Genetic mapping of the FACC gene and linkage analysis in Fanconi anaemia families


Abstract

Fanconi anaemia is an autosomal recessive disorder associated with increased chromosome breakage and progressive bone marrow failure. The gene for complementation group C (FACC) has been cloned and mapped to chromosome 9q22.3, but neither its genetic location nor the proportion of patients belonging to group C is known. We have used a polymorphism within the FACC gene to localise it within a 5 cM interval on chromosome 9q, bounded by D9S196/ D9S197 and D9S176. The genes for Gordin's syndrome and multiple self-healing squamous epitheliomata have also been mapped to this interval. Linkage analysis with the three highly informative microsatellite polymorphisms flanking FACC in 36 Fanconi anaemia families showed that only 8% of families were linked to this locus. This indicates that the genes for the other Fanconi anaemia complementation groups must map to one or more different chromosomal locations. The markers also allowed rapid exclusion of 56% of the families in our panel from complementation group C, thus substantially reducing the number of patients who need to be screened for FACC mutations.

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Materials and methods

A total of 36 families with a clinical diagnosis of FA were referred to the Regional Genetics Centre at Guy's Hospital for analysis. A summary of the geographical or ethnic origin of the families and their structure is given in the table. The clinical diagnosis was confirmed in all families by cytogenetic demonstration of hypersensitivity to the DNA cross linking agents DEB or MMC or both, as described by Cohen et al.1 Families in whom the index case was not hypersensitive to these agents were excluded from the linkage study.

The EcoRI RFLP in the FACC gene was typed in the parents of the 40 families of the CEPH collaboration using the polymerase chain reaction15 with the primer sequences described.8 The microsatellite polymorphisms D9S196, D9S197, and D9S17611 were typed in some CEPH persons for whom no data on these markers were available. Typing was done by end labelling one of each of the PCR primers with γ32P-ATP, followed by PCR and analysis on denaturing polyacrylamide gels.12 D9S196 and D9S197 were amplified together in the same reaction tube with 22 PCR cycles at an annealing temperature of 65°C, and D9S176 was amplified separately for 23 cycles and annealing at 63°C. All three markers can be analysed together on one lane of the gel as their allele sizes do not overlap (fig 1). Typing of persons in whom recombination was detected between closely linked markers was repeated to exclude laboratory error.

A linkage map of the region of interest on chromosome 9q was constructed using CRIMAP13 and used to map the position of the FACC gene relative to other markers. The map...
Table 1  Characteristics of the 36 FA families in the linkage panel

<table>
<thead>
<tr>
<th>Geographical/ethnic origin</th>
<th>No of families</th>
<th>No of affected</th>
<th>No of multiplex families*</th>
</tr>
</thead>
<tbody>
<tr>
<td>South African/Afrikaner</td>
<td>13</td>
<td>34</td>
<td>3</td>
</tr>
<tr>
<td>South African/Black</td>
<td>2</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Middle East/Arabic</td>
<td>5</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Brazil/Brasilian</td>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>East European</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>West European</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>UK/Asian</td>
<td>4</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>UK/White</td>
<td>4</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>98</td>
<td>19</td>
</tr>
</tbody>
</table>

* Multiplex implies more than one affected person or that the parents are related to each other or both.

Figure 1  Linkage analysis of the FACC locus in Fanconi anaemia families. The FACC locus is not linked to the FA phenotype in the family on the left. In the family on the right, the genotypes are consistent with, but do not prove, linkage.

was based on the dataset constructed by the Cooperative Human Linkage Centre. The FACC RFLP and flanking markers from the Genethon group were then typed in our panel of 36 families with Fanconi anaemia. Multipoint lod scores for each family between the markers D9S196/D9S197 (considered as a haplotype), D9S176, and the disease were computed using LINKAGE, with the disease locus placed at the maximum likelihood position of FACC as determined by the CRIMAP analysis. The proportion of families linked to FACC was estimated using a standard admixture analysis. Genotypic data for the microsatellite markers in the 36 FA families are available on request.

Results

Analysis of the FACC RFLP in the CEPH families and the FA families showed that FACC was located between D9S520 and D9S176, an interval of 8 cM, with odds of more than 106 over other positions. Its location in the 5 cM interval between D9S196/D9S197 and D9S176 was supported with odds of 2506:1. A map of these and other markers in the region is shown in fig 2. The availability of highly informative microsatellite markers which flank the FACC gene allowed us to address the question of what proportion of FA families might be linked to this locus.

