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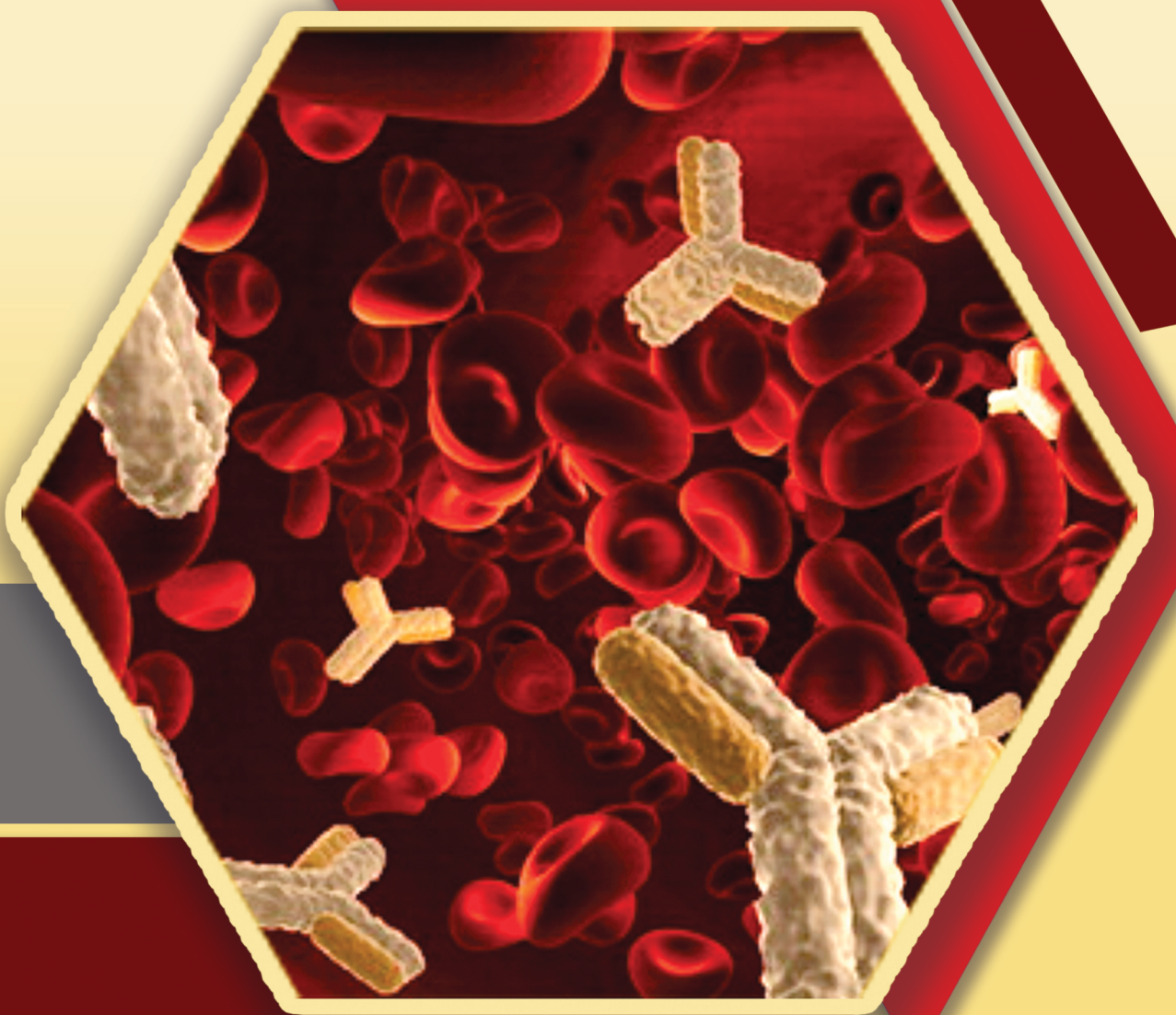

ICMR - NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY

Dr. H.M Bhatia oration was awarded to Dr. Kanjaksha Ghosh on 16th Feb 2018 on topic entitled “Dr. Bhatia's legacy how did we fare”. Dr. Sunil Parekh was the chief guest on this occasion.

On 19th Jan 2018, the team from World Federation of Hemophilia visited the Comprehensive Hemophilia Care Centre of KEM Hospital and NIIH, and discussed various issues of the “Humanitarian Aid” program and its impact on hemophilia care.

Under the Indo-Japan Collaborative project, Prof E Ito, Dr. T. Fujuwara & Prof. H Harigae of Tohaku University, Japan visited the Institute and delivered lectures

A workshop on Biomedical waste segregation was held at NIIH on 16th Feb, 2018, Dr. Sunil Kuyare, Assoc Prof, Dept of Microbiology, KEM Hospital delivered a lecture on “Biomedical waste segregation and its disposal”.

Swachh Bharat Abhiyan pakhwada of the Institute on 4th April, 2018.

