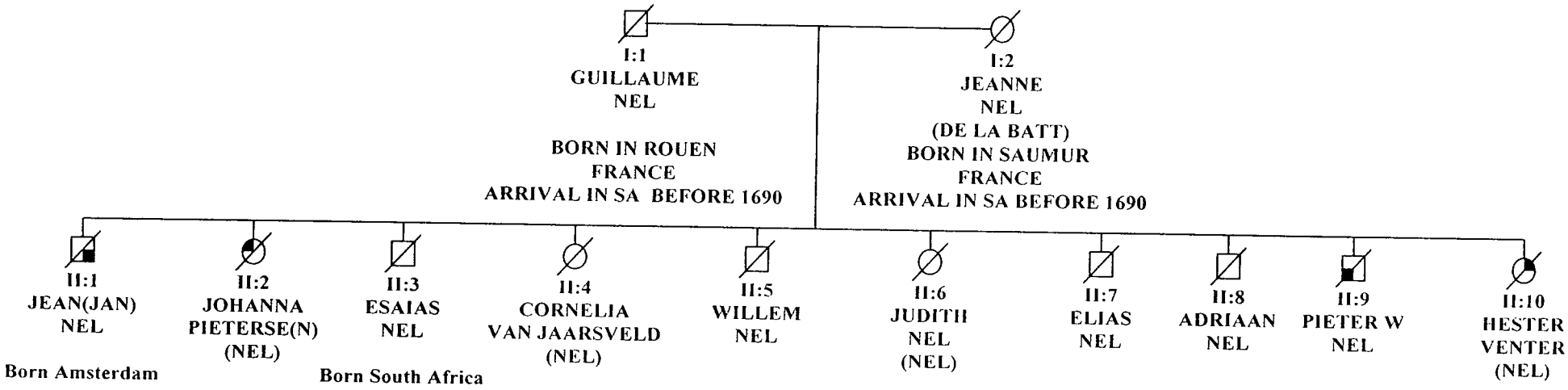

Figure 5. The children of Guillaume Nel and Jeanne de la Batt.

CHAPTER 5

THE GENEALOGY OF FANCONI ANAEMIA PATIENTS HOMOZYGOTIC FOR THE TYPE I AND TYPE II AFRIKANER MUTATIONS

Thomas Pearson[1], Neil Morgan[2], Alex Tipping[2], Lambertus P Kuyt[3], Christopher G Mathew[2], and Stander Jansen[1]

1 Division of Human Genetics, Department of Neurology, Faculty of Health Sciences, University of the Orange Free State, P.O. Box 339 Bloemfontein, 9300 South Africa

2 Division of Medical and Molecular Genetics, GKT School of Medicine, 8th Floor Guy's Tower, Guy's Hospital, London Bridge, London SE1 9RT, UK

3 Department of Human Genetics, Free University of Amsterdam, Van der Boechorststraat 7,1081-BT Amsterdam, The Netherlands

ABSTRACT

Fanconi anaemia (FA), a rare autosomal recessive genetic disorder, is known to occur at a higher than normal incidence amongst the white Afrikaans speaking population of South Africa. The disease is characterised by progressive bone marrow failure, starting predominantly in childhood, various phenotypic abnormalities as well as an increased sensitivity to the clastogenic agents diepoxybutane (DEB) or mytomycin C (MMC), resulting in chromosomal aberrations.

Initial genealogical investigations on 12 families affected by FA but with unknown mutational status, identified Guillaume Nel and/or his spouse Jeanne de la Batt as common ancestors. A founder effect, the cause of the high incidence of FA among this population group, was further

substantiated by results obtained through complementation studies and mutation screening, showing the predominance of complementation group A and mutation types I, II and III.

The existence of three mutation types among the descendants of Guillaume Nel and Jeanne de la Batt raised the possibility of more than one founder pair or family being involved. For this reason an additional genealogical investigation was carried out, limited to seven and six FA parents carrying respectively the Afrikaner types I and II mutations. Apart from Nel, the surname du Preez also featured prominently as founders for the FANCA Afrikaner type I mutation whereas either Venter or du Plessis are possibly founders of the FANCA Afrikaner type II mutation.

INTRODUCTION

The first ships to sail along Southern Africa's coast were expeditions send by Necho, the Egyptian Pharaoh, about six centuries BC[1]. These ships staffed by Phoenician sailors rounded the southern most point during a three-year journey. After Necho's expedition, two thousand years would elapse before Bartholomew Diaz rediscovered the southern point of Africa on his return journey to Portugal and called it the Cape of Good Hope[1].

The first European settlers setting foot on South Africa's soil were eight British men condemned to death during 1614. One of them died after an encounter with the Hottentots and four drowned six months later during an attempt to reach a British ship, ending the first endeavour to establish a settlement at the Cape of Good Hope[1]. In 1652 a Dutch party of 125 persons were successful in establishing a small settlement at the southern most point of Africa[2]. The second sizeable group of settlers, numbering 201, were the French Huguenots who arrived at the Cape between 1688-1692[3].

The Dutch East India Company, whose main purpose was trading with India, governed the expanding Cape colony from 1652 until 1795[4]. During this period a number of Germans working for the said Company, were also relocated to the Cape of Good Hope. The white Afrikaans speaking population of today, known as the Afrikaner, is mainly (94%) descendants of these three groups[5, 6]. Due to the small size of the settler population, intermarriage occurred frequently, thereby increasing the chances of introducing a single defective gene into their offspring. Familial hypercholesterolaemia (FH), Porphyria variegata (VP) and Fanconi anaemia (FA) are good examples of such a phenomenon, also known as a founder effect[7, 8, 9]. The incidence of FA in the Afrikaner is 1:22 000, compared to about 1:400 000 among South

African blacks and the rest of the world[10]. A founder effect has been postulated as the reason for the high incidence of FA among the white Afrikaans speaking population. This hypothesis is supported by the fact that the majority of South African white Afrikaner families carry only the Fanconi anaemia Complementation Group A (FANCA) defective gene.

Fanconi anaemia is an autosomal recessively inherited disease, as opposed to FH and VP, both showing an autosomal dominant inheritance pattern. FA is characterised by low birth weight, growth retardation, aplastic anaemia (often apparent within the first decade of life), variable congenital malformations (especially those of the radius, thumb or kidney), skin pigmentation disorders, and spontaneous chromosomal instability[11, 12]. Bone marrow failure may lead to death within a few months or years[13]. In affected individuals there is an increased incidence of malignancy, such as acute leukaemia, hepatoma, or squamous cell carcinoma. FA is phenotypically and genetically heterogeneous[14]. At least seven complementation groups, i.e., Fanconi Anaemia Complementation Group A (FAA), Group B (FAB), Group C (FAC), Group D (FAD), Group E (FAE) Group F (FAF), and Group G (FAG), have been described, representing seven different genes (FANCA – FANCG) on at least three different chromosomes[15].

The aim of this study was to verify that either Guillaume Nel or Jeanne la Batt was the founder of the Afrikaner type I mutation, based on molecular results, and if not, to identify a possible new founder for the Afrikaner type I mutation.

MATERIAL AND METHODS

Family selection

The selection of families used in this investigation was based on the presence of at least two of the three criteria used in diagnosing an affected member, i.e., cytogenetic, haematological and phenotypic characteristics. The mutation status of FA patients was used as a fourth criterion. Six families of FA patients belonging to Complementation Group A and homozygous for the most common Afrikaner mutation (type I), were selected. Adequate genealogical information was collected on seven parents carrying the Afrikaner FANCA type I mutation (deletion of exon12 to exon 31) whereas six FA parents were carriers for the Afrikaner FANCA type II mutation, a deletion stretching from exons 11 to 17.

Genealogical information

Genealogical information on the preceding four to five generations, dating back to 1880 - 1910, was obtained primarily from the families themselves. Information prior to this period was obtained from death notices and books on the genealogy of the Afrikaner. The Master of the Supreme Court keeps death notices as part of the documentation on the estates of deceased persons. Records of persons who died in the Free State province after 1960 are kept in Bloemfontein, whereas records of all persons who died in the old Transvaal province since 1975 are kept in Pretoria. Records prior to this date are kept at the State Archives of these provinces and cities.

This information was used to complement and find a connection with information recorded in the books on the genealogy of the Afrikaner. The Cyrillic 2® computer program was used to store all relevant data and for drawing family trees.

RESULTS

Mutation type I

In figure 1 all ancestors on the paternal side of CAH and who were born in South Africa, were identified, totalling 506 individuals. One thousand and sixty four persons were identified on the maternal ancestral line of AS, whereas 410 and 357 individuals, respectively, were identified as maternal and paternal ancestors of RB. In the case of LDV, 321 paternal ancestors were identified. Another 248 individuals formed the paternal ancestors of CM. Her maternal ancestors also totalled 248. The founder candidate for FANCA type I in South Africa must therefore be one of these 3154 individuals. It should, however, be kept in mind that it was impossible to trace all ancestral individuals. The only complete pedigree is on the paternal side of CAH, whereas the one on the maternal side of AS is about 95% complete. Information on the other five parents of FA patients homozygous for the type I mutation is more or less 70% complete.

Common Afrikaner surnames such as Bester, Beukes, Delport, Eksteen, Esterhuyzen, Hanekom, Greeff, Koekemoer, Kotze and others, could be excluded as candidate surnames because of their absence or infrequent occurrence among the 3154 individuals. In the selected seven FA parents, 10 Afrikaner surnames, namely Botha, Burger, du Preez, Helmes, Potgieter, Nel, Olivier, Cordier, Snyman and van der Merwe (Figs 1- 10) feature as ancestral surnames in at least five of the seven families.

The first possibility of a founder is Barend Burger or his wife Maria WS van der Merwe (Fig 1). It is only the father of RB and the mother of LC that cannot be traced back to him. This progenitor of the Burger surname in South Africa and his wife Maria WS van der Merwe, daughter of the first van der Merwe immigrant, had five children, and all of them had offspring (Table 1). These five children show linkage to the remaining five FA parents, implicating that they were all carriers of the type I mutation. This is highly unlikely, but not impossible. In addition, only five of the seven FA parents show ancestral links with Burger and van der Merwe, making them less certain contenders.

