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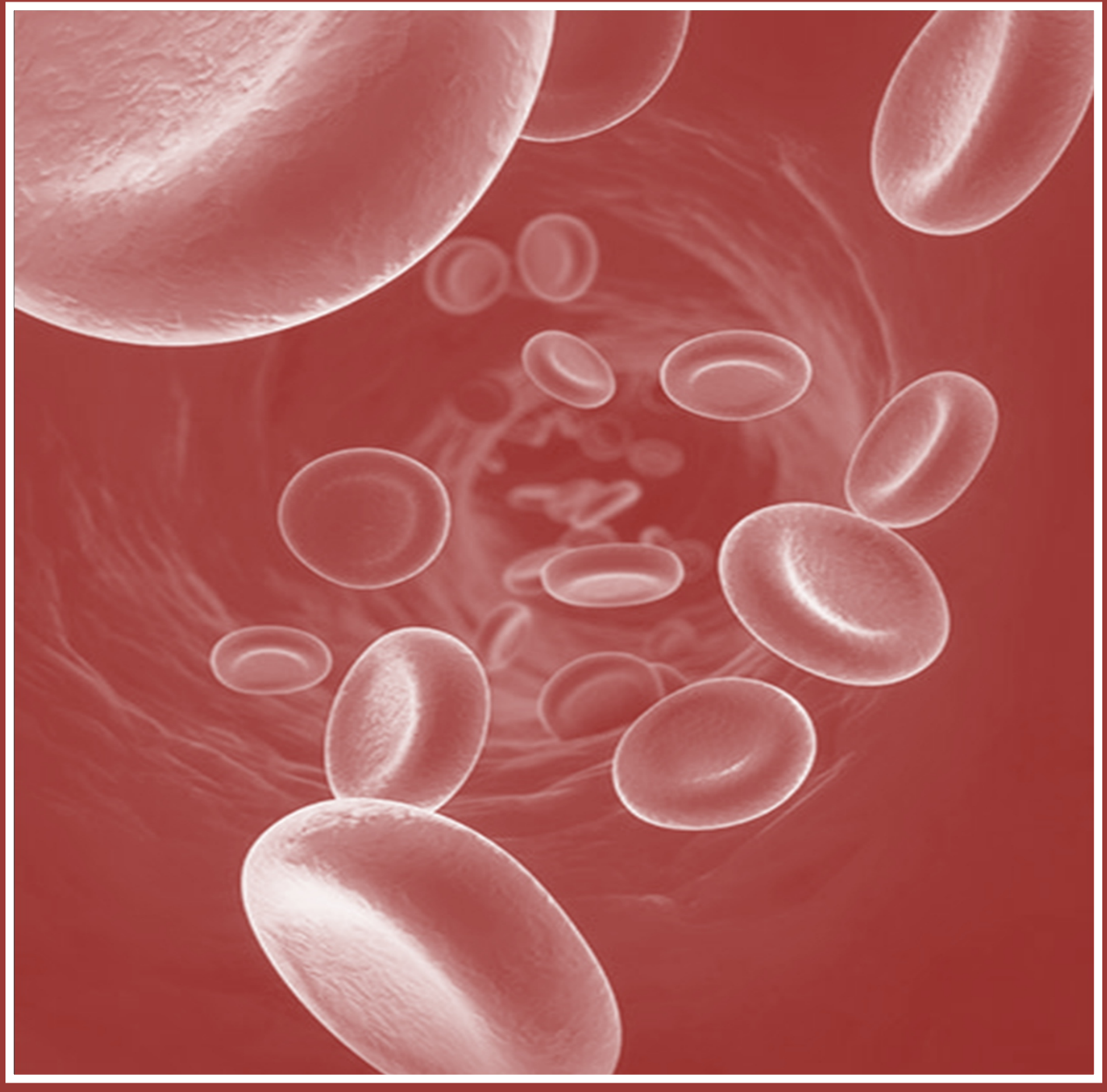

Pledge ceremony during Vigilance week

Essay Competition during Hindi Pakwada celebration

Fanconi anemia: A multispectrum disease

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Fanconi anemia (FA) is a rare inherited bone marrow failure disorder which occurs due to defect in a DNA damage repair pathway called Fanconi anemia pathway. Mutations in the FANC genes that encode DNA damage response proteins can result in genomic instability syndrome, a heightened predisposition to cancer, congenital physical anomalies, skin pigmentation abnormalities, and cellular sensitivity to ICLs. Bone marrow failure begins with tri-lineage depletion or may affect one or two lineage at early stage of life and then accelerates further to haematological malignancy or may lose control over cellular processes through complex developments and ultimately cause solid tumours. FA phenotype is associated with 21 genes and these proteins are actively involved in DNA repair through FA pathway. Molecular analysis for carrier detection through prenatal diagnosis and pre-implantation genetic diagnosis (PGD) methods is important for management of FA.

1. Introduction

Swiss paediatrician Guido Fanconi first described the disease Fanconi anemia in the 1920s. He put forward a case of a family with five children, in which, three brothers died of a severe condition that resembled pernicious anemia. In these three patients, disease manifested between the ages of five and seven years, and was associated with congenital microcephaly, café-au-lait spots, cutaneous haemorrhage, and hypoplasia of the testes, concurrent with a current-day diagnosis of FA [1].

FA is a recessive genetic disorder, with an estimated incidence of 1 in 360,000 live births and a carrier frequency of approximately 1 in 181 [2,3],occurs due to defect in any one of the 21 gene products (FANCA – FANCV) so far known to be involved in DNA damage repair pathway called FA pathway. FA pathway includes central event of FANCD2 monoubiquitination by upstream complex, which occurs in response to DNA damage. FANCI forms a complex with FANCD2, together recruit downstream complex proteins to the site of DNA damage, where they bring about the repair through homologous recombination as major repair pathway. FA cells are sensitive to ICLs like Mitomycin C (MMC; chemical name - [6-Amino-8a-methoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazireno{2',3':3,4}pyrrolo[1,2-a]indol-8-yl]methyl carbamate]) and Diepoxybutane (DEB; chemical name - (1,3- Butadiene diepoxide), which form mono and di-adduct formations on DNA strands,

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thus replication machinery gets stranded at such bulky adduct spots. Hence the machinery resorts for alternative mechanism, an error prone repair like Translesion synthesis (TLS) [4]. Therefore cancer predisposition is relatively common in FA patients, haematological severity can progress to acute myeloid leukemias via myelodysplatic syndrome or they may develop solid tumours of head and neck or oral cavity [5].

