

DIAGNOSIS OF FANCONI ANAEMIA (FA) IN DIZYGOTIC TWINS

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INTRODUCTION

FA is a rare autosomal instability syndrome characterized by progressive bone marrow failure, various congenital malformations, predisposition to malignancy and cellular hypersensitivity to cross linking agents because of inability to correct DNA damage. (Figure 1.A)

On the genetic level there is considerable heterogeneity in FA with 13 genes (A, B, C, D1, D2, E, F, G, I, J, L, M, N), currently recognised. The FA core complex containing the FA proteins (A/B/C/E/F/G/L/M) is required for the activation of the FANCD2 protein to a monoubiquitinated isoform (FANCD2-Ub), which interacts with DNA repair proteins leading to repair of the cross-link. In FA patients the FA pathway doesn't work properly. (Figure 1.B)

We report on a case of FA in dizygotic twins with characteristic congenital abnormalities and the same deletions of the FANCA gene.

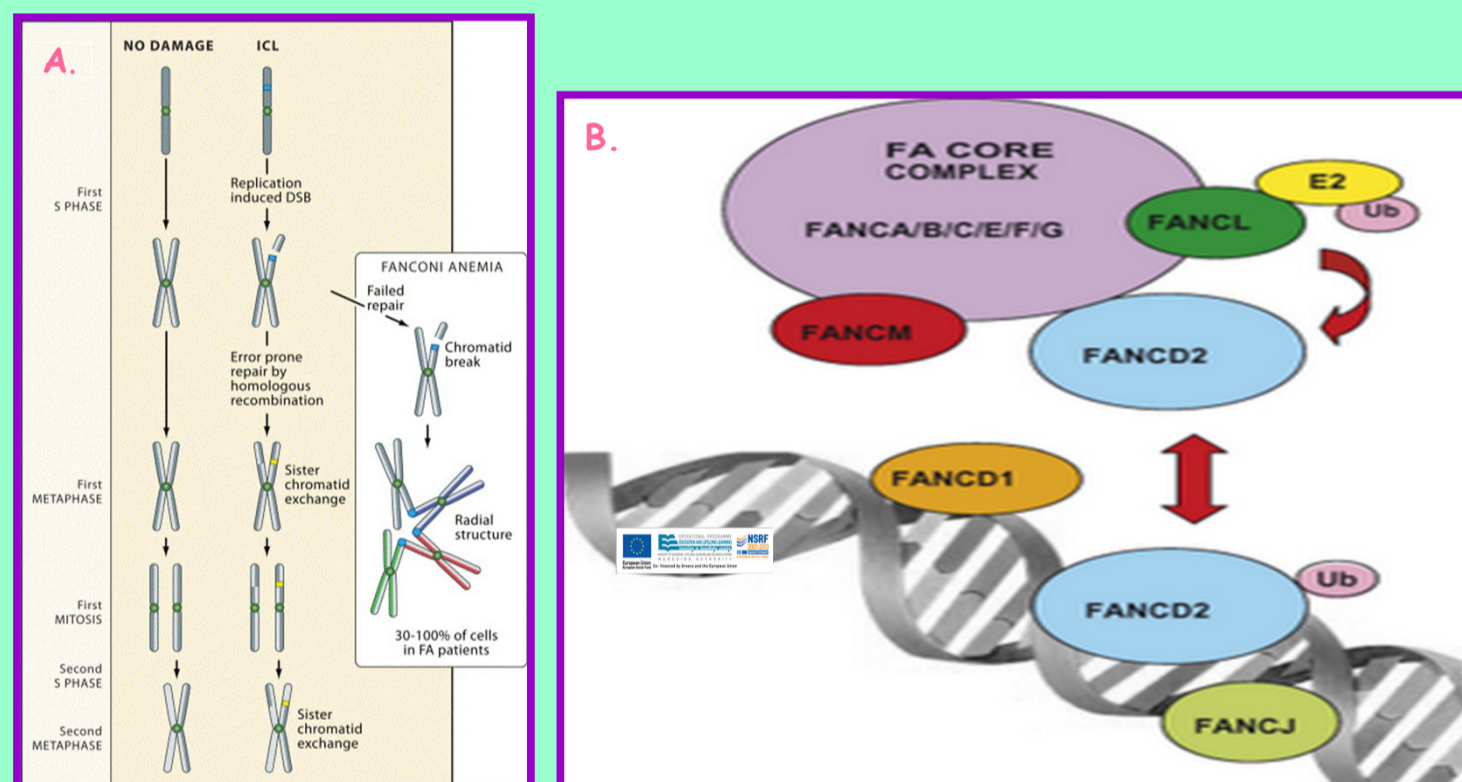


Figure 1: A. Chromosomal changes that can occur in response to DNA-Interstrand-Crosslink Damage. (adopted from Niedernhofer et al., Cell 123 : 1191-1198, 2005). B. An overview of the pathway. (adopted from Patel et al. , DNA Repair, 6 : 885-890, 2007)

PATIENTS

A twin boy and a girl, aged 6 years old, were referred to the Department of Medical Genetics for genetic investigation after the clinical suspicion of FA. Matched for age and sex donors were used as control.

METHODS

Peripheral blood samples were analysed with conventional cytogenetic techniques. For clastogen-induced chromosome damage MMC and DEB were added in two different sets of cultures. The final concentration for MMC was 3 µg/5ml and 5 µg/5ml and cells were cultured for 72 hours. DEB was added to the cultures 24 hours after initiation at a final concentration of 0,6 µg/5ml, thus exposing the cells to the chemical for 48 hours. A minimum of 150 metaphases per case were analysed. As FA positive was considered the case in which the percentage of breaks was 7-10 times higher as compared to control.