The second candidate pair, Harmen Potgieter and his wife Isabella Frederiks (Fig 2), had seven children of whom two daughters were childless (Table 1). Six FA parents can be traced back to only one of the Potgieter children (Johannes Potgieter), the seventh FA parent showing no connections. In this case J Potgieter is the common denominator rather than anyone of his two wives, since children of both wives show linkage to the six FA parents.

The possibility of an Olivier being the founder of FA in South Africa is also not without doubt. Two brothers, Ockert (Fig 3) and Hendrik, arrived in South Africa from the Netherlands during the 17th century. The paternal side of CAH shows linkage with Hendrik and his wife Beatrix Verwey but are not related to Ockert (OC), whereas the remaining six FA parents do have Ockert and his wife Aletta Verwey (sister of Beatrix) as common ancestors. This means that either brothers or both their spouses could have been carriers.

Figure 4 shows the relationship of six of the seven FA parents to a Botha ancestor, the paternal side of LDV being excluded. Their candidacy as founders is favoured by the fact that only two of their five children with a progeny show linkage to the FA parents, and that they are both of German origin, where the FA complementation group A is common. On the other hand, only six of the seven FA parents show linkage to the Botha/Kickers offspring.

Louis Cordier and his wife Francoise Martinet (Fig 5) are related to six FA parents through five of their six children. The mother of RB shows no relationship with the Cordier family, thereby excluding Cordier and/or Martinet as only founder possibilities. It also seems statistically disproportionate to have 83% offspring as carriers if one parent was a heterozygote.

The next five candidates, van der Merwe (Fig 6), Snyman (Fig 7), Helmes (Fig 8), Nel (Fig 9) and du Preez (Fig 10) appear as common ancestors of all seven FA parents. Van der Merwe seems unlikely since 10 of Schalk van der Merwe and his wife, Sophia Cloete's children are involved as ancestors, which is not to be expected from a single defective gene inherited in an

autosomal recessive pattern. The Dutch origin of van der Merwe, where Complementation Group A is scarce, is another negative point.

Christoffel Snyman, born in Bengal, and his wife Marguerite de Savoye, are also common ancestors to all seven FA parents. Nine children were born to this couple, five of whom are ancestors to the seven FA parents. In the refined pedigree (Fig 7) it can be seen that their daughter Elsje Snyman, married to Jacobus Botha, are common ancestors to six of the seven FA parents. The Snyman/de Savoye candidacy may only be the result of their relationship to Botha.

Hans Helmes and his wife Geertruy Willemse also feature prominently as possible founders of FA in South Africa (Fig 8), with 50% of their children being ancestors to all seven FA parents, which meets the statistical requirements for an autosomal recessive inheritance pattern. One factor not in their favour is that they are of Dutch origin, where Complementation Group C is the major gene defective in FA patients.

Nel and du Preez (figs 9 and 10) are the last two suggested candidates and both are common ancestors to all seven FA parents. Guillaume Nel and Jeanne de la Batt had 10 children, eight (80%) of whom are ancestors of FA parents. The minimum number of Nel children linking all seven FA parents, however, is four, i.e., Hester (married to Pieter Venter), Pieter Willem, Jean and Elias Nel (fig. 9). This fits the expected carrier frequency if Guillaume Nel or his wife Jeanne de la Batt were carriers of the FAA gene. The progenitors of both Nel and du Preez families immigrated to South Africa during the 17th century as part of the French Huguenots. Three of the six du Preez children are ancestors of the seven FA parents, statistically fitting the 50% criterion for Hercules du Preez or Cecilia D'Atis to be carriers (Fig 10). It becomes even more convincing when the pedigree analysis is limited to the descendants of Phillipe du Preez and Isabella Potgieter (Fig 10). They are common ancestors to six of the seven FA parents.

Mutation type II

Surnames such as Botha, van der Merwe and du Preez occur less frequently in the ancestry of type II mutation carriers than those for the type I mutation, and could therefore be excluded as founders of the FANCA type II mutation in South Africa. The most likely candidates are du Plessis and Venter (figs 11 and 12). Four of the six FA parents have Petrus Venter (baptized on 19 May 1726) and Martha du Plessis (baptized on 7 May 1730) as common ancestors. The other two FA parents share JADP and AEFDP as ancestors. The latter couple are descendants

of both du Plessis (Fig 11) as well as Venter (Fig 12), indicating a close familial relationship between these two surnames during the latter part of the 17th and beginning of the 18th centuries.

If the FANCA type II mutation was present in either Venter or du Plessis, as suggested, the link between types I and II is via Petrus Venter. Petrus Venter is the son of Pieter Venter, who was married to Hester Nel, a common ancestor of the FA type I mutation carriers. Hester Nel was the daughter of the Huguenots Guillaume Nel and Jeanne de la Batt (Fig 10).

DISCUSSION

Two approaches can be followed in the search for the origin of FA among the Afrikaner, keeping in mind that carriers are symptom free. The easier approach is to concentrate on familial relationships between FA families, and to determine who the ancestors of those individuals common to most FA families are. This approach was used in the first investigation. The second and a more time consuming approach used in this study, is to select only those FA families with a specific (type I and II) mutation, determine all their ancestors, and then search for common ancestors. Results obtained with this approach confirm our previous genealogical findings of Hester Venter, Jean Nel and Pieter Willem Nel as common ancestors of FA among the Afrikaner population of South Africa. However, other possibilities such as Botha, Helmes, Potgieter, Snyman, Olivier, van der Merwe, Burger, and especially du Preez, must also be considered as possible founders of the Δexon12-exon31 mutation (type I) in the FANCA gene.

The disadvantage of the second approach is the difficulty in pinpointing a common ancestor among the early generations of the 17th and 18th centuries, as illustrated in the various pedigrees depicted in Figures 1 to 10. The best example is probably the du Preez and Nel families. The Afrikaner type 1 mutation has not yet been described in the countries of origin of the majority of Afrikaners, such as the Netherlands and France, or in Italy. This may suggest a South African origin during the 17th or 18th century, rather than in Europe. If this is the case, than the chances are more likely for Phillipe du Preez or his wife Isabella Potgieter, as originator of the FANCA type I mutation. It should be kept in mind that the ancestors of LDV's great grandfather, PFB (Fig 10), are unknown, leaving the door open that all seven FA parents could be linked to this ancestral couple.

Two surnames, Venter and du Plessis, were identified as possible candidate founders for the type II FANCA mutation among Afrikaners (figs 11 and 12). Although these surnames differ

from those for the type I mutation, (Nel and du Preez), they are all related via Nel and Venter. This explains the occurrence of both type I and type II mutations among the descendants of Guillaume Nel and his wife Jeanne de la Batt in a previous study, at which time the mutation status of carriers was unknown.

Intermarriage among new European immigrants and local Caucasians occurred within the first generation after their arrival at the Cape. If the Afrikaner mutation originated in South Africa, it would therefore be difficult to identify the exact ancestral parents or even a single person among this group. The reason is the high number of consanguineous marriages that occurred in these early generations.

There is a tendency for Complementation Group A to predominate in FA patients of French Huguenot stock, be it in Canada, Germany or Italy. Although the people of Dutch origin were numerically the dominant Caucasian group in South Africa during the 17th century, this does not necessarily mean that they brought any of the common FANCA mutations to this country. This is further supported by the fact that the majority of present day Dutch FA patients have complementation group C (FANCC) mutations.

All those Germans who settled in South Africa during the 17th and 18th were also Protestants, the religion of the Huguenots. This religion was introduced to Germany during the religion wars, starting during the 16th century and ending during the 17th century, when Huguenots immigrated to Germany. Although the surname Venter is of German origin there is still a possibility that they are of Huguenot stock. This implies that all the possible founders of both the type I and II Afrikaner mutations originated from Huguenot stock, either directly (Nel, du Preez and du Plessis) or through a possible relationship (Venter). The Afrikaner comprises of 38% Dutch immigrants. The question may be asked whether the large number of Huguenots found among the ancestors of FA parents is a mere coincidence?

References

1. Hockly HE (1957): Cape of Good Hope. In "The story of the British settlers of 1820 in South Africa" eds Hockly, Juta & Co, Johannesburg. pp 1-12.
2. Botha MC, Pritchard J (1972): Blood group, gene frequencies an indication of the genetic constitution of population samples in Cape Town. S. Afr. Med. J. Suppl 1 April: 1-27.
3. Coertzen P (1988): Oortog en aankoms. In "Die Hugenote van Suid-Afrika 1688-1988". Tafelberg Uitgewers, Cape Town, pp 75-86.
4. Sleigh, D (1980): Jan Compagnie : the world of the Dutch East India Company. Tafelberg, Cape Town.

5. Pama C (1981): "The composition of the Afrikaner people." In: de Villiers CC, Pama C (eds). "Genealogies of old South African families." 2nd ed. Cape Town/Rotterdam: AA Balkema, pp.xviii-xxv.

6. Colenbrander HT (1902): "De afkomst der Boeren." Dordrecht: Algemeen Nederlandsch Verbond.

7. Kotze MJ, Langenhoven E, Warnich L, du Plessis L, Retief AE (1991): The molecular basis and diagnosis of familial hypercholestrolemia in South African Afrikaners. Ann Hum Genet 55:115-121.

8. Hift RJ (1999): The diagnosis of porphyria. S Afr Med J 89:10-14.

9. Dean G (1971): The Porphyrias: a story of inheritance and environment. 2nd ed., London: Pitman Medical.

10. Rosendorff J, Bernstein R, Macdougall L, Jenkins T (1987): Fanconi anemia: another disease of unusually high prevalence in the Afrikaans population of South Africa. Am J Med Genet 27: 793-797.

11. Glanz A, Frazer FC (1982): Spectrum of anomalies in Fanconi anemia. J Med Genet 19: 412-416.

12. Auerbach AD, Ghosh R, Pollio PC, Zhang M (1989a): Diepoxybutane test for prenatal and postnatal diagnosis of Fanconi's anemia. In Schroeder-Kurth TM, Auerbach AD, Obe G (eds). "Fanconi anemia: clinical, cytogenetic and experimental aspects." Berlin: Springer-Verlag, pp.71-82.