FA is managed by recovery of blood counts either through transfusion or by reducing cytotoxicity through antithymocyte globulin (ATG) administration with regimen of Cyclosporin A (CsA) or through hematopoietic stem cell transplant from HLA-matched donor [5]. In this article, we discuss the clinical presentations, FA pathway, diagnostic methods, and the treatment options available for FA.

2. Diagnosis of Fanconi anemia

2.1 Clinical diagnosis

Major congenital malformations are reported in approximately two-thirds of the patients, while one third is reported as not having congenital malformations. Most FA patients with congenital malformations are not diagnosed till the onset of hematologic abnormalities; delayed diagnosis might be due to lack of physician awareness of the phenotypic spectrum of FA. FA patients without congenital malformations frequently have alterations in growth parameters, like height, weight, or head circumference below the fifth centile. Other very common findings are skin pigmentation abnormalities, hypoplastic thenar eminence, microcephaly and / or microphthalmia. Increased awareness of the facial anomalies (Fig. 1c) as well as the complete spectrum of minor malformations seen in these patients should enable an earlier diagnosis to be made among patients without major congenital anomalies [6]. FA clinical presentations include various congenital anomalies like microcephaly, microphthalmia, abnormal thumbs or radii, and slow growth rate. Haematological signs can include early-onset aplastic anemia, myelodysplastic syndrome (MDS), acute myeloid leukaemia (AML) at an atypically young age, and one or more unexplained cytopenias of any cell lineage, including thrombocytopenia, neutropenia, and anemia[1]. Apart from bone marrow failure (BMF) and physical anomalies, presence of high levels of fetal hemoglobin (HbF) has been documented in several case reports describing FA [7] and other bone marrow failure syndromes [8]. Details of each of the clinical presentations of FA patients are summarized in Table 1.

Table 1 Clinical presentations observed in FA patients [Adapted from Auerbach AD, 2009]

Major and minor clinical presentations	
Skin	Café-au-lait spots hyper- and hypopigmentation (Fig. 1c)
Growth	Intrauterine growth retardation, short stature, endocrine abnormalities
Eyes	Microophthalmia, short or almond shaped palpebral fissures, ptosis, epicanthal folds, hypertelorism (Fig. 1c) and hypotelorism, strabismus, cataracts
Thumb and radius	Thenar hypoplasia, absence or hypoplasia of radius and/or thumb (Fig. 1c), floating thumb, bifid thumb, digitalized thumb/abnormal thumb placement
Other skeletal	Dysplastic or absent ulna, micrognathia, frontalbossing, spina bifida, Klippel-Feil, vertebral anomalies, absent clavicles, Sprengel's deformity, Perthes disease, congenital hipdysplasia/dislocation, scoliosis, ribabnormalities, clubfoot, sacral agenesis (hypoplasia), leg length discrepancy, kyphosis, brachydactyly, arachnodactyly, humeralabnormality, craniosynostosis
Kidney and urinary tract	Ectopic, horseshoe, rotated, hypoplastic orabsent, dysplastic, hydronephrosi hydroureter, urethral stenosis, reflux
Ears	Deafness (usually conductive), abnormal orabsent pinna, prominent ears, abnormally positioned ears (low set or posteriorly rotated), small or absent ear canals, absent tympanicmembrane, microtia, fused ossicles
Genital	Males: micropenis, penile/scrotal fusion, undescended or atrophic or absent testes, hypospadius, chordee, phimosis, azospermia. Females: bicornate uterus, aplasia or hypoplasiaof vagina and uterus, atresia of vagina, hypoplasic uterus, hypoplastic/absent ovary, hypoplastic/fused labia
Cardio-pulmonary	Patent ductus arteriosis, ventricular septaldefect, pulmonic or aortic stenosis, coarcation ofthe aorta, double aortic arch, cardiomyopathy, tetralogy of Fallot, pulmonary atresia
Gastrointestinal	Esophageal atresia, duodenal atresia, anal atresia, tracheoesophageal fistula, annular pancreas, intestinal malrotation, intestinal obstruction, duodenal web, biliary atresia, foregut duplicationcyst
Central nervous	Microcephaly, hydrocephalus, Bell's palsy, CNS arterial malformations, abnormal
system (CNS)	pituitary, absent septum pellucidum/corpus callosum, hyperreflexia, neural tube defection, Arnold-Chiari malformation, Moyamoya, single ventricle

