Molecular characterization of 44 Fanconi anemia patients by whole exome sequencing

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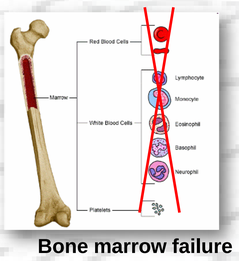
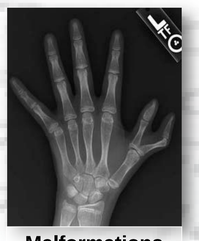
1.Fanconi Anemia (FA)

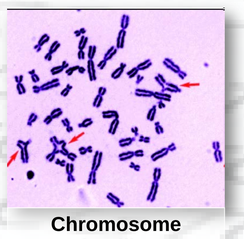
The FA is a rare autosomal and X-linked recessive genetic disease with a carrier frequency between 1/65 and 1/300



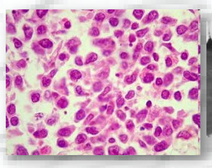
Bone marrow failure

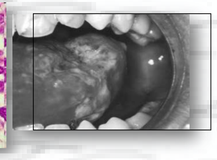


Malformations



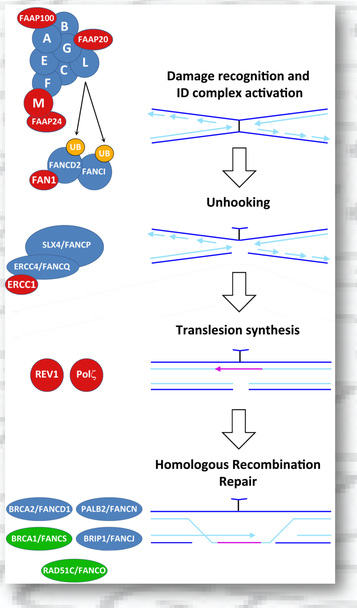
Chromosome fragility





Cancer predisposition (leukemia and HN-SCC)

2. FA genes and the DNA Interstrand Crosslink Repair

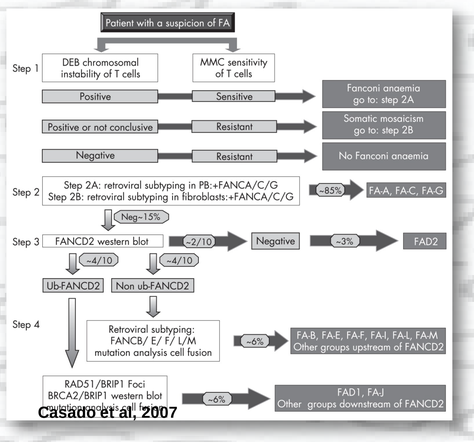


= FA genes (14)

= FA-associated genes (8)

= FA-like genes (2)

3.Current workflow for FA molecular analysis



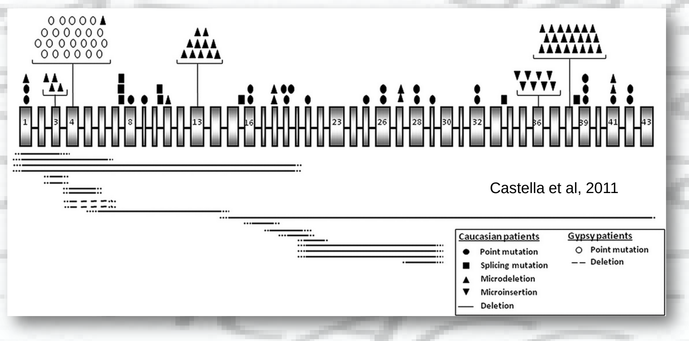
Casado et al, 2007

Problems:
- **Information only about the gene not the mutations.**
- **Retroviral subtyping can fail and is labor intensive.**
- **Problems with different batches of viral particles.**
- **No clear mutation hot spots and many private mutations.**
- **Some FA genes are very big such as FANCA, BRCA2, SLX4 or have pseudogenes (FANCD2)**