13. Gordon-Smith EC, Rutherford TR (1991): Fanconi anemia: constitutional aplastic anemia. Hematology 28:104-112.

14. Auerbach AD, Rogatko A, Schroeder-Kurth TM (1989b): International Fanconi Anemia Registry: Relation of clinical symptoms to diepoxybutane sensitivity. Blood 73: 391-396.

15. Joenje H, Levitus M, Waisfisz Q, D'Andrea A, Garcia-Higuera I, Pearson T, van Berkel CGM, Rooimans MA, Morgan N, Mathew CG, Arwert F (2000): Complementation Analysis in Fanconi Anemia: Re-assignment of the Reference FA-H patient to group A. Am J Hum Genet 67:759-762.

Table 1 Historical information on 10 candidate founder families and their offspring covering the first three generations

SURNAME OF ANCESTOR	COUNTRY OF ORIGIN	DATE OF ARRIVAL	CHILDREN			GRAND-CHILDREN	GREAT GRAND-CHILDREN	TOTAL DESCENDENTS
			TOTAL	SONS	NO OFF-SPRING			
NEL	FRANCE	1690	10	6	0	64	372	446
VAN DER MERWE	NETHERLANDS	1661	13	4	2 S+ 1 D	61	349	423
SNYMAN	BENGAL	1680	9	2	1 S	51	263	323
OLIVIER	NETHERLANDS	1701	12	5	2 S + 1 D	53	257	322
CORDIER	FRANCE	1688	7	3	1 S	39	232	278
BOTHA	GERMANY	1685	8	4	3 D	44	195	246
POTGIETER	GERMANY	1665	7	5	2 D	39	174	220
HELMES	NETHERLANDS	1671	6	1	1 S	28	168	202
BURGER	GERMANY	1691	5	4	0	33	154	192
DU PREEZ	FRANCE	1688	6	3	0	34	152	192

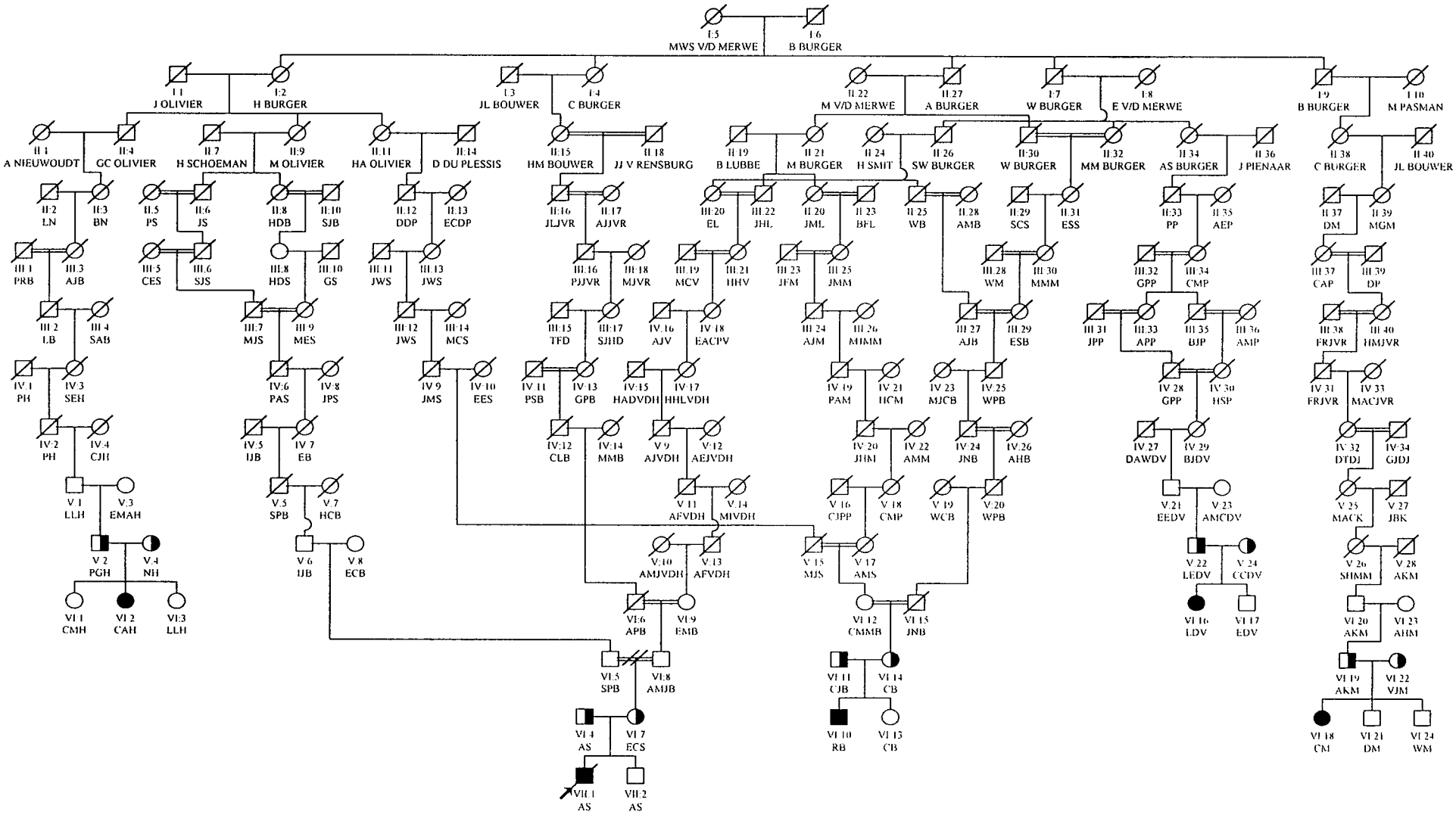

Figure 1: Possible segregation of the FANCA Afrikaner type I mutation if Barend Burger or his wife Maria van der Merwe was the founder of this mutation

□ Male; ○ Female, ■ ● Affected with Fanconi anaemia, ◧ ◑ Carrier of a FANCA mutation.

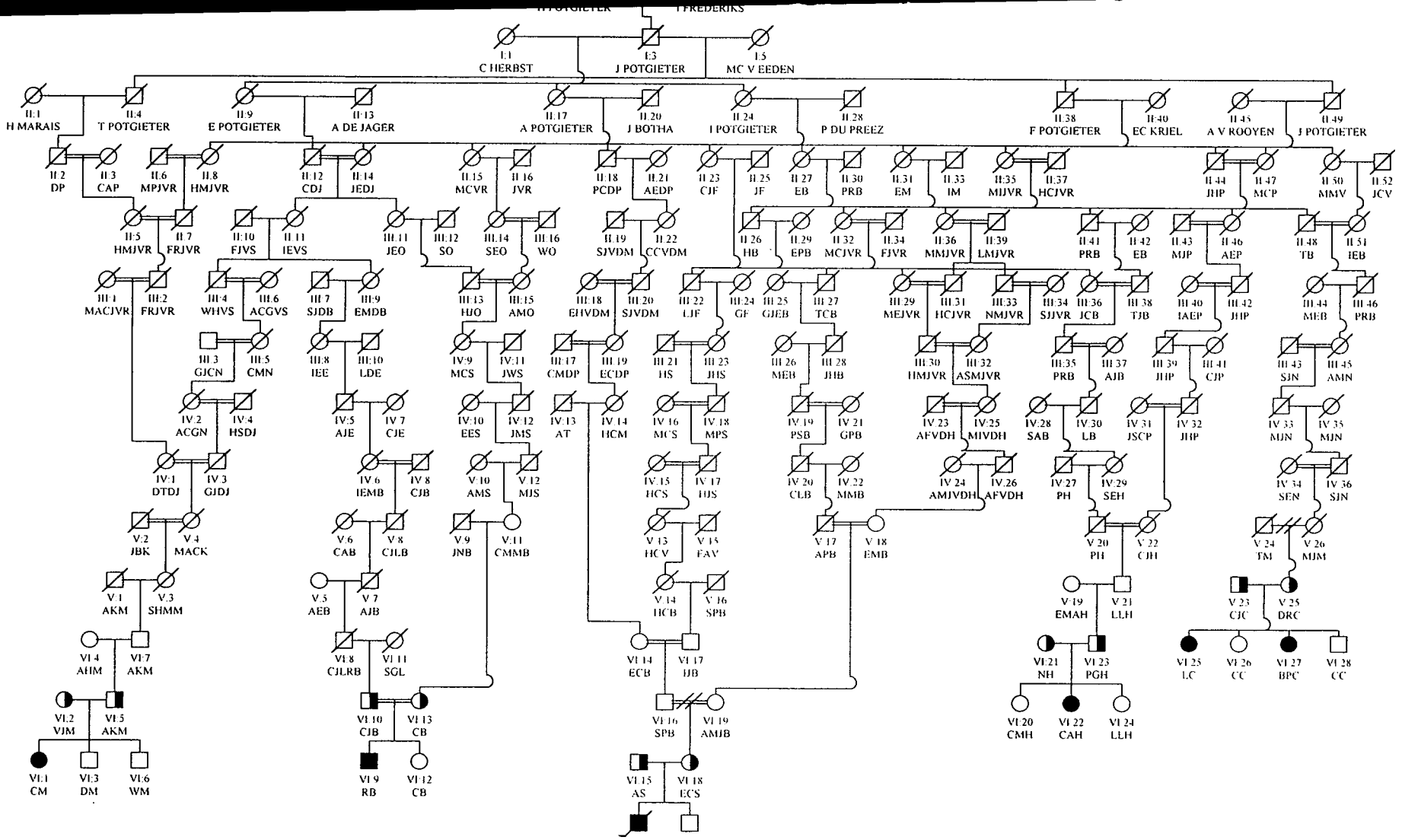

Figure 2: Possible segregation of the FANCA Afrikaner type I mutation if Harmen Potgieter or his wife Isabella Frederiks was the founder of this mutation

□ Male; ○ Female, ■ ● Affected with Fanconi anaemia, ◨ ◑ Carrier of a FANCA mutation.