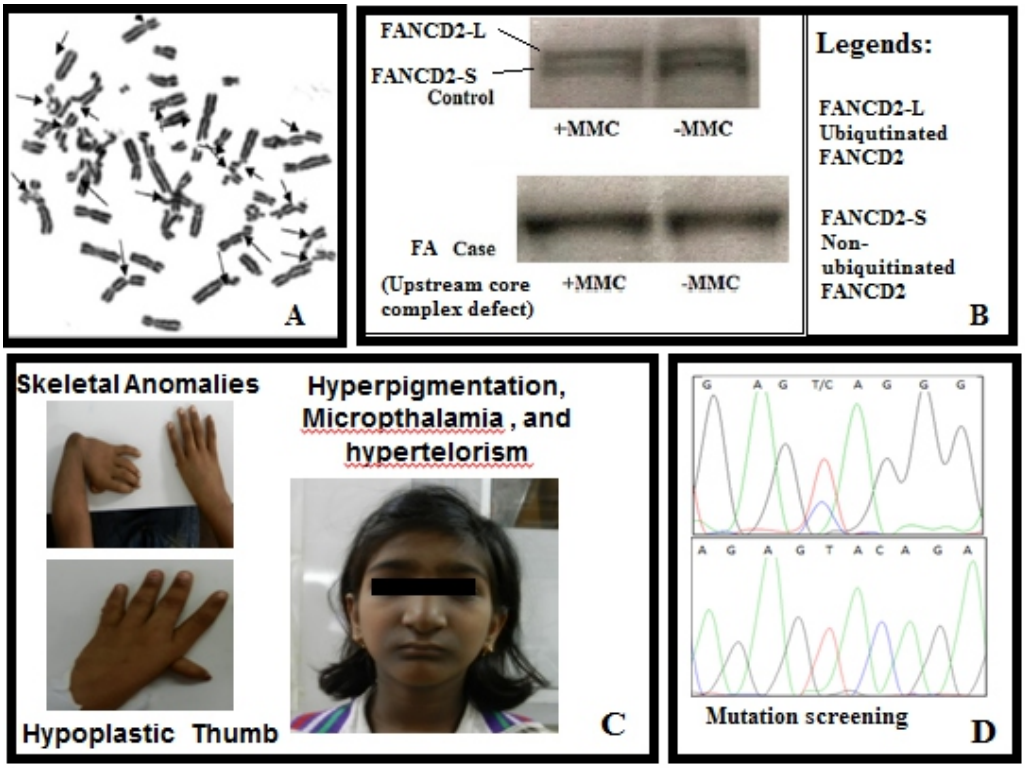

Fig. 1 Diagnosis of Fanconi anemia based on cellular sensitivity to ICLs (A), Immunoblotting assay for FANCD2 monoubiquitination (B), Clinical presentations (C), and mutation screening(D).

2.2 Laboratory investigations

2.2.1 Chromosomal breakage test

Sensitivity to ICLs forms basis for diagnosis of FA by chromosomal breakage test and it has still been considered as gold standard for the diagnosis. Phytohemagglutinin induced peripheral blood cultures from patients and controls are cultured for 72 hours as per standard cytogenetic test. ICL induced and spontaneous peripheral blood cultures are set up for each patient along with age matched control with same set of flask as set up for patients. Chromosomal breaks are scored as summation of chromatid breaks or gaps, radial formations (tri-radial chromosome, tetra-radial formation) (Fig. 1A). In FA patients chromosomal breaks are increased eight to twenty times [6, 9]. Number of chromosomal breaks induced by MMC and DEB depends on concentration of DNA damaging agent, optimal and reliable concentration should therefore be set up for the investigation.

2.2.2 FANCD2 immunoblot

Although positive chromosomal breakage test for suspected cases is sufficient, start of molecular analysis requires confirmation of exact defect in FA pathway i.e., defect in upstream complex or downstream complex component. The key event in FA pathway is monoubiquitination of FANCD2, upon DNA damage, by upstream core complex. Using FANCD2 immunoblotting method, stimulated T-lymphocytes are tested for the occurrence of the ubiquitinated isoform of FANCD2. Patients with high frequency chromosomal breakage and absence of ubiquitinated form of FANCD2 reveal FA in cases where this isoform is lacking either of the functional subunits of upstream core complex. Primary antibodies raised against a

domain of FANCD2 is used, which can detect both monoubiquitinated and non-ubiquitinated forms. Thus, patients with chromosomal breakage test positive and non-ubiquitinated form or monoubiquitinated FANCD2 form can help differentiate upstream or downstream complex gene defect respectively. [10]

2.2.3 Cell Cycle Arrest by Flow cytometry

Cell cycle arrest by flow cytometry is carried out on PHA-stimulated peripheral blood lymphocytes from FA patients as compared with controls. Cells derived from FA patients spontaneously accumulate chromosomal breaks and exhibit marked sensitivity to ICLs. Flow-cytometry estimates the G2/M phase events, which is observed to be increased in FA patients after 72 hr in vitro culture, compared to untreated or control cells.

In the presence of DNA crosslinks, the double helix cannot be unwound, and the replication forks stall to repair the crosslink before the replication continues [11]. The S-phase checkpoint ensures stability of replication forks and relieves the effect of replication stress on the genome. Notably, the same kinases that control the FA signalling during DNA damage response (e.g. ATR, CHK1) are essential for the S-phase checkpoint. Thus, it is conceivable that FA proteins show a functional role in the intra-S checkpoint response where these proteins participate in the suppression/restart of DNA synthesis [12].

3. Molecular genetics of FA and genotype-phenotype correlation

Earlier complementation group assignment for FA patients was done by cell fusion, positional cloning using micro-cell mediated chromosome transfer, and complementation analysis by introduction of cDNA plasmid that expresses a normal copy of the defective gene [13]. Transduction of fibroblasts, lymphoblastoid cell lines from FA patients using retroviral vector containing the cDNA for wildtype *FANC* gene and assessing the reversal of chromosomal breakage repair confirms the complementation group for the patient. Exact molecular mutations underlying the pathogenesis has now been detected by direct sequencing or advanced sequencing methods like targeted exome sequencing and whole genome sequencing [14, 15].

Till date 21 gene products are known to be involved in FA pathway. With the most recent additions, there are now 21 confirmed FA genes; *FANCA, FANCB, FANCC, BRCA2/FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCI, BRIP1/FANCJ, FANCL, FANCM, PALB2/FANCN, RAD51C/FANCO, SLX4/FANCP, ERCC4/FANCQ, RAD51/FANCR, BRCA1/FANCS, UBE2T/FANCT, XRCC2/ FANCU,* and *MAD2L2/REV7/FANCV*. The FANCC gene was identified in 1992. Subsequent discoveries of the *FANCA*, *FANCG*, *FANCE*, *FANCF*, and *FANCD2* genes followed. *FANCB* is located on X-chromosome, rest others are located on autosomes. So this disorder is inherited in X-linked or autosomal recessive pattern. FA-A, FA-C, and FA-G are major complementation groups represented by FA patients all over world with frequencies of 60%, 15%, and 10% respectively [1]. List of *FANC* genes with their length, encoded protein, molecular weight and functions is given in Table 2.