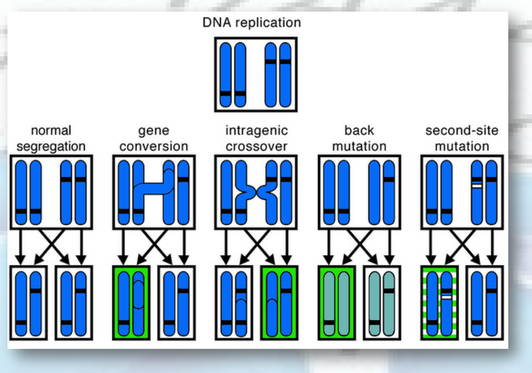
4.Analysis by WES of 44 FA patients

- 27 Patients were “full FA” patients with no somatic mosaicism detected
- 17 patient were mosaic
- 27 patients were already subtyped
- In 10 patients several FA genes had been disregarded by retroviral subtype analysis but the affected gene was still unknown
- 7 Patients were completely unsubtyped.
- Commercial enrichment kit SureSelectXT All Exon, V5 50,621,018 bp V5 of Agilent was used with a Illumina HiSeeq 2500 sequencing system.

5. Challenges for our approach



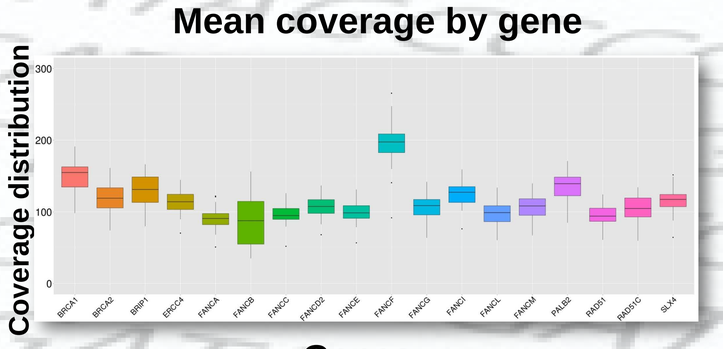
20-30% of the patients have large deletions involving FANCA gene. WES cannot detect this kind of mutations and a specific technique (MLPA) is used instead.



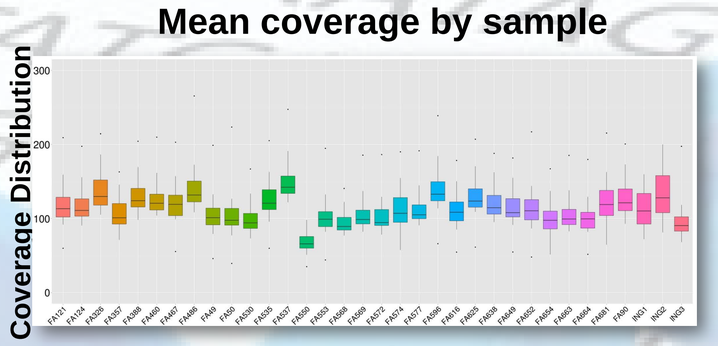
15-20% of FA patients are mosaic and depending on the levels of repopulation of the bone marrow, one mutation can be undetectable in DNA from peripheral blood (PB).