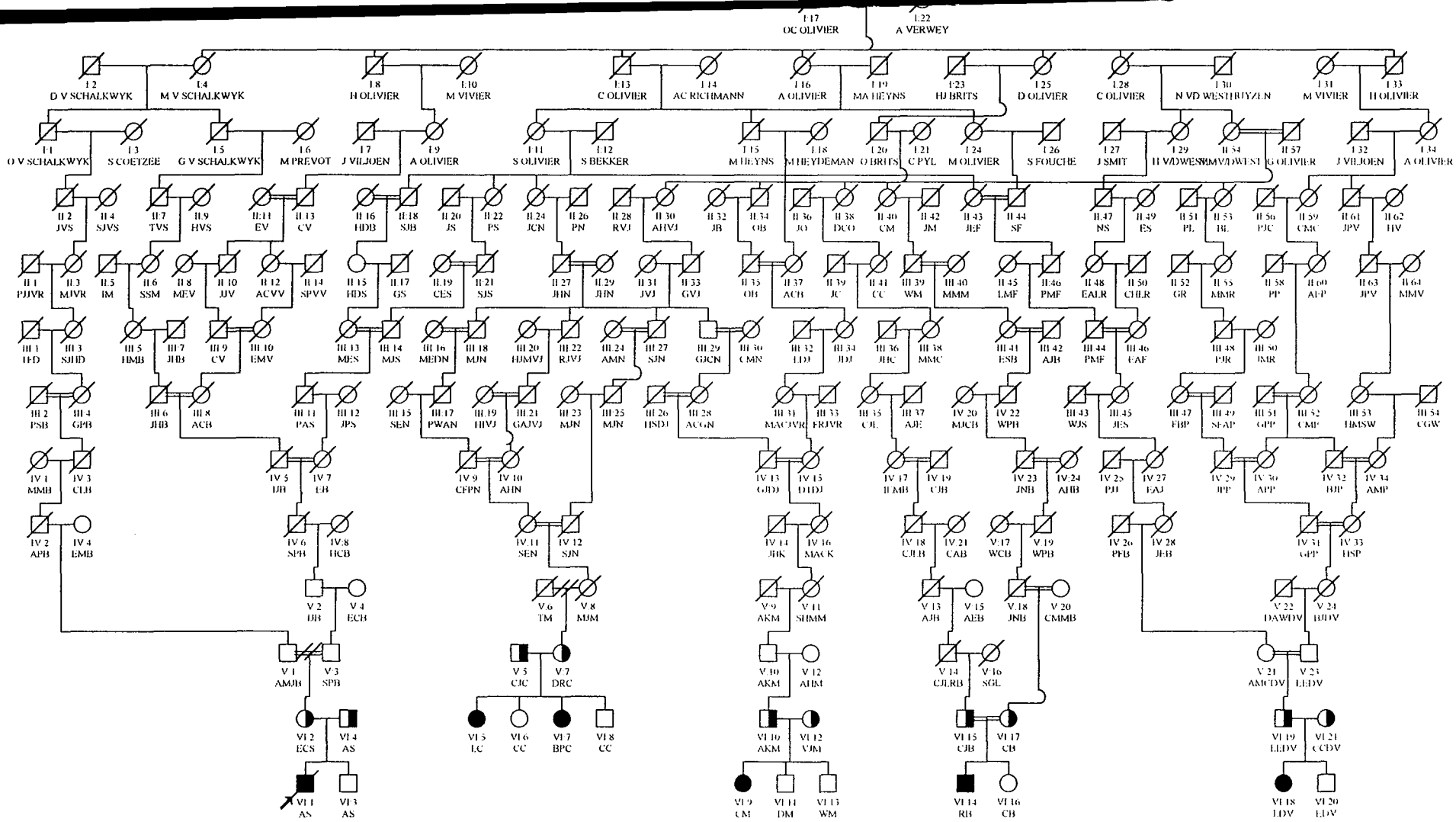

Figure 3: Possible segregation of the FANCA Afrikaner type I mutation if Ockert C Olivier or his wife Aletta Verwey was the founder of this mutation

□ Male; ○ Female, ■ ● Affected with Fanconi anaemia, ◧ ◑ Carrier of a FANCA mutation.

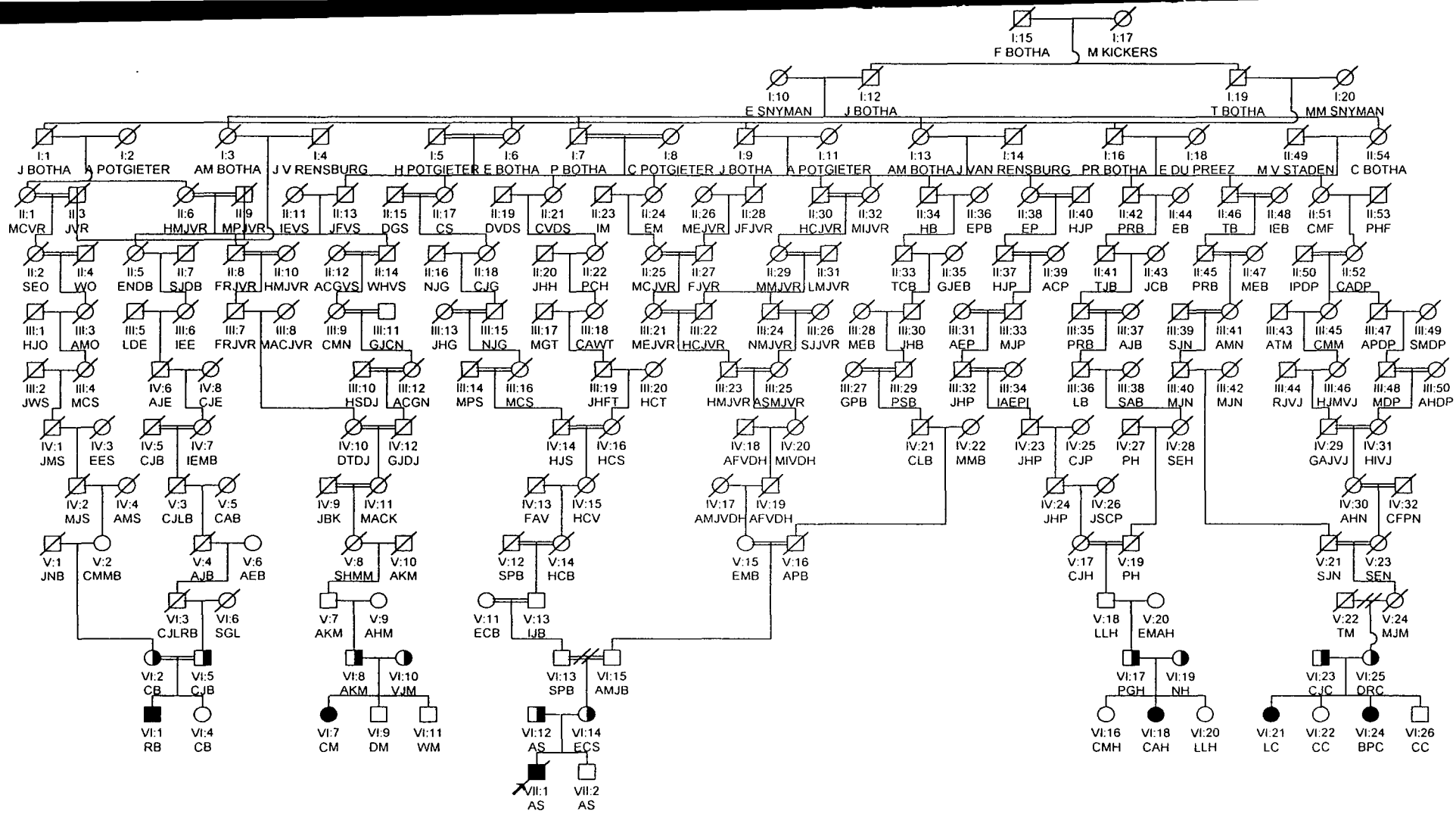

Figure 4: Possible segregation of the FANCA Afrikaner type I mutation if Frederick Botha or his wife Maria Kickers was the founder of this mutation

□ Male; ○ Female, ■ ● Affected with Fanconi anaemia, ◨ ◑ Carrier of a FANCA mutation.