Table 2: Location, length, frequency, functions and molecular weights of FANC genes

Gene	Gene length (bp)	Frequency	Location	Protein size (kDa)	Function
FANCA	5451	60%	16q24.3	163	FANCA and FANCG form a complex in the cytoplasm, through N-terminus of FANCA, then FANCC joins the complex, and phosphorylation of FANCA would induce its translocation into nucleus.
FANCB	3008	2%	Xp22.31	95	Involved in formation of upstream core complex
FANCC	4585	15%	9q22.3	63	Involved in formation of upstream core complex
FANCE	2554	3.5%	6p21.3	60	Involved in formation of upstream core complex
FANCF	4269	2%	11p15	42	Involved in formation of upstream core complex
FANCG	2631	10%	9p13	68	Involved in formation of upstream core complex
FANCL	1698	0.4%	2p16	52	E3 Ubiquitin Ligase activity for ubiquitination of FANCD2
FANCJ	6048	1.7%	17q23.2	140.8	BRIP1. Homologous recombination and translesion synthesis
FANCN	4003	2.1%	16p13.3	131.2	PALB2. Homologous recombination
FANCO	2591	Rare	17q21	42.1	RAD51C. Homologous recombination
FANCP	7307	Rare	16p13.3	200	SLX4. Nuclease scaffold
FANCQ	6765	Rare	16p13.12	104.4	ERCC4 protein encoded by this gene forms a complex with ERCC1 and is involved in the 5' incision made during nucleotide excision repair
FANCR	2449	Rare	15q15.1	36.9	RAD51 plays an important role in homologous strand exchange, a key step in DNA repair through homologous recombination
FANCS	7094	Rare	17q21.31	207.7	BRCA1. Homologous recombination and fork stabilization

(Table continue on next page)

Gene	Gene length (bp)	Frequency	Location	Protein size (kDa)	Function
FANCT	922	Rare	1q32.1	22.5	UBE2T. Upstream core complex. E2 Ubiquitin Ligase activity for FANCD2 ubiquitination.
FANCU	3067	Rare	7q36.1	31.9	XRCC2is involved in the repair of DNA double-strand breaks by homologous recombination and it functionally complements Chinese hamster irs1, a repair-deficient mutant that exhibits hypersensitivity to a number of different DNA-damaging agents.
FANCV	1860	Rare	1p36.22	24.3	REV7 Homolog. a subunit of DNA polymerase ζ. Involved in translesion DNA synthesis (TLS), Pol∂ is capable of synthesizing directly acrosstemplate DNA lesions; specifically inserting nucleotides opposite DNA adducts, abasic sites, and, of particular interest in FA, DNA crosslinks. Through this process, the cell can tolerate the effects of DNA lesions during replication, effectively guarding against genomic instability.
FAAP20	1413	-	9q13.11	19.8	
FAAP100	3627	-	17q25.1	93.4	
FANCD1	1986	5%	13q12.3	380	Homologous recombination and fork stabilization
FANCD2	5219	4.7%	3p25.3	155, 162	FANCD2 forms a complex with FANCI. Monoubiquitinated ID2 localizes to chromatin where it associates with several established DNA repair proteins, including BRCA1/FANCS, BRCA2/FANCD1, and RAD51/FANCR.
FANCM	7111	0.3%	14q21.2	250	The FANCM anchor complex, comprising FANCM, FAAP24, FAAP16/MHF1, and FAAP10/MHF2, promotes the chromatin recruitment of the FA core complex.
FANCI	4743	2.8%	15q25-16	140	Forms complex with FANCD2 and recruits several DNA repair proteins, including CtIP, FAN1, FANCP and FANCP.

FA is more common among people of Ashkenazi Jewish descent, the Roma population of Spain, and black South Africans. The birth incidence of FA in Bantu-speaking populations of sub-Saharan Africa population is higher than actual incidence rate, which suggests that the FANCG deletion is an ancient founder mutation in this population. A deletion mutation in the FANCG gene (c.637_643delTACCGCC) is present in 82% of FA patients in the black populations of Southern Africa. These patients originated from South Africa, Swaziland, Mozambique, and Malawi [16]. Callen E et al., 2005 have identified FANCA c.295C>T mutation leading to FANCA truncation and FA pathway disruption, in only Spanish Gypsies as it was not found in other Gypsy patients with FA from Hungary, Germany, Slovakia, and Ireland. Haplotype analysis by Callen et al. (2005) showed that all the Spanish Gypsy patients shared the same haplotype and concluded that the high incidence of FA among Spanish Gypsies is due to an ancestral founder mutation in FANCA that originated in Spain less than 600 years ago[17]. Whitney M A et al. (1994) identified a common mutation in the FANCC gene, which accounts for a majority of FA cases in Ashkenazi Jewish individuals. FANCC IVS4 + 4 AT allele was found on 83% of Jewish FA patients, indicating that it is indeed responsible for most cases of FA among Ashkenazi Jews [18].

Genotype-phenotype correlation studies for FA have been reported with distinct parameters of phenotypic outcome by different research groups. There have also been reports describing sibling with same mutations showing distinct phenotypes. Attempts made for genotype-phenotype correlation for FA by different teams of researchers have no unanimous or striking conclusive fruition. Compared to controls, accelerated shortening of telomere repeats or enhanced loss of telomere signals of FA lymphocytes and serially propagated FA fibroblasts have been frequently reported [19, 20]. Multiple genotypes and multiple clinical phenotypes connected by a singular cellular phenotype, i.e., high degree of heterogeneity at the molecular and phenotypic levels are in marked contrast to the FA cellular phenotype which is surprisingly uniform. Majority of cellular phenotypes like sensitivity to ICLs and atmospheric oxygen can be safely predicted from any of the genetic alterations in any of the genes of FA [21]. However, there is no distinct association which could be made out so far due to various reasons [19, 22].

4. FA and cancer

Hematologic abnormalities occur in virtually all patients with FA at a median age of 7 years (range: birth to 41 years). Thrombocytopenia is often associated with elevated levels of fetal hemoglobin and macrocytosis and usually preceded onset of anemia or neutropenia. Some patients present myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) without prior diagnosis of aplastic anemia (AA). The cumulative incidence of MDS or AML was found to be 33% till the age of 40 years. Based on a survey of FA patients performed by Rosenberg et al., the median age of onset of leukemia was 11.3 years [23].

