A COMMON FANCONI ANEMIA MUTATION IN BLACK POPULATIONS OF
SUB-SAHARAN AFRICA

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Fanconi anemia (FA) is a genetically heterogeneous chromosomal instability syndrome associated with multiple congenital abnormalities, aplastic anemia, and cancer. We report that a deletion mutation in the \emph{FANCG} gene (c.637_643delTACCGCC) was present in 82% of FA patients in the Black populations of Southern Africa. These patients originated from South Africa, Swaziland, Mozambique and Malawi. The mutation was found on the same haplotype, and was present in 1% of controls from the Black South African population. These data indicate that the birth incidence of FA in this population is greater than 1/40,000, which is much higher than previously supposed, and suggest that the \emph{FANCG} deletion is an ancient founder mutation in Bantu-speaking populations of sub-Saharan Africa. Diagnostic screening is now possible by means of a simple DNA test.

**Introduction**

FA is rare in most populations with a prevalence of about 1 in 300,000, but carrier frequencies of 1/90 have been observed in Ashkenazi Jews\(^1,2\) and in the Afrikaner population of South Africa\(^3\). FA is genetically heterogeneous, arising from mutations in one of at least 11 different genes (\emph{FANCA}, \emph{FANCB}, \emph{FANCC}, \emph{FANCD1/BRCA2}, \emph{FANCD2}, \emph{FANCE}, \emph{FANCF}, \emph{FANCG}, \emph{FANCI}, \emph{FANCI} and \emph{FANCL}). Almost all Ashkenazi FA cases are caused by the c.711+4A>T (IVS4+4A>T) mutation in \emph{FANCC}\(^1\), whereas 80% of Afrikaner cases have one of two large deletions in \emph{FANCA}\(^4\). Clinical studies indicated that FA is rare in Black South Africans, with a homozygote prevalence
of 1/476,000⁵. We investigated the molecular basis of FA in this population to inform future patient management strategies.

**Methods**

FA patients were ascertained by the Departments of Hematology/Oncology, Pediatrics and Human Genetics at Hospitals attached to the Universities of the Witwatersrand, Free State and Pretoria. Clinical diagnoses were confirmed by demonstrating increased chromosome breakage with diepoxybutane or mitomycin C as described previously.⁶ Blood samples were taken from informed, consenting FA patients, and clinical data on age of onset, severity of hematological symptoms, survival, incidence of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and somatic abnormalities⁶ obtained. Controls were anonymised paternity specimens.

Lymphoblastoid cell lines were established from 4 Black South African FA patients and screened for the presence of the known FA proteins by Western blotting.⁶ The 14 exons of the *FANCG* gene [OMIM: 602956; mRNA: AJ007669.1; genomic: NT_008413] were amplified and sequenced from forward and reverse strands on an ABI 377 automated DNA sequencer.⁶ A PCR assay was designed to detect the common 7 bp deletion in exon 5 of the *FANCG* gene. Amplification was carried out with primers 637-643delF CCC AGG GAT TGA AGG ATG TC, and 637-643delR GCA TGA GAC TGG AGG ACC AC, with 30 cycles of 94°C (1 minute), 60°C (1 minute) and 72°C (1 minute). Products were sized by electrophoresis on a 12.5% polyacrylamide gel. A single nucleotide polymorphism (SNP) in the *FANCG* gene, c.84+77A>C, was genotyped by amplification with primers IVS1+77A>C/F: GGT TTG CAG TGG ATT TAC CCC ATC
and FANCG 1R: CTT TGG TCA AGC TCA GTC CC, followed by DNA sequencing or by digestion with Fok1 and sizing of the 53 bp and 46 bp alleles by electrophoresis as above.

Clinical phenotype data was collected from Black South African FA patients as described, and compared with previously published clinical data from 245 European patients of known complementation group.6 Statistical testing was carried out6 using Splus5 (MathSoft Inc, Seattle, USA).

Results and Discussion

Western blot analysis of known FA proteins in extracts from cell lines from 4 Black South African FA patients was carried out for an initial assessment of complementation groups. This established that the FANCG protein was undetectable in all 4 cell lines tested (data not shown). DNA sequencing of the exons of the FANCG gene revealed that all 4 patients were homozygous for a 7 bp deletion in exon 5 (Fig. 1a), c.637_643delTACCGCC, that is predicted to produce a truncated protein of 217 amino acid residues (Y213K + 4X). A simple PCR mutation assay was devised, which detects a 105 bp fragment in the wildtype gene and a 98 bp fragment from the deleted allele (Fig. 1b). DNA samples from a total of 40 unrelated Black FA patients were then screened for the FANCG deletion. The mutation was present in 33/40 (82.5%) patients (all except two being homozygotes), and in 64/80 mutant alleles (80%). Most mutation-carrying patients were from the Sotho, Tswana, Venda and Zulu tribes from South Africa, but two were from Swaziland, two from Mozambique and one from the Tumbuka tribe of Malawi. The same mutation was independently detected in a Portuguese FA patient (EUFA939) who
was homozygous for the deletion, and whose parents originated from the Black population of Mozambique, formerly a Portuguese colony.