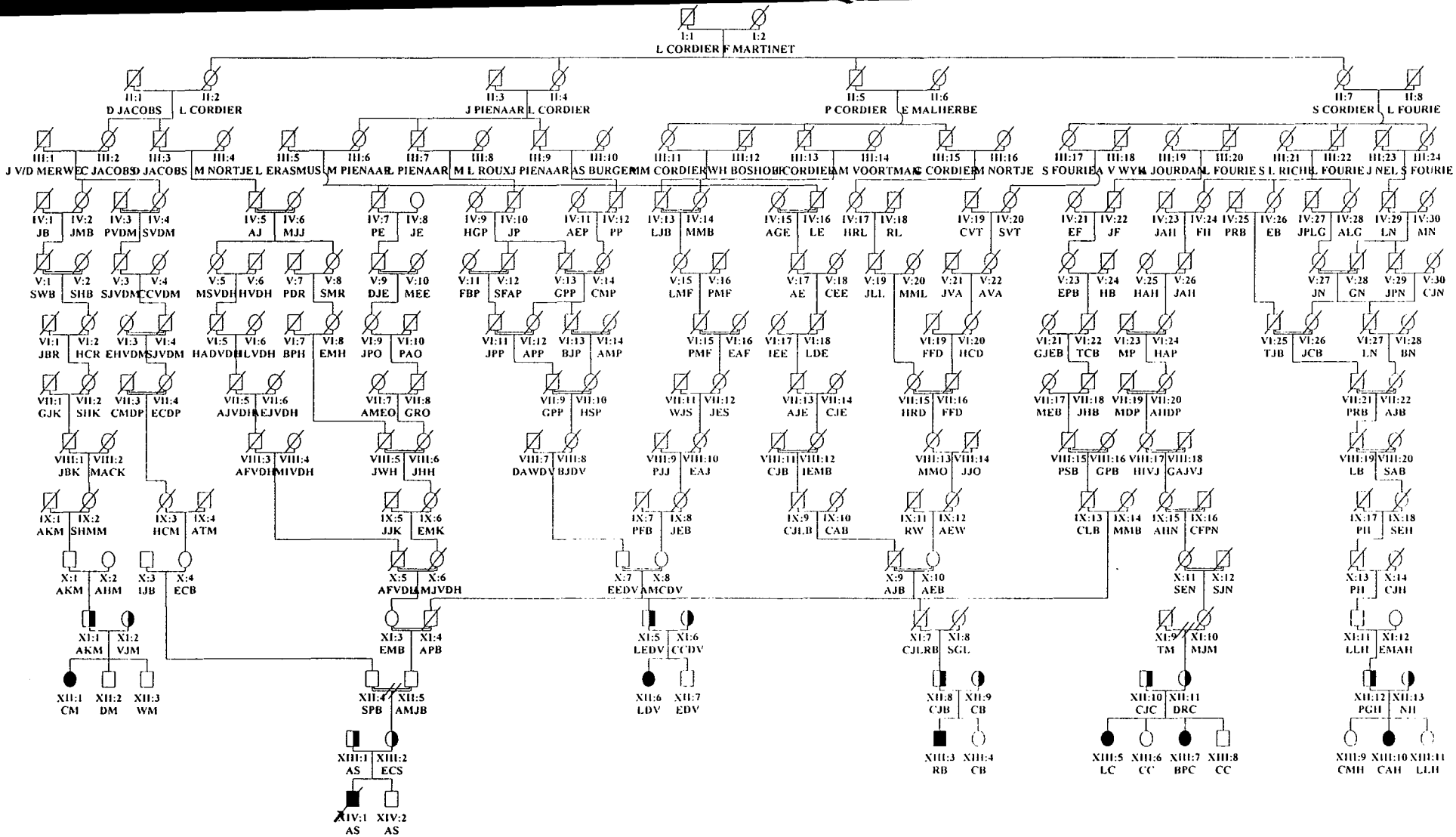

Figure 5: Possible segregation of the FANCA Afrikaner type I mutation if Louis Cordier or his wife Francoise Maritinet was the founder of this mutation

□ Male; ○ Female, ■ ● Affected with Fanconi anaemia, ◨ ◑ Carrier of a FANCA mutation.

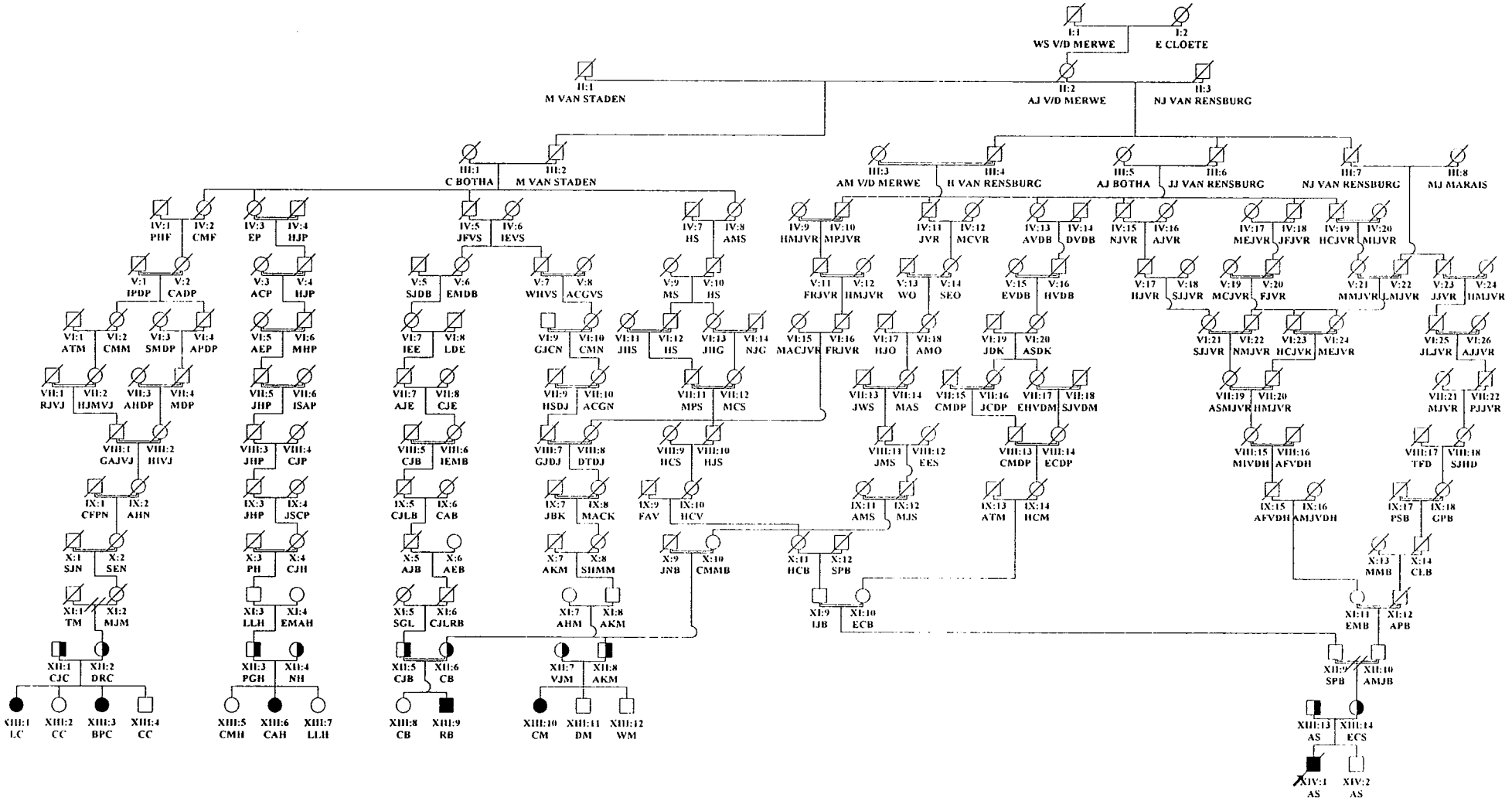

Figure 6: Possible segregation of the FANCA Afrikaner type I mutation if Willem van der Merwe or his wife Elsje Cloete was the founder of this mutation

□ Male; ○ Female, ■ ● Affected with Fanconi anaemia, ◨ ◑ Carrier of a FANCA mutation.

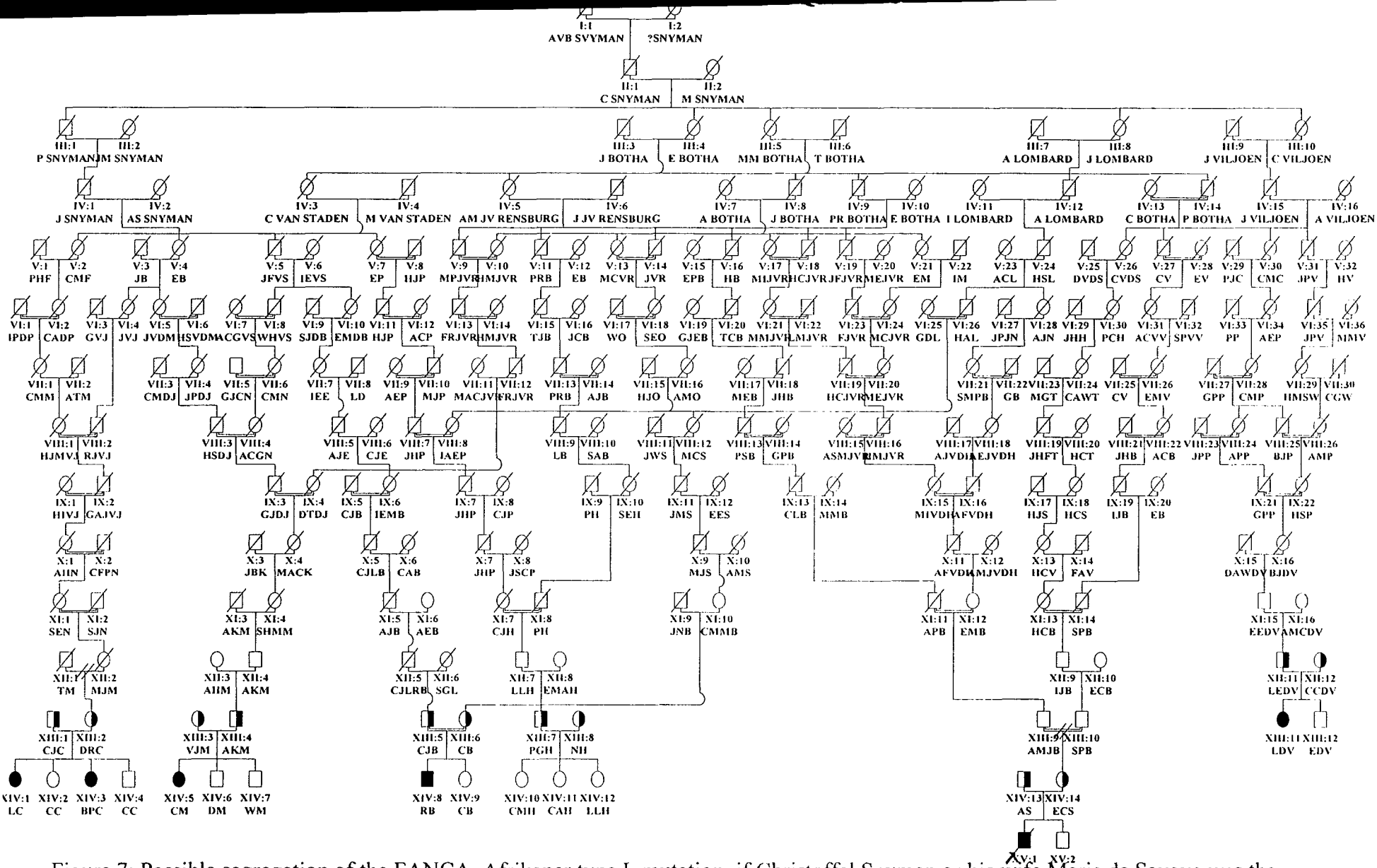

Figure 7: Possible segregation of the FANCA Afrikaner type I mutation if Christoffel Snyman or his wife Maria de Savoye was the founder of this mutation

□ Male; ○ Female, ■ ● Affected with Fanconi anaemia, ◧ ◑ Carrier of a FANCA mutation.