6. WES Mean Coverage

Mean coverage by gene

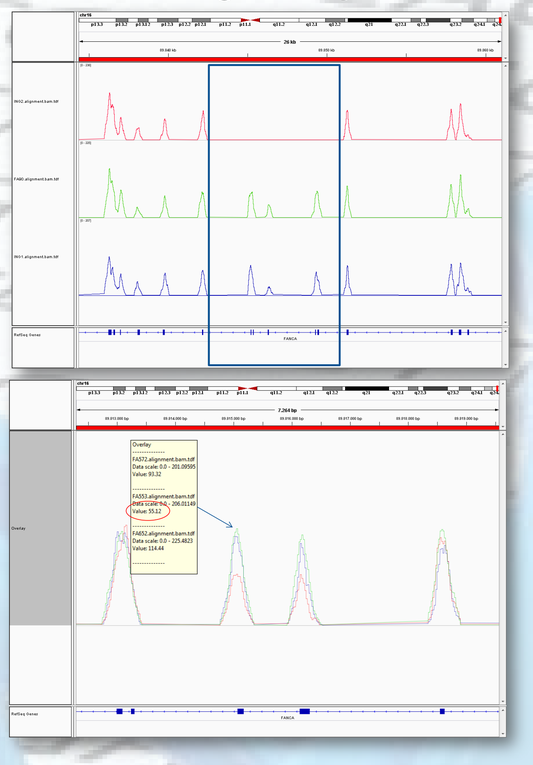


Mean coverage by sample

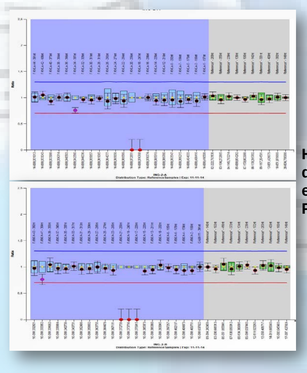


7. Detecting large deletions by coverage analysis

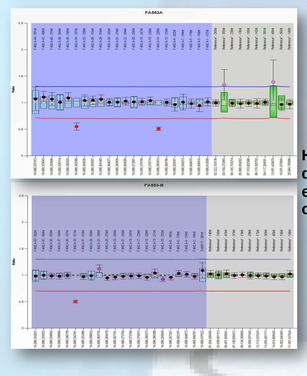
Coverage analysis



MLPA confirmation



Homozigotic deletion from exon 16 to 20 of FANCA



Heterozigotic deletion of both exon 32 and 33 of FANCA

8. Results

Patients with all mutations found						
Code	Sex	Subtype	DNA	Gene	1st Mutation	2nd Mutation
FA530	F	ND	PB	FANCA	Exón 36; c.3558insG; p.R1186Efs*28; Savino, 2003	Homozygous
FA535	M	A	PB	FANCA	Exón 13; c.1115_1118delTTGG; p.V372Afs*42; FA/Breast cancer consortium, 1996	Exón 13; c.1100G>T; p.S367I; Not Reported; Condel: N - MLPA negative
FA537	F	A	PB	FANCA	Exón 38; 3788_3790delTCT; p.F1263del; Rockfeller DB	Homozygous
FA569	F	No A, B, C, maybe G	PB	FANCA	Exón 8; c.790C>T; p.Q264*; Savino, 1997	Exón 36; c.3558dupG; p.R1186Efs*28; Savino, 1997
FA577	F	A	PB	FANCA	MPLA: deleción del Exón 3	Homozygous
FA596	M	A	PB	FANCA	Exón 13; c.1115_1118delTTGG; p.V372Afs*42; FA/Breast cancer consortium, 1996	Exón 3; c.233_236delTTGA; p.T78Ifs*16; Castellá, 2011
FA616	M	A	PB	FANCA	Exón 2; c.163C>T; p.Q55*; Wijker et al, 1999	Homozygous
FA625	M	A	PB	FANCA	Exón 12; c.1033G>T; p.E345*; Not Reported	Exón 4; c.295C>T; p.Q99*; Callen, 2004
FA638	F	A	FB	FANCA	Exón 28; c.2641C>T; p.Q881*; Castellá, 2011	Exón 35; c.3448dupC; p.L1150Pfs*65; Not Reported
FA649	M	A	PB	FANCA	Exón 37; c.3762G>T; p.E1255*; Not Reported	Exón: 33; c.3335T>G; p.V1112G; Not Reported; Condel: D
FA652	M	A	PB	FANCA	Exón 21; c.1860dupC ; p.Y621Lfs*9; Not Reported	i10 c.893+2T>C ; Splicing Not Reported
FA654	M	A	PB	FANCA	Exón 39; c.3913C>T; p.L1305F; Rockfeller DB	Exón 41; c.4124_4125delCA; p.T1375Sfs*49; Rockfeller DB
FA718	F	ND	PB	FANCA	Exón 23; c.2128A>T p.R710*	i38; c.3828+1G>T splicing
ING1	ND	A	PB	FANCA	Exon 29; c.2837_2838insT; p.Ser947Phefs4*; Not Reported	Homozygous
ING2	ND	A	PB	FANCA	ND, Analisis del Coverage: deleción Exones 16-20 - MLPA confirmado.	Homozygous
ING3	ND	A	PB	FANCA	ND, Analisis del Coverage: deleción Exones 1-6	Homozygous
FA357	F	No A,B,C,G,E,F,D2	PB	FANCD2	Exón 3; c.98dupA; pK34Efs*6; Not Reported	Homozygous