These diverse geographic and tribal origins suggest that the mutation predated the arrival of Bantu-speakers in Southern Africa around 400 AD. If so, we would expect it to occur on the same haplotype defined by DNA polymorphisms within or very close to *FANCG*. All patients homozygous for c.637_643delTACCGCC were also homozygous for the C allele of a single nucleotide polymorphism in intron 1 of *FANCG*, c.84+77A>C (Fig. 1c), whereas the frequency of the C allele in Black South African controls was 0.22. This is consistent with a single origin for the *FANCG* mutation. In view of the prevalence of the deletion in African FA patients, we then determined its carrier frequency in unrelated Black South African controls. The deletion was present in 3/300 (1%) of controls (95% CI 1/35 - 1/475), indicating an expected birth incidence of 1/40,000. The true incidence of FA would be higher since this mutation accounts for 80% of FA alleles in the population, and is therefore likely to be about ten-fold higher than that reported from clinical ascertainment of FA in Black school children and estimates of population size from a population census.5

We also investigated the clinical phenotype of Black South African patients homozygous for the *FANCG* deletion. Detailed clinical information was available from 20 patients, and this was compared to data from 20 European FA-G patients and from all 245 European FA patients described previously.6 There was no significant difference in age of diagnosis (mean age 6.51 years), survival, or rates of AML and MDS (1 MDS and 1 AML in 20 patients) in Black FA-G patients compared to either European FA-G or all European FA groups. However, the mean number of severe hematological indices (2.53),
was significantly greater in Black FA-G patients than in all European FA patients (1.55, \( p = 0.0009 \)), although not significantly different from European FA-G patients (2.07, \( p = 0.42 \)). Differences in socioeconomic conditions and healthcare provision may also influence hematological status. Black FA-G patients had a significantly higher frequency of distal radial ray abnormalities, growth retardation and eye abnormalities than all European FA patients, but similar rates of somatic abnormalities to European FA-G patients (Table 1).

The presence of the c.637_643delTACCGCC mutation in different tribal groups from South Africa, and in Malawi and Mozambique suggests that it represents a founder mutation in the Bantu-speaking peoples of sub-Saharan Africa, consistent with the genetic homogeneity of many Bantu-speaking populations.\(^7\) It may therefore contribute to FA in other African countries such as the Congo, Angola, Tanzania, Zambia, and Zimbabwe. Founder mutations in \textit{FANCG} also have been described for European, South American and Japanese populations\(^8-10\). The high carrier frequency of the deletion in the Black South African population suggests that FA is much commoner than previously estimated, and may be underdiagnosed in Africa because of a combination of lack of specialist healthcare in rural areas, and other more prevalent health problems. A relatively sophisticated chromosomal breakage test is required to confirm the diagnosis of FA, but since the mutation accounts for the majority of FA cases in this population, a simple DNA assay may now permit more widespread testing for FA in Southern Africa.
REFERENCES


Table 1  Frequency of somatic abnormalities in Black South African patients homozygous for the FANCG deletion (c.637-643delTACCGCC) compared with FA patients in the European study⁴

<table>
<thead>
<tr>
<th>Site of abnormality</th>
<th>South African</th>
<th>European (FA-G)</th>
<th>European (All FA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FANCG del</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% (n = 20)</td>
<td></td>
<td>% (n = 20)</td>
<td>% (n = 241)</td>
</tr>
<tr>
<td>Skin</td>
<td>85</td>
<td>70</td>
<td>70.4</td>
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<tr>
<td>Radial ray</td>
<td>85</td>
<td>50 (p = 0.04)*</td>
<td>46.9 (p = 0.002)*</td>
</tr>
<tr>
<td>Growth retardation</td>
<td>90</td>
<td>75</td>
<td>58.0 (p = 0.01)*</td>
</tr>
<tr>
<td>Kidney</td>
<td>15</td>
<td>20</td>
<td>21.6</td>
</tr>
<tr>
<td>Urogenital</td>
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<td>5</td>
<td>11.6</td>
</tr>
<tr>
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<tr>
<td>Gastrointestinal</td>
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<td>12.9</td>
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<tr>
<td>CNS †</td>
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<td>0</td>
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</tr>
<tr>
<td>Deafness</td>
<td>5</td>
<td>5</td>
<td>10.3</td>
</tr>
<tr>
<td>Eye</td>
<td>85</td>
<td>65</td>
<td>55.3 (p = 0.002)*</td>
</tr>
</tbody>
</table>

* South African FA-G patients were compared with FA-G patients in the European study using Fisher’s exact test, and with all FA patients in the European study using the X² test.

† CNS = central nervous system.
FIGURE LEGEND

Figure 1

A. Sequence of part of exon 5 of the *FANCG* gene showing the c.637_643delTACCGCC
mutation (637-643del).

B. PCR assay for the *FANCG* deletion. Samples from African FA patients; the final two
lanes are a heterozygote and a normal control. The normal allele is 105 bp in length and
the mutant allele 98 bp.

C. Sequence of part of intron 1 of the *FANCG* gene showing the c.84+77A>C SNP
(IVS1+77A>C).
Figure 1

A  637-643del

B

C  IVS1+77A>C

105 bp

98 bp
A common Fanconi Anemia mutation in black populations of sub-Saharan Africa

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