Presence of bone marrow clonal cytogenetic abnormalities becomes increasingly common with age. The actuarial risk of identifying clonal cytogenetic abnormalities at the time of marrow failure is 67% by 30 years of age [23]. Among the most frequent

clonal abnormalities observed are duplications and triplications of the long arm of chromosome 1, gains of portions of the long arm of chromosome 3, and monosomy 7 or loss of material from the long arm of chromosome 7. Deletions of 5q, 11q, rearrangements of 6p, and gains of chromosomes 8 and 21 have also been noted by different groups [23]. In addition, AML in FA patients rarely involves chromosomal rearrangements observed in non-FA patients.

In addition to the extraordinarily high frequency of AML in FA patients (actuarial risk of 52% for the development of MDS and/or AML by 40 years of age) [23], the high incidence of non-hematologic malignancy in FA patients is especially striking because of the predicted early death from hematologic causes associated with the syndrome (median estimated survival is 23 years; actuarial risk of death from hematologic causes is 81% by 40 years of age). Thus patients are unusually young when they develop cancer, and the incidence of malignancy probably would be considerably higher if patients had a longer life expectancy [23]. Most of the non-hematologic tumors in FA patients are squamous cell carcinomas (SCC) especially of the head and neck and anogenital regions [23]. Fanconi anemia patients have a 500- to 700-fold higher incidence of head and neck SCC than the general population and a 14% cumulative incidence of head and neck SCC by the age of 40 years [23].

5. FA pathway, its activation and turning off, and functions

One major function of the FA-BRCA pathway is to orchestrate the repair of DNA ICLs. ICLs pose a direct physical block to DNA replication and RNA transcription and result in cellular cytotoxicity and chromosome structural aberrations if not properly repaired.

5.1 FA pathway

ICL repair mediated by the FA proteins occurs in three phases. In the first phase, the FA proteins FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, and FANCL, as well as the Fanconi anemia associated proteins FAAP20 and FAAP100, assemble to form the FA core complex (Fig. 2). Upon DNA damage, the FA core complex is recruited to chromatin where it interacts with constitutively chromatin localized UBE2T/ FANCT [24, 25]. The FANCM anchor complex, comprising FANCM, FAAP24, FAAP16/MHF1, and FAAP10/MHF2, promotes the chromatin recruitment of the FA core complex [26-29]. Together, the FA core complex and UBE2T/FANCT constitute an active multi-subunit E2/E3 ubiquitination enzyme complex. The RING domain-containing FANCL subunit functions as the E3 ubiquitin ligase while UBE2T/FANCT functions as the E2 ubiquitin conjugating enzyme [30-32]. This complex catalyzes the second phase of the pathway, the conjugation of a single ubiquitin moiety (mono-ubiquitin) to K561 of FANCD2 and K523 of FANCI [33-35]. FANCD2 and FANCI form a heterodimer known as ID2 [36]. Ubiquitin is a 76-amino acid protein that is post-translationally attached to target proteins covalently. Monoubiquitin functions as a molecular signal that regulates diverse cellular processes including the targeting of proteins to distinct sub-cellular locations and the promotion of protein–protein interactions [37].

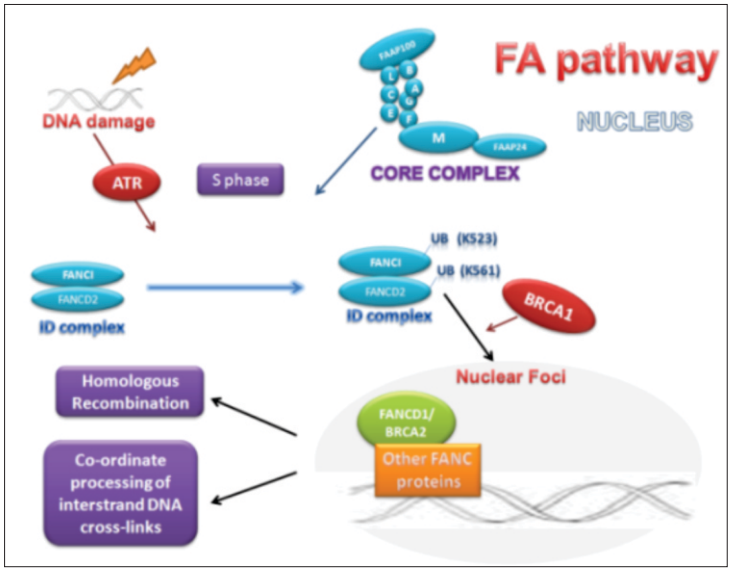

Fig. 2 Fanconi anemia pathway

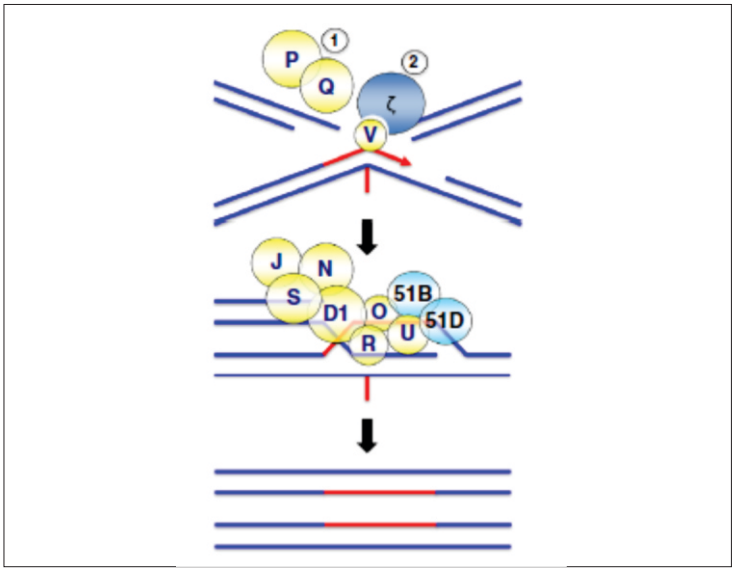

Fig. 3 Downstream steps of FA pathway (Adapted from Mamrak N.E. et al., 2016)