NIIH staff undertaking Swachh Bharat Abhiyan pledge on 4th April, 2018

DARC: Structure, Function and Disease Pathophysiology

Roshan Shaikh

Introduction

Duffy antigen/chemokine receptor (DARC), also known as Fy glycoprotein (FY) or CD234 (Cluster of Differentiation 234), is a protein that is encoded by the DARC/Fy gene in humans. The Duffy antigen is located on the surface of red blood cells, and is named after the patient in which it was first discovered. The protein, encoded by the gene, is a glycosylated membrane protein and a non-specific receptor for several chemokines. It is also the receptor for the human malarial parasites *Plasmodium vivax*, *Plasmodium knowlesi* and simian malarial parasite *Plasmodium cynomolgi*.

The FY gene is located on Chromosome 1q22-23. This antigen has other names in the literature including Duffy antigen, Fy antigen, and glycoprotein D (GPD).

The duffy blood group system was first described by Cutbush M et al, 1950. The antigen Fy^a was first identified during an investigation of a haemolytic transfusion reaction in 43 years old multi-transfused patient named Mr. Duffy. A year later in 1951, Fy^b was discovered in a patient two days post delivery of her third child. The remaining Duffy antigens (Fy3, Fy4, Fy5 and Fy6) were discovered later (about 20 years), but of these only Fy3 is considered to be clinically significant.

Genetics and Biochemistry:

The DARC gene is located on chromosome 1. It consists of two exons distributed over 1.5 kb of genomic DNA. The gene contains two coding exons separated by an intron. Exon 1 encodes only the first seven amino acids of the Duffy glycoprotein. There are two gene products: the major is of 336 amino acids, called beta and the minor is of 338 amino acids called alpha.

The Fy /Fy polymorphism is a result of a single nucleotide change in the second exon. Fya encodes Gly42 while Fyb encodes Asp42. The nucleotide change responsible for Fy(a-b-) phenotype is a T>C mutation in the promoter region of the gene occurring at 67 nucleotides upstream of the Open Reading Frame (Figure 1). This mutation brings about a nucleotide change in the GATA-1 binding motif (TTATCT) of erythroid specific transcription factor, eTFII to TTACCT. This prevents the binding of eTFII to transcription start site, thus preventing expression of the gene in erythroid tissues (Tournamille et al, 1995). Individuals with such a mutation will lack the Fy antigen on the RBC, however the antigen will be expressed on the non-erythroid cells. This is owing to the fact that the transcription machinery in non-erythroid cells uses different transcription start site as compared to that of erythroid cells.

Roshan Shaikh, Senior Research Fellow,
Department of Transfusion Medicine, ICMR - National Institute of Immunohaematology,
13th Floor, New Multistoreyed Building, KEM Hospital Campus,
Parel, Mumbai 400 012. Email: roshan2orayan@gmail.com

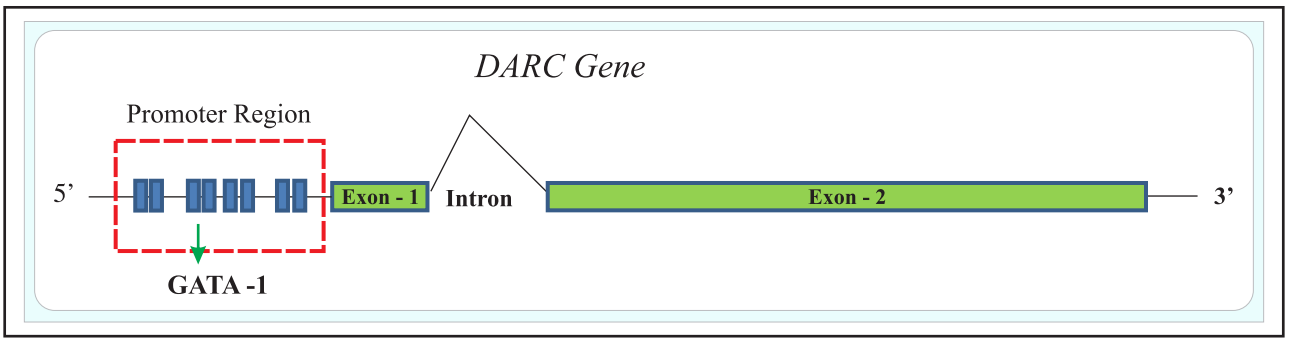

***Fig.1** : Schematic representation of the Duffy blood group gene Fy. The discontinuous box indicates the promoter region harbouring the GATA-1 site. The gene consists of 2 exons, interrupted by an intron*

The Duffy glycoprotein is also the receptor for a wide variety of chemokines like interleukin-8 (IL-8), melanoma growth stimulating activator (MGSA), CXCL1, CCL5 and CCL2 . The duffy antigen is composed of 336 amino acids with a molecular mass of around 36-46 kDa. The antigenic determinants reside in an acidic glycoprotein (gp-Fy), which spans the membrane seven times and has an extra-cellular N-terminal domain and an intra-cellular C-terminal domain (Figure 2).