FA383	F	FANCD2	FB	FANCD2	Exón26; c.2444G>A; p.R815Q; reported Kalb et al., 2007;	i37; c.3777+1G>A
FA531	F	NO A,C,G,E,F,D2	PB	FANCD2	Exón 26; c.2444G>A; p.R815Q; reported Kalb et al., 2007;	Homozygous.
FA326	F	No A,C,G,E,F,D2	FB	FANCG	Exón 13; c.1642C>T; p.R548*; Ahuerbach, 2003	Exón 5; c.566dupA; p.E190Gfs*45; Not Reported
FA486	F	G	PB	FANCG	Exón 13; c.1642C>T; p.R548*; Ahuerbach, 2003	Homozygous
FA287	F	No A,B,C,G,E,F,D2,J. Normal RAD51 Foci	PB	FANCJ	Exón 7; c.751C>T; p.R251C; Chandrasekharappa,2013	Exón 7; c.975_977delA; p.S278fs*10; Not Reported
FA104	F	No A,B,C,G,E,F,D2,J. Normal RAD51 Foci	PB	FANCQ/ERCC4	Exón 11; c.2065C>A; p.R689S; Bogliolo, 2013	Exón 8: c.2371_2398dup28; p.I800Tfs*24; Bogliolo, 2013
FA49	M	A; Sibling: FA50	LCL	FANCA	c.1083+2T>C; splicing; Not Reported	MLPA: deletion Exons 21-29
FA50	M	A; Sibling: FA49	LCL	FANCA	c.1083+2T>C;splicing; Not Reported	MLPA: deletion Exons 21-29
FA90	F	A	LCL	FANCA	Exón 36; c.3558insG; p.R1186Efs*28; Savino, 2003	Exón 5; c.472insC; p.Hist158Profs*23; Not Reported
FA331	M	No A,C,G	PB	FANCA	Exón 40; c.3982A>G; p.T1328A; Rockfeller DB	Exón 33; c.3263C>T; p.S1088F; Rockfeller DB
FA550	F	A; Sibling: FA551	FB	FANCA	Exón 38; 3788_3790delTCT; p.F1263del; Rockfeller DB	Exón 41; c.4130C>G; p.S1377*; Ameziane, 2008
FA551	M	A; Sibling: FA550	FB	FANCA	Exón 38; c.3788_3790delTCT; p.F1263del; Rockfeller DB	Exón 41; c.4130C>G; p.Ser1377*; Ameziane, 2008
FA553	M	A	FB	FANCA	Exón 13; c.1115_1118delTTGG; p.V372Afs*42; FA/Breast cancer consortium, 1996	MLPA: deletion Exons 32-33
FA568	F	A	PB	FANCA	Exón 39 ; c.3913C>T; p.L1305F; Rockfeller DB	Exón 13; c.1115_1118delTTGG; p.V372Afs*42; FA/Breast cancer consortium, 1996
FA609	M	A	PB	FANCA	Exón 1 c.65G>A; p.Trp22X	Homozygous
FA707	F	ND	PB	FANCA	Exón 33; c.3263C>T; p.S1088F; Rockfeller DB	MLPA: Deletion Exons 15-17
FA574	M	No A,C,G; no ub-FANCD2	PB	FANCB	Exón 3; c.786G>T; p.K262N; Condel: N	X-Linked
FA158	M	ND	PB	FANCD1/BRCA2	c.8488-1G>A; IVS19-1 G to A; Howlett, 2002	Homozygous
FA663	F	ND	PB	FANCD1/BRCA2	Exón 16; c.7796A>G; p.E2599G;Not Reported; Condel: D	Exón 10; c.1814_1815insA; Pro606Thrfs*8; rs80359310 - BIC: Clinically relevant