Monoubiquitinated ID2 functions in the recruitment of several DNA repair proteins, including CtIP, Fanconi anemia associated nuclease 1 (FAN1), SLX4/FANCP, and ERCC4/FANCQ. SLX4/ FANCP and the ERCC4/FANCQ endonuclease catalyze the unhooking of the ICL, enabling translesion DNA synthesis (TLS) beyond the ICL by the multi-subunit TLS polymerase Polζ, and one subunit of which isREV7/FANCV (Fig. 3). During the final phase of ICL repair, the FA proteins - BRCA2/FANCD1, BRIP1/FANCJ, PALB2/FANCN, RAD51C/FANCO, BRCA1/FANCS, RAD51/FANCR, and XRCC2/FANCU - function cooperatively to repair the remaining broken duplex via homologous recombination (HR) (Fig. 3). HR is predominantly a conservative and error-free process whereby DNA damage is repaired using a homologous DNA template, typically the sister chromatid. RAD51/FANCR is the major eukaryotic HR repair protein. RAD51/FANCR forms nucleoprotein filaments on 5′-3′ resected single-stranded DNA and catalyze homologous pairing and DNA strand invasion and exchange. Many of the downstream FA proteins, e.g. BRCA2/ FANCD1, PALB2/FANCN, and RAD51C/FANCO, are known to facilitate RAD51 function. Disruption of the FA-BRCA pathway leads to defective Homologous recombination (HR) and an increased dependence on the typically error-prone non-homologous DNA end-joining (NHEJ) repair pathway [1].

5.2 Activation and Turning off of the FA pathway

Fanconi pathway is activated and functions primarily in the S phase of the cell cycle in response to DNA replication fork arrest. FANCD2 is monoubiquitinated at the G1/S boundary, remains monoubiquitinated throughout S phase, and becomes deubiquitinated at the end of S phase as the synchronized cell population enters G2. Replication fork arrest leads to the local generation of single-strand DNA, perhaps through helicase unwinding of the DNA duplex. Single-strand DNA binds RPA (Replication Protein A) and then ATR (Ataxia Telangiectasia and RAD3-related protein) and ATR Interacting Protein (ATRIP). After recruitment to the stalled fork, ATR phosphorylates multiple substrates, leading to additional cell cycle checkpoint and DNA repair responses. This may occur through the

direct phosphorylation of one or more sub-units of complex 1[38].

Inappropriate activation of the FA pathway in undamaged cells may lead to harm by promoting mutagenic TLS. Ubiquitin specific peptidase 1 (USP1) has been identified as deubiquitinating (Dub) enzyme which regulates the FA pathway. Dub enzymes are cysteine proteases that cleave peptide and isopeptide linkage immediately after the glycine-glycine sequence (i.e., the C terminus of ubiquitin). USP1 activity peaks at the end of S phase, perhaps accounting for the observed deubiquitination of FANCD2 in late S phase [38].

5.3 F unctions of FA pathway

FA pathway does not only participate in DNA damage repair but is also actively involved in detection and processing of stalled replication forks. FANCJ and FANCM have DNA helicases activities [39, 40]. A few components of core complex associate with BLM, topoisomerase III, and replication protein A. This helps stalled replication machinery to co-operate in DNA unwinding [41-43]. FA downstream complex co-localizes with NBS1, a subunit of MRE11/RAD50/NBS1 (MRN) complex which has 3'- 5' exonuclease activity and it is required for DNA processing prior to repair through HR [44, 45].

FA pathway requires the 3'-5' exonuclease activity of the RAD50, MRE11, and NBS1 complex to expose the 3' ends on either side of the double strand breaks, this process requires a BRCA1. During S phase, DNA cross-links result in a replication fork arrest. In the process of repair, the cross-links are excised by an endonuclease leading to the generation of DSB intermediates. The DSB can then be repaired by HR. Monoubiquitinated FANCD2 associates with BRCA1, BRCA2, RAD51, PCNA, and NBS1 and is required for the relocalization of BRCA2 to chromatin and the subsequent formation of RAD51 foci [38]. This process can result in point mutations due to the mis-incorporation of nucleotides.TLS may offer an alternative to HR in order to re-establish replication forks or may work in conjunction with HR [38,4].

6. Treatment options, prevention of disease by prenatal diagnosis and preimplantation genetic diagnosis

The Fanconi anemia consensus guidelines suggest initiation of packed RBC transfusion when the hemoglobin level is 8 g/dL or less and platelet concentrates transfusion when the platelet count is ≤ 30,000/mcL. Febrile episodes when neutrophil count less than 1000/mcL is managed by broad spectrum parenteral antibiotics. In the same time patients with an HLA-matched healthy related donor should be offered stem cell transplant. Preparation for stem cell transplant has been most successful with low-dose cyclophosphamide-based conditioning regimen, and the survival is more than 70%; with an alternative donor, the survival is less than 40%. Hematopoietic stem cells can be obtained from bone marrow, cord blood, or peripheral blood [23].

Patients without a matched sibling donor should be offered treatment with androgens, usually oxymetholone at 2-5 mg/kg/day [23]. The treatment options and their benefits for those who does not get suitable donor has been shown in Table 3 [23].

