

Cytogenetic and Molecular investigation of Greek patients referred for Fanconi Anaemia.

N. Selenti¹, A. Kolialexi¹, A. Kattamis¹, E. Fryssira¹, S. Kitsiou¹, V. Papadakis²,
K. Roka², S. Polychronopoulou², E. Kanavakis¹ and A. Mavrou¹

1. Department of Medical Genetics, Athens University School of Medicine ,
2. Department of Pediatric Hematology - Oncology, Aghia Sophia Children's Hospital, Athens Greece

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 molecular 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 does not work properly. (Figure 1.B)

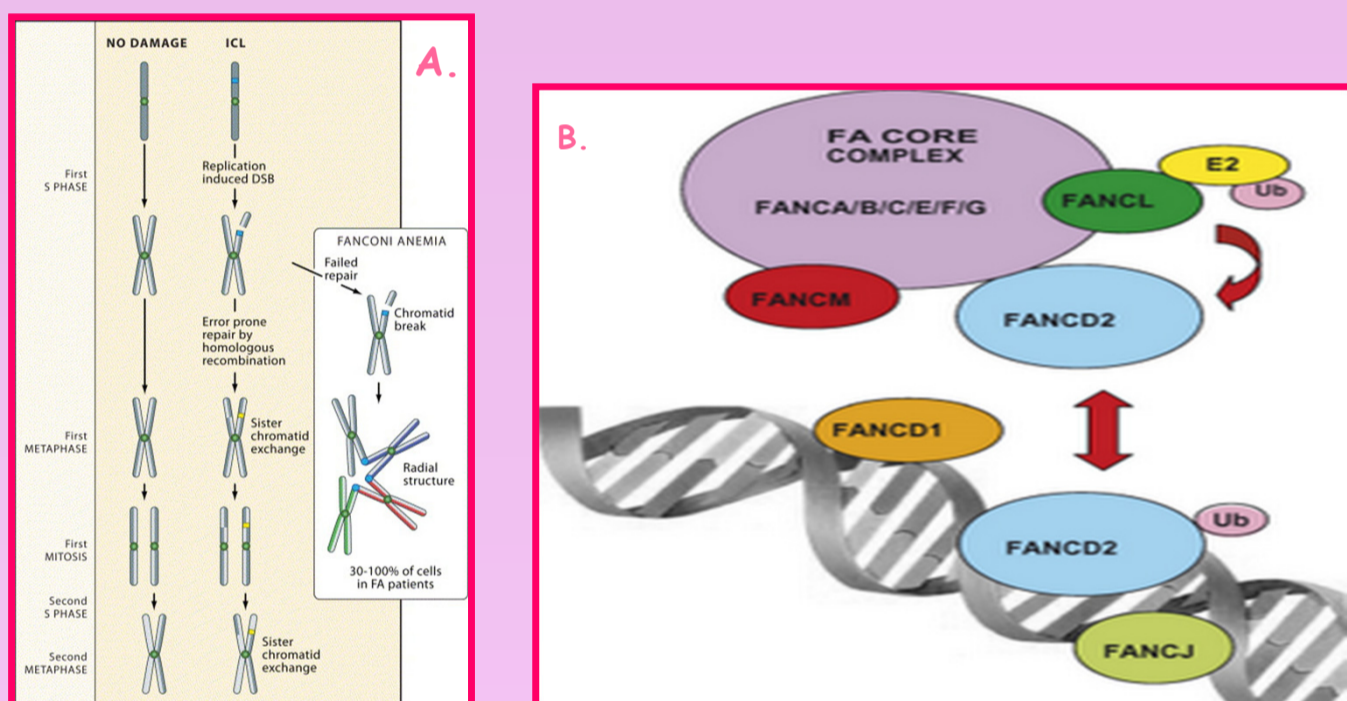


Figure 1: A. Chromosomal changes that can occur in response to DNA-Interstrand-Crosslink Damage. (adopted from Niedernhofer et al., Cell 123 : 1191-1198, 2005). B. An overview of the pathway. (adopted from Patel et al. , DNA Repair, 6 : 885-890, 2007)

PATIENTS

We report on 97 children ,aged 2 months-14 years , who were referred to the Department of Medical Genetics from 2007-2010 for genetic investigation after the clinical suspicion (myelodysplasia and/or congenital anomalies) of FA. Peripheral blood samples were analysed originally with cytogenetic techniques. If the results were positive molecular analysis followed. Matched for age and sex controls were used. Carrier detection was performed in family members of FA patients.

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

Cytogenetic testing

Induced breaks and radial formations were detected in 7/97 patients with both clustogens (Figure 2). 3/7 FA patients presented with congenital anomalies and 4/7 had aplastic anaemia.

Molecular testing

DNA from the 7 patients were subsequently tested for deletions of the FANCA gene. Molecular analysis showed that 5/7 belonged to the FA A subtype. Two were dizygotic twins, compound heterozygous for the same deletions (Figure 3). 16 family members were tested for carrier detection and 6 were identified as heterozygous for deletions of the FANCA gene.



Figure 2 : Metaphase spread from a FA patient, after exposure to MMC, exhibiting multiple chromosomal breaks and radial formations.

FANCA GENE	FATHER	MOTHER	1 st TWIN	2 nd TWIN	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 : Dizygotic twins, 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

7% of patients, referred for FA investigation, were FA positive and of those 71 % belonged to the FA A subtype. Accurate and timely diagnosis is important in order to implement appropriate therapy, bone marrow transplantation and genetic counselling.