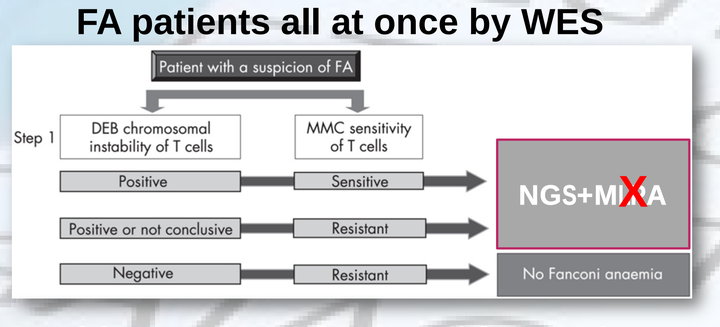
Patients with only one mutation found						
Code	Sex	Subtype	DNA	Gene	1st Mutation	2nd Mutation
FA166	F	A	LCL	FANCA	Exón 36; c.3558dupG; p.R1186Efs*28; Savino, 2003	ND; MLPA Negative
FA388	F	A	PB	FANCA	Exón 13; c.1115_1118delTTGG; p.V372Afs*42; FA/Breast cancer consortium, 1996	ND; MLPA Negative
FA460	F	A	PB	FANCA	Exón 37; c.3763G>T; p.E1255*; Not Reported	ND; MLPA Negative
FA572	F	no A,C,G,D2,E,F	PB	FANCA	Exón 27; c.2574C>G; p.S858R; Tamary, 2000	ND; MLPA Negative
FA124	F	No A/C/G.	PB	FANCD2	Exón 13; c.1068T>A ; p.Y356*; Not Reported	ND
FA664	M	ND; Sibling: FA681	PB	FANCD2	Exón 3; c.226_228delA; p.K77Rfs*7; Not Reported	ND
FA681	M	ND; WB: No FANCD2 band ; Sibling: FA664	PB	FANCD2	Exón 3; c.226_228delA; p.K77Rfs*7; Not Reported	ND
FA121	M	E	PB	FANCE	Exón 4; c.929insC; p.V311Sfs*1; Not Reported	ND

Full Fanconi
Mosaic

PB: Periferal Blood; FB: primary fibroblasts; LCL: Lymphoblastoid cell line

9. Proposed improved pipeline

Subtype and molecularly characterize FA patients all at once by WES



This strategy is extensible to other genetically heterogeneous BMF syndromes.

- We identified the gene and both mutations in 36/44 (81,8%) FA patients (26 A, 1 B, 2 D1, 3 D2, 2 G, 1J and 1 Q)
- We identified 79/87 mutant alleles (90,8%) including full FAs and mosaics.
- By WES + MLPA we were able to detect at least 1 mutation in all cases (100%)
- At least 5 out of 9 patients presented mutations in genes (2 A, 1 D2, 1 G and 1J) that were previously disregarded by retroviral subtyping.
- PB is not an ideal DNA source to characterize mosaic patients: only one mutation was found in 4/11 PB samples (36%) from mosaics, while for full FA patients only 3 out of 23 (13%) PB DNA samples were not fully characterized.
- Primary fibroblasts should be used as default DNA source for molecular analysis of all FA patients by WES.

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