Table 3 Treatment options, their response and benefits

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Table 3 Treatment options, their response and benefits

Treatment options	Response and Benefits
Treatment response	>50% patients respond with improved blood counts
Side effects	virilization, liver dysfunction, and liver tumours (3% of reported patients with Fanconi anemia)
Alternative therapeutic options	immune suppressive therapy with ATG and CsA or high-dose cyclophosphamide
Cause underlying the disease	Immune-mediated dysfunction. This was proven in-vitro by improvement in the colony number in tissue culture after removal of lymphocytes, and their addition to normal marrow inhibits hematopoiesis.
Benefits of ATG	Depletion of immune compete cells and contains various antibodies recognizing human T-cell epitopes, many directed against activated T cells or activation antigens
Benefits of CsA	A more selective inhibitory effect on T-lymphocytes, suppressing early cellular response to antigenic and regulatory stimuli. By blocking expression of nuclear regulatory proteins, it leads to reduced T-cell proliferation and activation with diminished release of cytokines such as interleukin-2 and interferon-gamma.
Combination of ATG and CsA	The current standard therapy in severe aplastic anemia. Overall response is achieved in about two thirds of patients; the cumulative incidence of relapse among responders is approximately 20–30% and clonal evolution occurs in about 10–15% of cases.
Risk of Leuekmias and MDS	Leukaemia has been reported in approximately 10% of patients, and MDS has been reported in about 6% of patients, primarily in teens and young adults, some of whom may not have had a preceding phase of aplastic anemia
Risk of solid tumours	Solid tumours have been reported in close to 10% of patients, often in young adults who may never have had aplastic anemia. The most common tumours are liver adenomas and hepatomas, primarily in patients who had aplastic anemia that was treated with oral androgens. Head and neck cancer and squamous cell carcinoma.

Allogeneic stem cell transplantation from an HLA-matched sibling is curative therapy in most patients who undergo this procedure [23]. Survival expectancy and treatment regimens are tabulated in Table 4.

Table 4 Life expectancy, survival rate frequency and alternative treatment regimens for allogeneic stem cell transplantation

Allogeneic stem cell transplantation (HLA-matched sibling)		
Survival rate and period		77%, 5 years
Survival rate for minimally transfused patients		80–90%
Alternative Treatment regimens	**Radiation-free conditioning regimens for heavily transfused patients**	Cyclophosphamide + ATG improved the tolerability and allowed for engraftment in heavily transfused patients
	Alternate stem cell donor source	Matched unrelated, umbilical cord for those FA patients who lacked an HLA-matched related donor or failed to respond to immune suppressive therapy (IST) or have recurrent disease after IST.

Prenatal screening or preimplantation genetic diagnosis is essential for families with FA proband to avoid further birth of carrier and affected children in the families. Genetic counsellors and physician should closely work for FA families for the same [46].

7. Experience at NIIH

FA research is one of the major research areas in our institute. The studies for FA are conducted at Cytogenetics department and ours is the only laboratory working on FA patients in India. Our laboratory has systematic work-up strategy which includes chromosomal breakage investigation, western blotting for FANCD2 monoubiquitination detection and molecular diagnosis. The suspected FA cases are referred to us from all over India. Apart from FA diagnosis, we have also been undertaking research study on FA which could provide insights into human development and the genesis of cancer due to implication of FA pathway in DNA damage repair. Our recent studies on a cohort of FA patients with median age of 8.5 years and a few adolescent cases (~6%) have also been diagnosed with progression to haematological malignancies and solid tumours. Chromosomal breakage investigation on peripheral blood and FANCD2 monoubiquitination detection by Immunoblotting assay are adequate techniques to correctly diagnose the FA in suspected cases. Upstream gene defect (~95.3%) is more prevalent than downstream gene defect (~4.6%) among the registered Indian FA cases at NIIH. FA-A is the major complementation group (~58.7%), followed by FA-G (~21.7%) [47, 48] and FA-C and FA-D2 (~2.17% each) complementation groups. FA-L, although rare complementation group as per world literature, is emerging as a frequent complementation group in Indian FA patients with 9.38% frequency [Unpublished data]. Further investigation on this is underway at our department and it would be most interesting to study it as FANCL has E3 ubiquitin ligase activity which ubiquitinates FANCD2 and further downstream cascade of events are necessary to recruit downstream complex at the site of DNA damage for the repair. We also carried out pre-natal diagnosis for FA families in which FA proband was diagnosed with exact molecular lesion of FANC gene underlying the pathogenesis [49].

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NIIH HAPPENINGS

Department of Pediatric Immunology & Leukocyte Biology

Dr Manisha Madkaikar, Scientist F

1. Invited to attend "India Africa Health Sciences Meet (IAHSM)" held at Vigyan Bhavan, New Delhi on 1st to 3rd September, 2016.
2. Invited to deliver a lecture on "Primary Immunodeficiency-Indian Scenario"' for International CME on Allergy and Immunology organized by Seth G S Medical College on 21st October 2016.
3. Invited to deliver a Key Note address on "Infections and Immunity: High tech Revelations" for NCPID (National Conference of Paediatric Infectious Diseases) held at Pune from 22nd -23rd October 2016.
4. Invited to deliver a lecture on "Tests in Immunology/ Immunodeficiency" for the 20th Annual Conference of the Pediatric Hematology Oncology Chapter of Indian Academy of Pediatrics, Mumbai held on 4th November 2016.
5. Invited to deliver a talk on 'Primary Immunodeficiency Disorders – Indian Experience' at "Autoimmune/drugs/viruses related lymphadenopathies / lymphoid proliferations" workshop organized by Santokhba Durlabjee Medical Hospital, Jaipur for Indian Society of Hematology and Transfusion Medicine conference on 9th November 2016.
6. Invited as scientific advisory committee member of National Institute of Research in Reproduction held on 15th -16th December 2016.

Department of Hematogenetics

Dr Malay Mukherjee, Scientist E

1. Attended PRC meeting to finalize common protocols for the Task Force Project on Hemoglobinopathies held at ICMR, New Delhi on 30th September 2016.
2. Attended "Tribal Health Research Forum Meeting" held at NIRTH, Jabalpur from 1st to 2nd November 2016 and presented the work done at NIIH, Mumbai.

Dr Anita Nadkarni, Scientist E

1. Attended 2nd workshop in molecular diagnostics held at CMC Vellore on 3rd September 2016 and delivered a lecture entitled "Molecular Diagnosis of Thalassemia".
2. Attended 37th Annual conference of Maharashtra Chapter of the Indian Association of Pathologist and Microbiologist [MAPCON] held at D Y Patil University School of Medicine on 23rd September 2016 and delivered a lecture entitled "Genetic analysis and prenatal diagnosis of hemoglobinopathies".
3. Attended 5th Annual Conference of Indian Society of Transfusion Medicine (ISTM) TRANSMEDCON held at Bhopal from 18th to 20th November, 2016 and delivered a lecture entitled "Molecular genetics and Prenatal diagnosis of Hemoglobinopathies:.
4. Attended 'CME in Haematology' organized by Haematology department of TNMC and Nair hospital on 4th December, 2016 and delivered a lecture entitled "HPLC for Identification of Hb variants".