Molecular investigation was performed using the Multiplex Ligation-dependent Probe Amplification (MLPA) technique to detect deletions of the FANCA gene which account for more than 65% of Fanconi Anaemia cases. PO31/32 is the commercially available kit that was used.

RESULTS

Induced breaks and radial formations were detected in both patients in over 90 % of metaphases analyzed. (Figure 2) MLPA identified the same deletions involving exons 1-5 and 7-17 of the FANCA gene in both patients. Parental molecular testing revealed that the mother was heterozygous for deletions of exons 1-5 and the father for deletions of exons 7-17. (Figure 3)

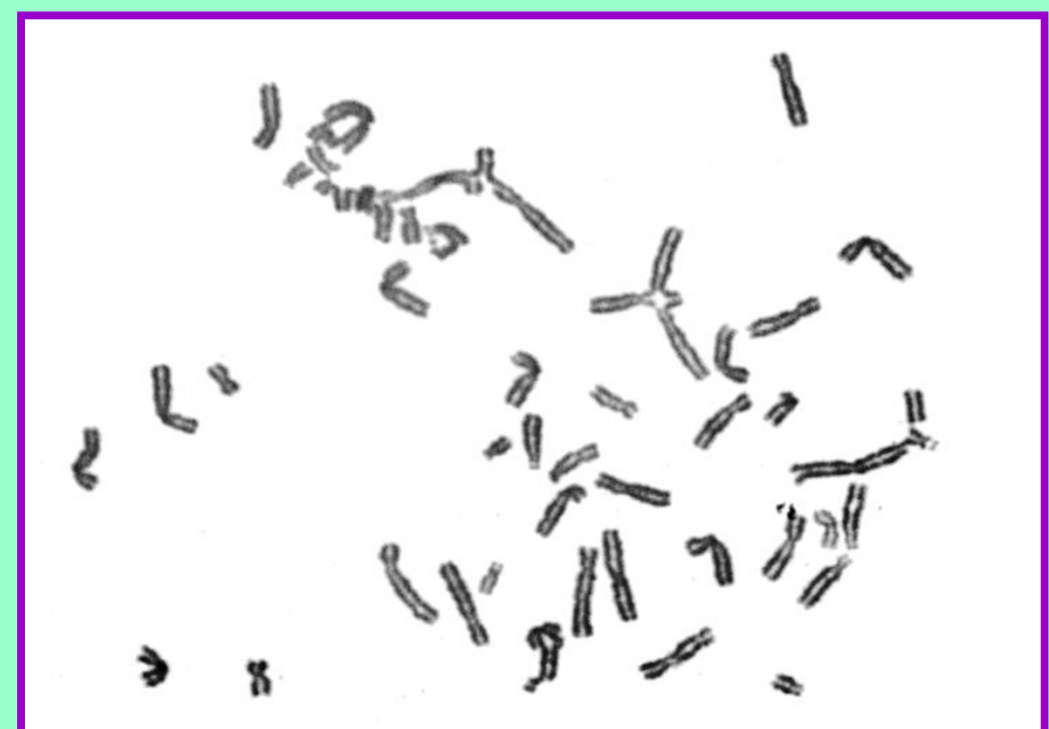


Figure 2 : Metaphase spread from the child 1, after exposure to MMC, exhibiting multiple chromosomal breaks and radial formations.

| FANCA GENE | FATHER | MOTHER | CHILD1 | CHILD2 | CONTROL |
|------------|--------|--------|--------|--------|---------|
| exon 01 | 0,52 | 0,95 | 0,52 | 0,52 | 0,97 |
| exon 02 | 0,56 | 0,94 | 0,54 | 0,54 | 0,99 |
| exon 03 | 0,53 | 1,1 | 0,55 | 0,55 | 1 |
| exon 04 | 0,55 | 1 | 0,48 | 0,48 | 1,08 |
| exon 05 | 0,55 | 1,18 | 0,57 | 0,57 | 1,12 |
| exon 06 | 0,99 | 1,08 | 0,99 | 0,99 | 0,99 |
| exon 07 | 0,98 | 0,4 | 0,42 | 0,42 | 0,9 |
| exon 08 | 1,1 | 0,36 | 0,6 | 0,6 | 0,94 |
| exon 09 | 1,12 | 0,51 | 0,52 | 0,52 | 0,98 |
| exon 10 | 0,98 | 0,5 | 0,53 | 0,53 | 0,96 |
| exon 11 | 0,97 | 0,6 | 0,52 | 0,52 | 1,1 |
| exon 12 | 0,91 | 0,48 | 0,48 | 0,48 | 1,08 |
| exon 13 | 1,1 | 0,42 | 0,54 | 0,54 | 0,94 |
| exon 14 | 1 | 0,49 | 0,54 | 0,54 | 1 |
| exon 15 | 0,97 | 0,63 | 0,48 | 0,48 | 1,08 |
| exon 16 | 0,95 | 0,54 | 0,47 | 0,47 | 1,1 |
| exon 17 | 1,11 | 0,58 | 0,48 | 0,48 | 0,99 |
| exon 18 | 1,12 | 0,91 | 1 | 1 | 0,95 |
| exon 19 | 1 | 0,96 | 0,98 | 0,98 | 0,98 |
| exon 20 | 1,1 | 0,99 | 0,99 | 0,99 | 1,12 |

Figure 3 : Child 1 and child 2, compound heterozygotes for FANCA gene deletions. Deletion of exons 1-5 is of paternal origin and del of exons 7-17 is of maternal.

CONCLUSION

It's noteworthy that although the siblings were dizygotic twins, they inherited the same deletions from their parents and were both compound heterozygotes for deletions of exons 1-5 and 7-17 of the FANCA gene.