Linkage between the FACC locus, as defined by the haplotype D9S196/D9S197—D9S176, and Fanconi anaemia was determined in the 36 families with a cytogenetically confirmed diagnosis of FA. There was no evidence for linkage overall, with a total lod score of −38·8 at the FACC locus. Independent segregation of the FACC markers from the FA phenotype was observed in 20 of the 36 families in the panel, but some smaller families were consistent with linkage (see fig 1 for examples). A standard admixture test was used, under the assumption of genetic heterogeneity, to calculate the proportion of linked families. The best estimate was found to be 8%, with 95% confidence limits of 0–35%. Our linkage panel included 13 families from the Afrikaner population of South Africa, which has the highest reported incidence of FA (1 in 22 000) in the world. It has been suggested that this might result from a founder effect, in which case the population would be likely to be relatively homogeneous with respect to FA complementation group. The total lod score for linkage of the FACC locus to FA in this group of families was −8·4. There were no families of Ashkenazi Jewish origin in our panel, a high proportion of whom are from group C.

The three highly informative markers which flank FACC and which can be analysed together in the same lane of a sequencing gel provide a rapid screen for exclusion of FA families from complementation group C. In fig 1, for example, the family on the left can be excluded from group C since the son, who is unaffected clinically and who does not show increased chromosome breakage, has inherited the same haplotype as his affected sister. The haplotypes in the family on the right are consistent with linkage to FACC, and cannot therefore be excluded from group C.

Discussion

A major goal of the Human Genome Project is to establish a physical map of all known genes and DNA segments. The creation of a
Figure 2  Linkage map of markers ordered with odds of greater than 10^2:1 in the CEPH families. Physical locations are taken from the Genome Database, The Johns Hopkins University.

The meiotic map of these genes is a critical step in that process. We have used an RFLP within the FA group C gene to place this disease gene on the genetic map. Linkage analysis in the CEPH and FA families has shown that the gene lies within a 5 cM interval bounded by D9S196/D9S197 proximally and D9S176 distally. This region of the genome appears to be particularly rich in genes involved in DNA repair and cancer since it also contains the genes for xeroderma pigmentosum complementation group A,\(^8\) and the interval containing FACC is currently under intensive investigation by research groups attempting to clone the genes for Gorlin's syndrome\(^9\) and multiple self-healing squamous epitheliomata.\(^20\) Although the degree of overlap of clinical presentation between these three disorders is very limited, the recent demonstration that mutations in the receptor tyrosine kinase gene RET can give rise to phenotypes as dissimilar as multiple endocrine neoplasia type 2 and Hirschsprung's disease\(^22\) suggests that the FACC gene should not be excluded as a candidate for Gorlin's syndrome or ESS1 solely on phenotypic grounds.

Our analysis suggests that mutations in the FACC gene are responsible for the Fanconi anaemia phenotype in only a small minority of FA families, the best estimate being 8%. Thus the genes which specify the other complementation groups must map to one or more other loci, and it is not necessary to invoke the hypothesis of intragenic complementation to explain the cellular heterogeneity in FA. This is in contrast to the situation in ataxia telangiectasia, where the genes in virtually all families and complementation groups map to the same locus on chromosome 11q23.\(^23\) Our estimate of 8% agrees well with the results we have obtained from mutation analysis of the FACC gene in FA patients. We have screened the full FACC coding sequence by chemical cleavage, and detected mutations in 2/30 (7%) of unrelated patients\(^6\) (Gibson et al, manuscript in preparation). Whitney et al,\(^7\) also using chemical cleavage, detected mutations in 4/17 patients, but one of these was a complementation group A, and the mutation in this patient was later shown to be a coding sequence polymorphism.\(^24\) Thus, combining our data with that of Whitney et al,\(^7\) a total of five out of 47 FA patients (10-6%) had mutations in the group C gene. The higher figure of 14% observed by Verlander et al\(^8\) is likely to be a consequence of the high proportion of Ashkenazi Jewish patients in their study, the majority of whom (75%) had a splice mutation in intron 4 of the FACC gene.\(^7\) Our linkage data from the 13 African families suggest that if this population is homogeneous with respect to complementation group, it will not be from group C.

The polymorphic markers within and around the FACC gene provide a rapid means of exclusion of FA patients from group C in families where the index case has at least one sib. Typing of five to six FA families with the markers can be done within about 24 hours on a single polyacrylamide gel, and has more than halved the number of patients that we have had to take through the labour intensive process of screening the 14 exons of FACC for mutations.

The chromosomal location of the genes for the other FA complementation groups is unknown. The possible linkage of the FA-A gene to chromosome 20q\(^1\) could be resolved by carrying out complementation analysis on sufficient FA families with multiple affected persons to assemble a panel of group A families for linkage analysis. This approach has been initiated by means of a European collaborative group (EUFAR) founded to promote research into Fanconi anaemia.

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2 Strathdee CA, Duncan AMV, Buchwald M. Evidence for
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