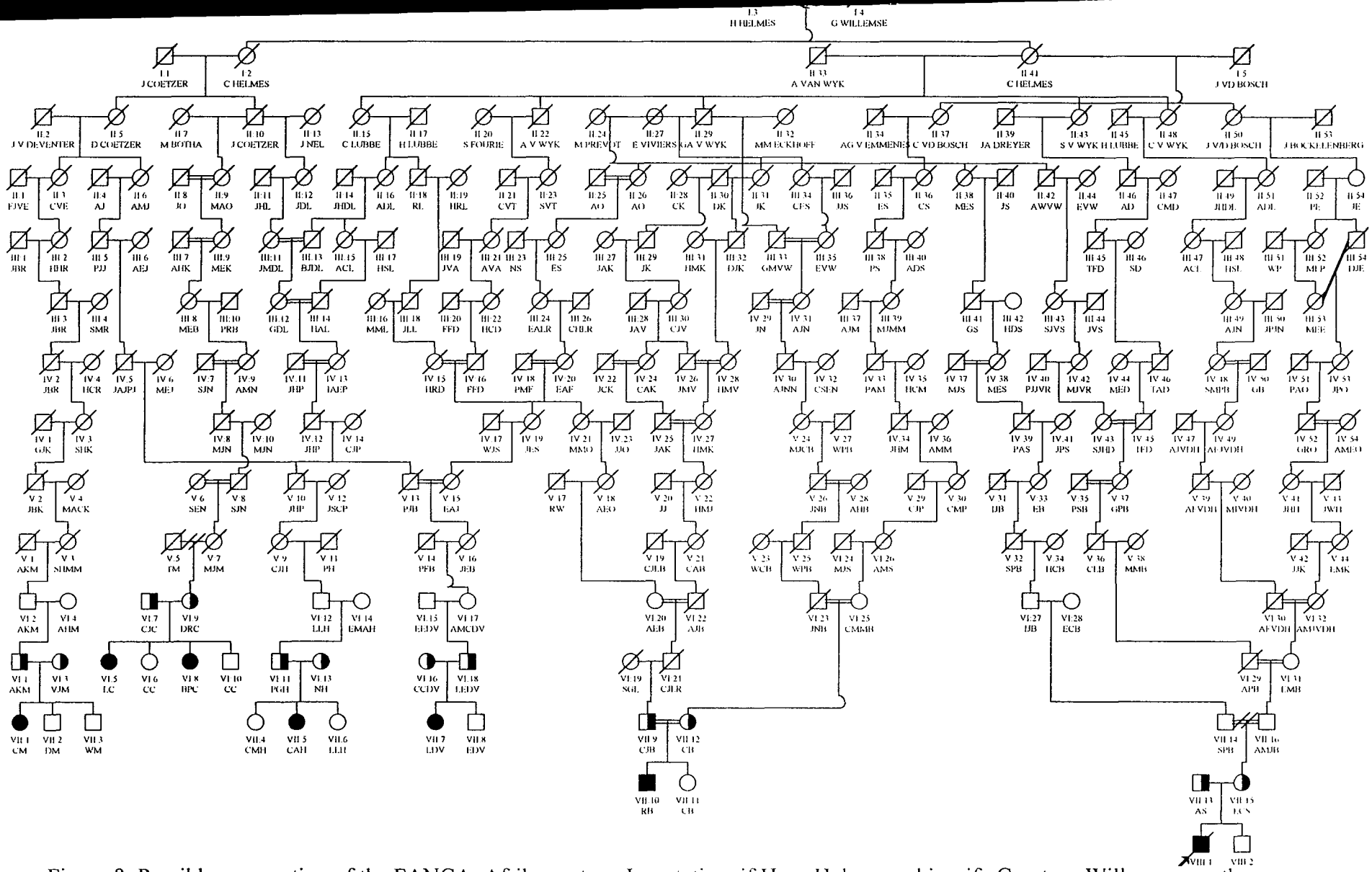

Figure 8: Possible segregation of the FANCA Afrikaner type I mutation if Hans Helmes or his wife Geertruy Willemse was the founder of this mutation

□ Male; ○ Female, ■ ● Affected with Fanconi anaemia, ◨ ◑ Carrier of a FANCA mutation.

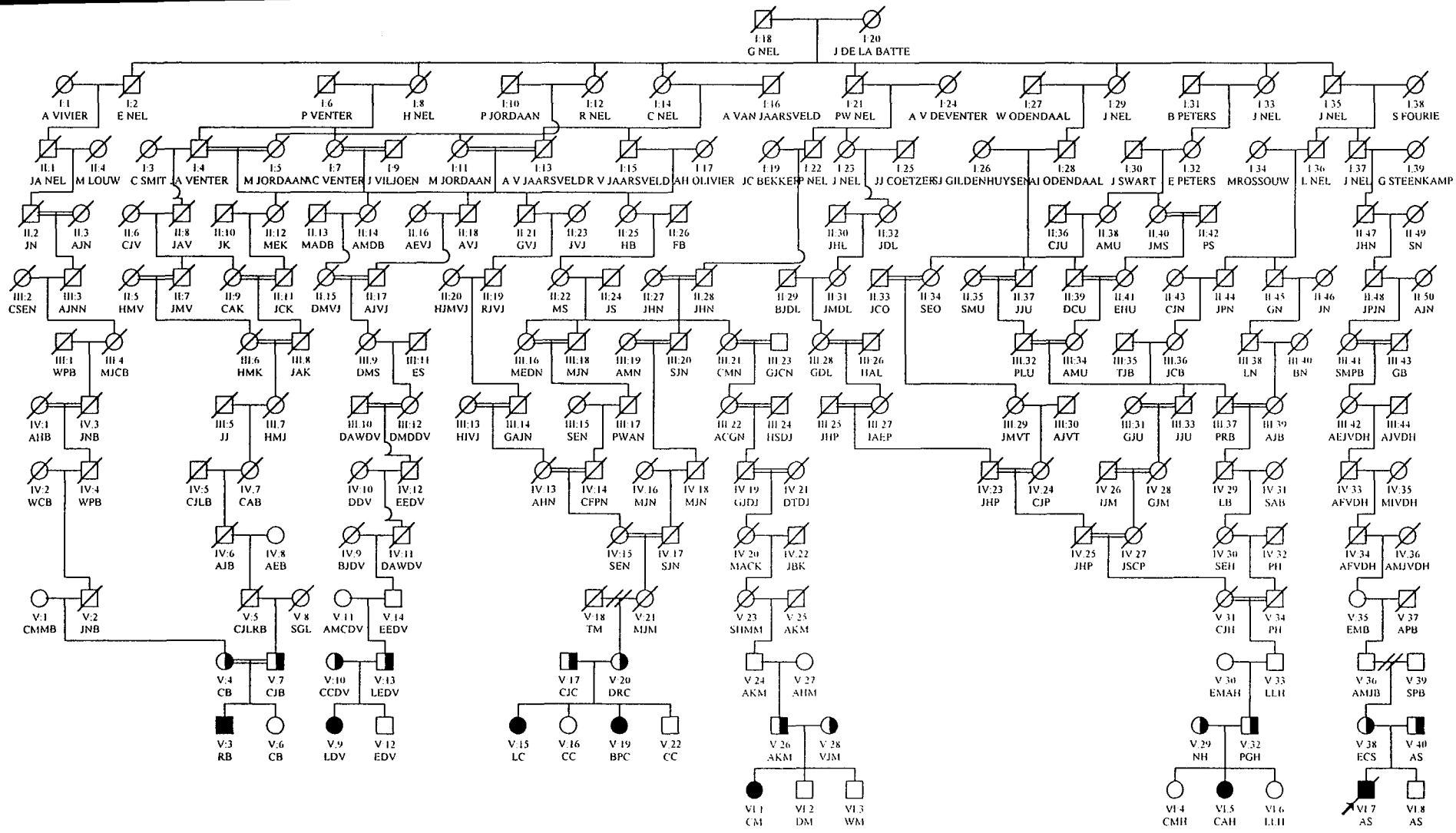

Figure 9: Possible segregation of the FANCA Afrikaner type I mutation if Guillaume Nel or his wife Jeanne de la Batt was the founder of this mutation

□ Male; ○ Female, ■ ● Affected with Fanconi anaemia, ◨ ◑ Carrier of a FANCA mutation.