Dr Prabhakar Kedar, Scientist D

1. Presented a paper entitled "Red Cell Pyruvate Kinase Deficiency In India: An Update" in the 57th Annual Conference of ISHBT held at Jaipur on 10th to 13th November 2016.
2. Undergone a short term training at Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United kingdom under ICMR International fellowship for Senior Biomedical Scientist from 16th November to 1st December 2016.

Dr Prashant Warang, TA

Presented a poster entitled "Hereditary haemolytic anaemia caused by red cell Pyrimidine 5' Nucleotidase deficiency association with Gilbert's syndrome: A clinico-genetic study" at 57TH Annual Conference of Indian Society of Haematology and Blood Transfusion held at Jaipur from 10th to 13thNovember 2016.

Department of Transfusion Medicine

Dr Ajit Gorakshakar, Scientist F

1. Attended India Africa Health Summit organized by ICMR at Vigyan Bhavan, New Delhi from 1st to 3rd September and displayed a poster entitled "RDB kit for screening for common Indian beta thalassemia mutations and abnormal hemoglobins.
2. Participated in "The Abhyas Mahotsav" organized for Biotechnology students by K J Somaiyya College, Vidhyavihar as a Community Group activity on 16th September and delivered a talk entitled "Transfusion Medicine – Basic Facts".
3. Attended the pre conference workshop "Laboratory Testing in Pediatric Hematology" of the 20th Annual Conference of the Pediatric Hematology Oncology Chapter of Indian Academy of Pediatrics on 4th November 2016 at Wadia Hospital for Children, Mumbai and delivered a talk entitled "Laboratory Testing for Hemoglobinopathies".
4. Attended 5th Annual Conference of Indian Society of Transfusion Medicine held at Bhopal from 18th to 20th November 2016 and awarded best abstract for the paper entitled "Genotyping of the high frequency antigen Vel among Indian blood donors".
5. Delivered a talk entitled "Clinical Importance of extended phenotyping of blood donors" at Bharati Vidyapith Hospital, Pune on 24th November 2016.
6. Participated in "The Vigyan Yagnya" an exhibition organized by K J Somaiya College, Mumbai, in December 2016 where posters related to the activities and diagnostic tests carried out at NIIH were displayed for school and college students.

Dr Swati Kulkarni, Scientist C

1. Attended 41st Annual Conference of Indian Society of Blood Transfusion and Immunohaematology held at Pune from 26th to 28th August 2016 and delivered a lecture entitled "Rare Donor Registry".
2. Attended 5th Annual Conference of Indian Society of Transfusion Medicine held at Bhopal from 18th to 20th November, 2016 and delivered a lecture entitled "The Conundrum over D".

Harita Gogri, SRF: Awarded first prize for oral presentation at 5th Annual Conference of Indian Society of Transfusion Medicine held at Bhopal from 18th to 20th November, 2016 for the paper entitled "Molecular genotyping of Human Neutrophil Antigen-3 among Indian blood donors".

Roshan Shaikh, SRF: Awaeded first prize for poster presentation at 5th Annual conference of Indian Society of Transfusion Medicine held at Bhopal from 18th to 20th November, 2016 for the paper entitled "Distribution of α-thalassemia in tribal population from Chiplun, Maharashtra".

Department of Hemostasis

Dr Shrimati Shetty, Scientist E

1. Elected as member of National academy of Medical Sciences for the year 2016 in the 56th Annual Conference of National Academy of Medical Sciences (India) held at All India Institute of Medical Sciences, Raipur from 21st to 23rd October 2016.
2. Attended Expert Group Meeting on hemophilia and rare diseases held at ICMR, New Delhi on 2nd December.

The following Staff and Students attended and made oral/poster presentations in the 57th Annual Conference of the Indian Society of Haematology & Blood Transfusion (ISHBT) - Haematocon - 2016 held at Jaipur from 10th to 13th November 2016.

Sharda Shanbhag, TA: Case of somatic mosaicism in a severe HB family detected by allele specific PCR.

Nikesh Kawankar, TA: Molecular basis of 10 patients with factor XI deficiency from India: Identification of 4 novel mutations and a common mutation.

Rucha Patil, PDF: Diversity and complexity of immediate acting inhibitors in congenital haemophilia patients.

Tejasvita Gaikwad, PDF: A Predication Model For Estimating Warfarin Dose In Indian Patients.

Aniket Prabhu Desai, SRF: Impaired fibrinolysis as a cause of venous thrombosis in Indian patients: A comprehensive study.

Rutuja Deshpande, SRF: Genetic testing is mandatory to identify all congenital Antithrombin deficiency cases.

Sneha Yadav, JRF: Clinical and Laboratory evaluation of thrombotic microangiopathies.

Darshana Mirgal, PhD Student: In-vitro investigation to modulate the function of activated protein C (APC): Towards an alternative therapeutic option for haemophilia

Puja Soni, PhD Student: Global hemostasis assays for monitoring bypassing agent therapy in hemophilic patients with inhibitors.

Department of Cytogenetics

Dr V Babu Rao, Scientist E

1. Delivered a guest lecture entitled "Cytogenetics in Hematology", in Haematology CME-2016, held at TN Medical College and BYC Nair charitable Hospital, Mumbai on 3rd December 2016.

Mrs Anita Mukherjee, PS to Director and Mr Sunil Rasam, Section Officer visited RMRC Belgaum from 5th to 8th December 2016 to prepare EFC document for Chandrapur centre.

Vijay Padwal, ALIO attended two days advanced training workshop for ICMR library and information professionals held at NIV Pune from 24th to 25th November 2016.

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Dr.Soumya Swaminathan, Secretary, DHR and DG, ICMR addressing the SAC meeting through skype

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