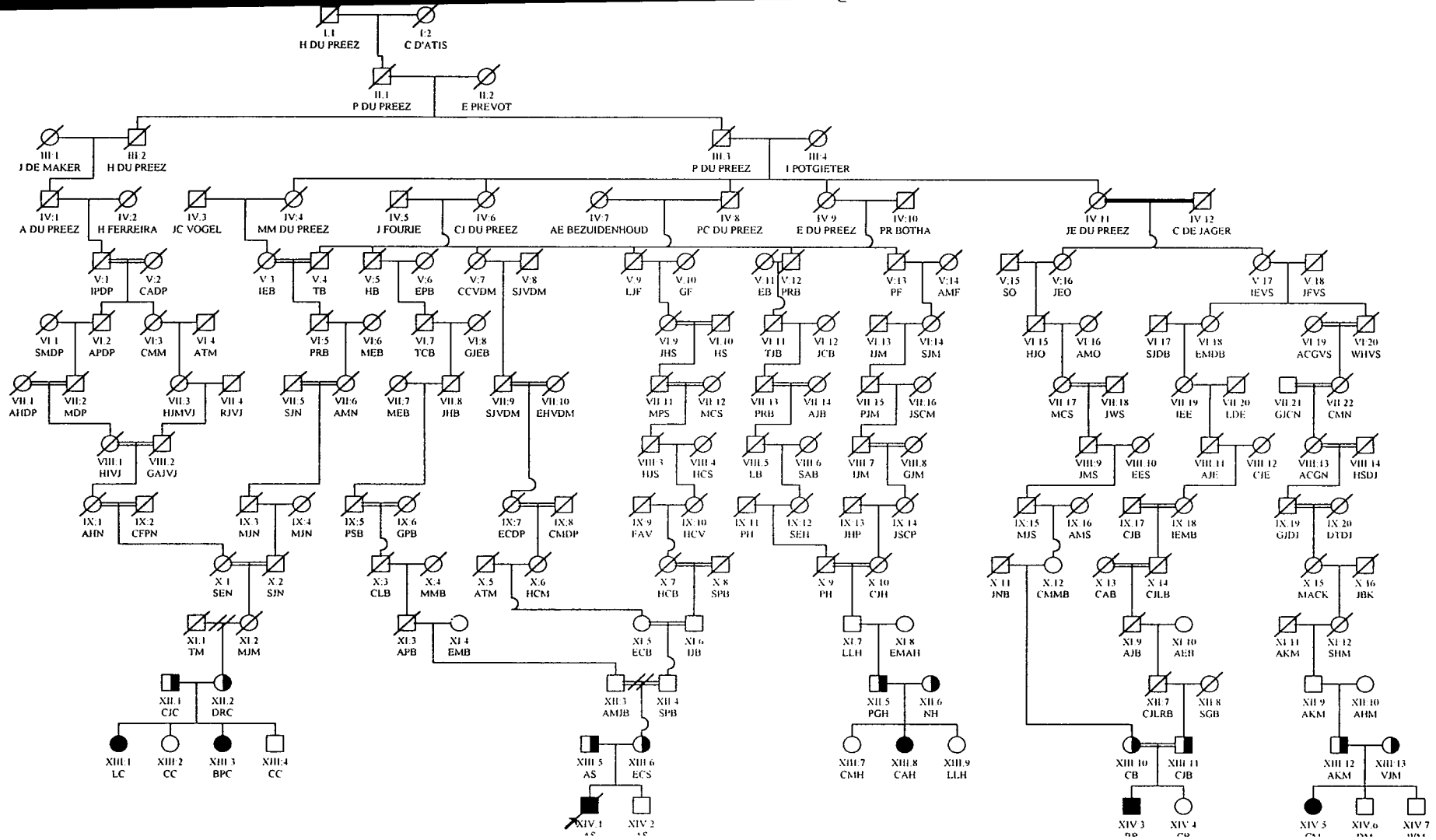

Figure 10: Possible segregation of the FANCA Afrikaner type I mutation if Hercule du Preez or his wife Cecilia D'Atis was the founder of this mutation

□ Male; ○ Female, ■ ● Affected with Fanconi anaemia, ◨ ◑ Carrier of a FANCA mutation.

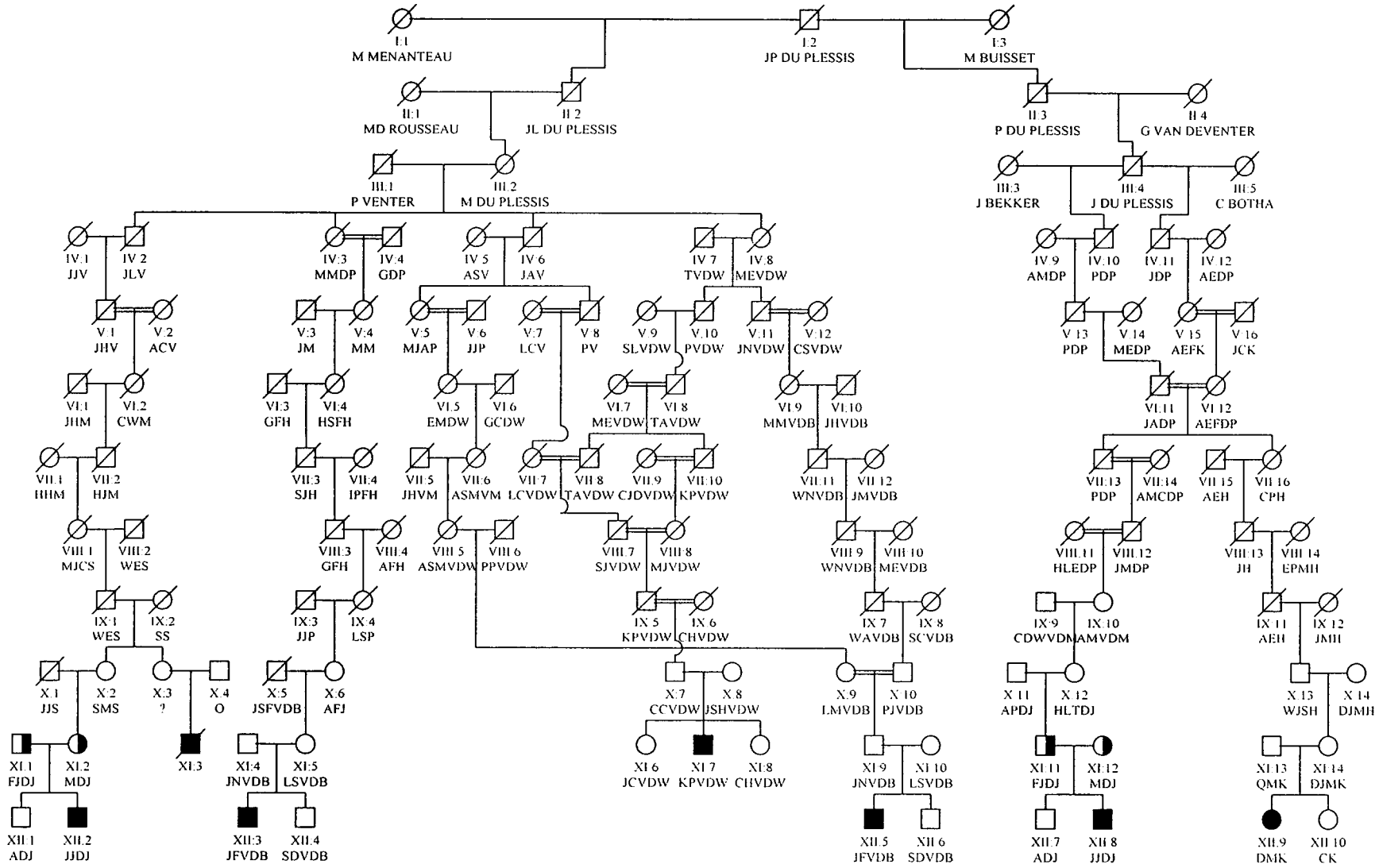

Figure 11: Possible segregation of the FANCA Afrikaner type II mutation if Jean Prieur du Plessis was the founder of this mutation

□ Male; ○ Female, ■ ● Affected with Fanconi anaemia, ◧ ◑ Carrier of a FANCA mutation.

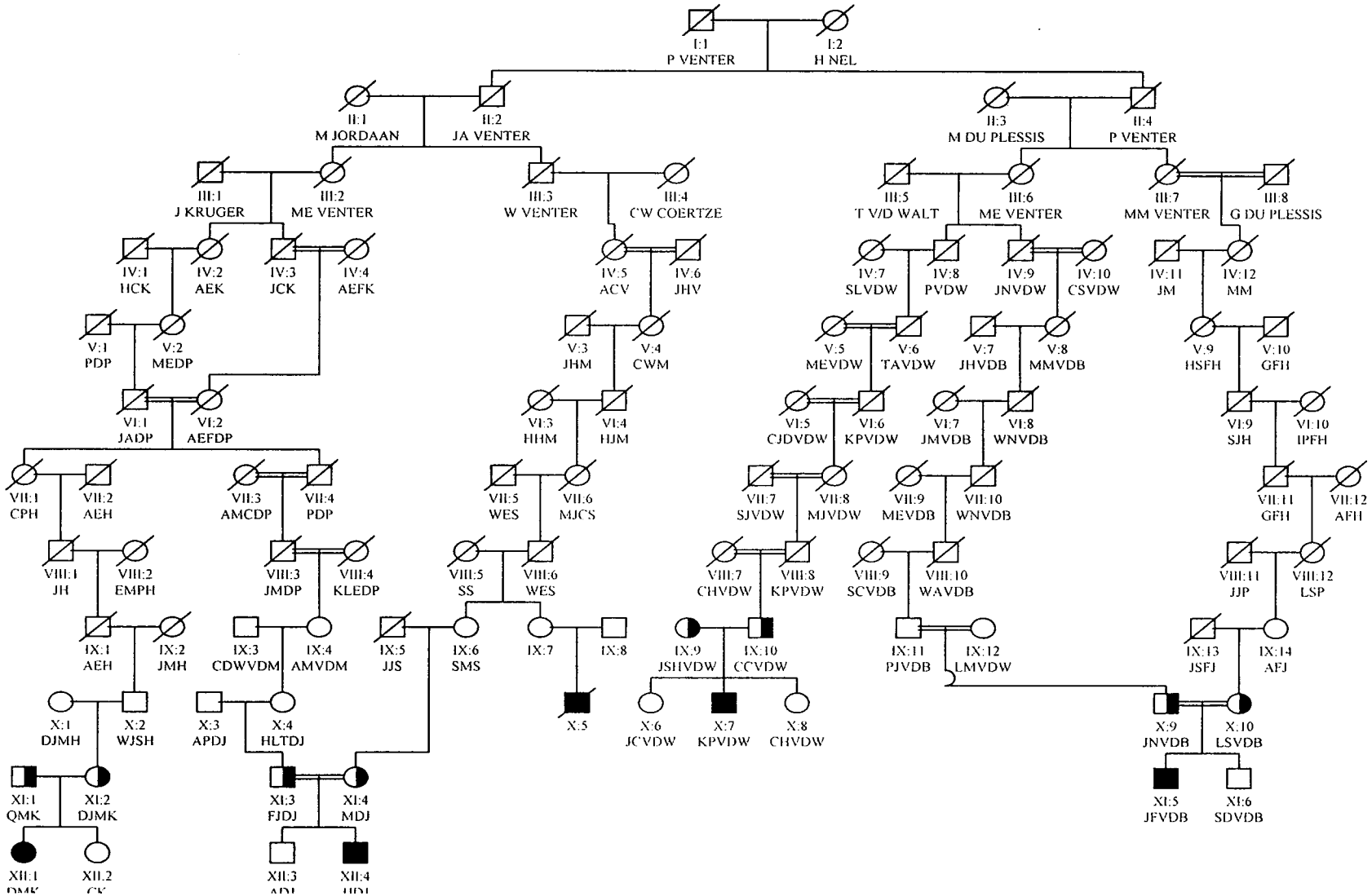

Figure 12: Possible segregation of the FANCA Afrikaner type II mutation if Pieter Venter or his wife Hester Nel was the founder of this mutation

□ Male; ○ Female, ■ ● Affected with Fanconi anaemia, ◨ ◑ Carrier of a FANCA mutation.

CHAPTER 6

DISCUSSION AND CONCLUSIONS

The diagnosis of Fanconi anaemia, an autosomal recessive disease, depends on the presence of certain haematological and phenotypic characteristics. Due to the variable expression of these two criteria, the hypersensitivity of patients' lymphocytes against bifunctional alkylating agents is used as a third criterion. Marx et al.[1] and Auerbach et al.[2] indicated that this cytogenetic sensitivity test can be used to differentiate between FA homozygotes and heterozygotes, as well as between heterozygotes and controls. Since other investigators had difficulties in repeating these results, and since most of the patients and family members used by Marx et al. were available to me, my primary aim was to establish reference values for DEB sensitivity, and secondly to establish by statistical analysis the resolution of this technique in my hands. I was indeed fortunate to have had 25 FA patients and their families to be included in this project.

Although a slight increase in DEB-induced chromosomal aberration yield occurred in obligate carriers, it was too small to distinguish them from controls. This technique allows differentiation with confidence between FA homozygotes on the one hand, and heterozygotes and controls on the other hand, which is in accordance with the findings of almost all laboratories, both nationally and internationally. My suggestion is that clastogen-induced chromosomal breakage only be used as a diagnostic tool in combination with phenotypic and haematological findings, and that the term "non-FA" should be abandoned.

Dokal et al.[3] reported two brothers with identical phenotypic and haematological features, both being compound heterozygotes for the FANCC mutations, L554P and ΔG322. However, the cells of only one of them revealed sensitivity to DEB during repetitive tests[3]. In this study a difference in DEB-sensitivity was also observed between the different sibs in three separate families. In the first family a fourfold difference in aberration yields were obtained in two FA siblings, whereas in the second family the values for one affected child were double those of the other. It must be emphasized that the lymphocyte cultures of all members of each family were set up simultaneously, using the same DEB stock solution, and hence, the same diepoxybutane concentration.

In addition, Kwee et al. described a patient where about 40% of his cells were responsive to bifunctional agents, and the case reported by Auerbach et al. showed chromosomal aberrations in only 20% of cells[4, 5]. The hypothesis was put forward that at some stage in the lives of these patients a "de novo arising of clastogen resistant cells" occurred[4]. The present study reports two South African FA patients where 39% and 42% of cells, respectively, were found to be responsive to diepoxybutane. The first explanation for this phenomenon is that DNA repair is influenced by certain external factors. The second and perhaps more plausible hypothesis is that all DNA repair mechanisms are linked, which would mean that one gene, in certain instances, could take over the functions of the defective gene in a specific FA patient. Treatment options other than bone marrow transplant or gene therapy might become available for Fanconi anaemia if this should be the case. In these patients the diagnosis of Fanconi anaemia is difficult although they present with the phenotypic and haematological features of Fanconi anaemia[5, 6]. Therefore, "to be FA or not to be FA, that is the question" in these patients. Cytogenetic breakage studies using alkylating agents still remain a useful and important tool in the diagnosis of Fanconi anaemia, irrespective of complementation group, although the patient's specific mutation can be traced to at least seven complementation groups and where two defective genes, FANCA and FANCC, account for 79% of cases[7].

The distribution of the genes defective in Fanconi anaemia patients is 66% for FANCA, 12.7% for FANCC, 4.3% for FANCB as well as FANCD, and 12.7% for the remaining genes, FANCE, FANCF and FANCG[8]. A founder effect was described in the Ashkenazi Jews, with mutation IVS4 + 4A→T as founder mutation. In the present investigation the majority of white FA homozygotes are Afrikaans-speaking (Afrikaners), which is similar to the observation by Rosendorff et al (1987) [9]. Complementation analysis revealed that all the Afrikaner Fanconi anaemia patients on whom screening have been carried out, belong to Fanconi anaemia Complementation Group A. Since the Afrikaner population is primarily a mixture of Dutch, German and French settlers, it sounds reasonable to suspect that the founder mutation should come from an ancestor belonging one of these ethnic groups. In Europe the majority (67%) of Dutch Fanconi anaemia patients belong to complementation group C, whereas in Germany, especially the Ruhr area, and France, FANCA gene defects predominate. The first genealogical investigation carried out on Afrikaner FA families, indicated Guillaume Nel and/or his wife Jeanne de la Batt, both from French Huguenot stock, as a possible founder of a mutation in the FANCA gene. At that stage the mutation status of patients' parents was still unknown. Later on three major mutations were detected among the parents. A deletion

stretching from exons 12 to 31, the type I mutation, was found in 66,7%, of FA parents. Mathew et al. detected the type II mutation, which comprises a deletion from exons 11 to 17, in 17 % of chromosomes analysed. In this study 11% of Fanconi anaemia parents were found to be carriers of this specific mutation.

In the first genealogical investigation only 12 FA families were used which is a small proportion of the total Afrikaner FA patients. The geographical distribution of these 12 FA patients, either from the Free State (7) or from the former Transvaal (5) could also influence the ratio of occurrence of the Afrikaner mutations in these families. If, for example, the occurrence of the Afrikaner type II mutation is slightly higher among residents of the former Transvaal, i.e. the minority of patients used in the first genealogical study, then a slightly lower percentage of occurrences of the type II mutation could be expected.

The main purpose of the second genealogical investigation was to verify the results of the first, by tracing back the origins of type I (seven) and type II (six) carrier parents. Guillaume Nel and/or his wife Jeanne de la Batt again turned out to be possible founders of the FANCA Afrikaner type I mutation, and three of their four children previously involved showed linkage to the seven type I carrier FA parents. However, Hercule du Preez and/or his wife Cecile D'Atis have now also become founder candidates for the FANCA type I mutation. It is interesting to note is that all the possible candidates identified as possible founders of the FANCA type I mutation, are from Huguenot stock.

The six FA parents carrying the FANCA Afrikaner type II mutation, link to either Petrus Venter or his wife Martha du Plessis. Whereas Venter is from German origin, du Plessis is of French Huguenot stock. It is interesting to note that in those countries with French Huguenots in their ancestry, such as South Africa, Canada or the Ruhr area of Germany, the majority of Fanconi anaemia patients belong to complementation group A, whereas FANCC tends to be the defective gene in other areas or countries. In Italy, another European country with a high prevalence of complementation group A patients, the French connection is not Huguenots, but the French governed a major part of present-day Italy until the late 18th century[10]. The major mutations in the FANCA gene arise as ALU mediated recombination errors, as described by Levran et.al and Morgan et al.[11, 12]. A correlation occurs between countries with strong genealogical connections to France and a high prevalence of complementation group A Fanconi anaemia patients. The question may be asked whether this correlation is by chance or whether the French are indeed responsible for the high prevalence of Fanconi anaemia in

certain countries? The presence or absence of the Afrikaner type mutations among the populations of France and Germany will greatly contribute to solving this question.

FUTURE RESEARCH

Further investigations should be directed towards the following:

1. statistical analyses on the genealogy of parents carrying the Afrikaner type I and type II FANCA mutations.
2. the genealogy of the Afrikaner type III mutation carriers.
3. search for a more reliable test to detect the type II mutation among the carriers
4. establishing the carrier frequency of the type I and type II mutations among the Afrikaner.

References

1. Marx MP, Smith S, Heyns AduP, Van Tonder IZ (1983): Fanconi's Anemia: A Cytogenetic Study on Lymphocyte and Bone Marrow Cultures Utilizing 1,2:3,4-Diepoxybutane. Cancer Genet. Cytogenet. 9: 51-60.
2. Auerbach AD, Adler B, Changanti RSK (1981): Pre- and Postnatal diagnosis and carrier detection of Fanconi anaemia by a Cytogenetic method. Pediatrics 67 (1): 128 - 135.
3. Dokal I; Chase A; Morgan NV; Coulthard S; Hall G; Mathew CG; Roberts I (1996) Positive diepoxybutane test in only one of two brothers found to be compound heterozygotes for Fanconi's anaemia complementation group C mutations. Br. J. Haematol. 93(4): 813-6.
4. Kwee ML, Poll EHA, van de Kemp JJP, de Koning H, Eriksson AW, Joenje H (1983): Unusual response to bifunctional alkalyting agents in a case of Fanconi anaemia. Hum. Genet. 64:348-387.
5. Giampietro PF; Auerbach AD; Elias ER; Gutman A; Zellers NJ; Davis JG (1998): New recessive syndrome characterized by increased chromosomal breakage and several findings which overlap with Fanconi anemia. Am. J. Med. Genet. 16; 78(1): 70-5.

6. Milner RDG, Khallouf KA, Gibson R, Hajianpour A, Mathew CG (1993): A new autosomal recessive anomaly mimicking Fanconi's anaemia phenotype. Arch. Dis. Child. 68: 101-103.
7. Buchwald M, Carreau M (2000): Genetic basis of Fanconi's anemia. In: "Aplastic Anemia" edt. Schrenzenmeier H, Bacigalupo A, Cambridge University Press, UK, p338-354.
8. Alter BP (2000): Clinical features and diagnosis of Fanconi's anemia. In: "Aplastic Anemia" edt. Schrenzenmeier H, Bacigalupo A, Cambridge University Press, UK, p319-337.
9. Rosendorff J, Bernstein R, Macdougall L, Jenkins T (1987): Fanconi Anemia; Another Disease of Unusually High Prevalence in the Afrikaans Population of South Africa. Am. J. Med. Genet. 27:793-797.
10. Savoia A, Zatterale A, Del Principe D, Joenje H (1996):Fanconi anaemia in Italy: high prevalence of complementation group A in two geographic clusters. Hum. Genet. 97(5): 599-603.
11. Morgan NV, Tipping AJ, Joenje H, Mathew CG (1999): High frequency of large intragenic deletions in the Fanconi anemia group A gene. Am. J. Hum. Genet. 65(5): 1330-41.
12. Levran O, Doggett NA, Auerbach AD (1998): Identification of Alu-mediated deletions in the Fanconi anemia gene FAA. Hum. Mutat., 12(3): 145-152.