Apart from RBC, presence of DARC is also observed on various other cell types. These include endothelial cells of post capillary venules, epithelial cells of renal collecting ducts, epithelial cells of pulmonary alveoli and Purkinje neurons of the cerebellum (Peiper et al, 1995). The

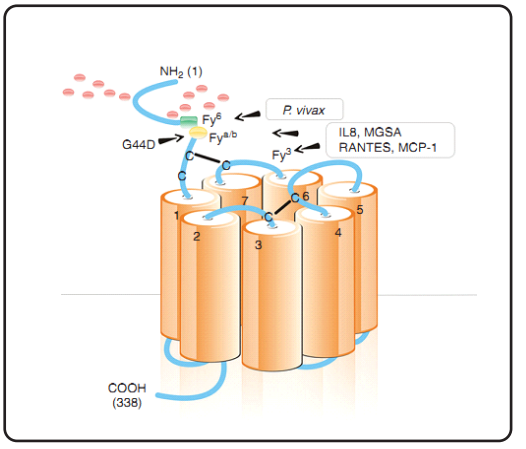

***Figure 2:** Glycoprotein Duffy: Functions. Adopted from Mallison G et al (1995)*

Duffy glycoprotein is also expressed in colon, spleen, thyroid, and thymus (Chaudhuri A et. al, 1993; Neote K et al, 1994).

There is a striking similarity between duffy antigen and G-protein coupled superfamily receptors. DARC is a member of the superfamily of chemokine receptors and the receptor for the human malarial parasite, *Plasmodium vivax*, and the simian malarial parasite, *Plasmodium knowlesi*. The parasite-specific binding site, the binding site for chemokines, and the major antigenic domains are located in overlapping regions at the exocellular N-terminal terminus (Figure 2).

Polymorphisms:

The Duffy system is defined by three alleles namely FY*A and FY*B which encode two antithetical antigens, Fya and Fyb. The Fy alleles give rise to four phenotypes: Fy(a+b-), Fy(a-b+), Fy(a+b+), and Fy(a-b-). The FY*0 allele (Duffy Null phenotype) results from the absence of Fy antigen on RBCs due to erythroid transcription silencing (i.e. absence of duffy antigen from RBC membrane). FY null is the major allele in Africans and occurs rarely in other populations. The frequency of the Duffy phenotypes varies in different populations (Table 1). The racial variation in the

Red Cell phenotype	Genotype	Frequencies (%)			
		Caucasians (Dean L, 2005)	Blacks (Dean L, 2005)	Chinese (Dean L, 2005)	Indian (Makroo et al 2013)
Fy(a+b-)	Fy^a/Fy^a or Fy^a/Fy	20	10	91	42.1
Fy(a-b+)	Fy^b/Fy^b or Fy^b/Fy	32	20	0.3	12.3
Fy(a+b+)	Fy^a/Fy^b	48	2	8.7	45
Fy(a-b-)	Fy/Fy	Very rare	68	0.0	0.3

Table 1: Comparison of phenotype frequencies of common antigens of Duffy blood group system in different ethnic groups

distribution of Duffy antigens is a result of a positive selection pressure as the absence of Duffy antigens on red blood cells (RBCs) makes the RBCs more resistant to invasion by a malarial parasite.

There is another Fy(a-b-) phenotype identified in Caucasians that results from silent alleles. These alleles are generated by four different molecular mechanisms associated with stop codon caused by either single point mutations or deletion in exon 2. The Fy(a-b-) phenotype due to FY*X identified in Caucasians is a results of concomitant SNPs in exon 2 of the FY gene at nucleotide positions (265C>T) and (298 G>A) which encode the amino acid changes Arg89Cys and Ala100Thr respectively. Although the 265C>T SNP is the critical mutation responsible for the Fy*X phenotype. The Ala100Thr was shown not to affect the expression of the cell-surface Fy protein level, whereas Arg89Cys affects the translation and/or stability of the protein that results in an unstable protein and a reduced level of protein on the RBC. Fy*X has one-tenth the level of Fy antigens. This explains why Fy*X may not be detected by the routinely used standard agglutination assays, so that it may appear as an Fy(b-) phenotype

Disease Pathophysiology:

Duffy antigen has long been only considered as the receptor for *P vivax* binding to RBC. Recent studies have identified additional roles in other health and disease conditions.

DARC T-46C polymorphism has been shown to be associated with higher allergic diathesis (Vergara C et al, 2008). There is also an elevated level of IgE in Fy(a-b-) individuals. African descendents who harbour the silencing mutation are more prone to severe asthma. The preclinical data suggest a strong link between Duffy antigen and coagulation. Based on the study by Mayr FB et al, 2009, Africans have an attenuated procoagulation response as compared to Caucasians.

DARC antigen plays a pivotal role in hematopoiesis. Reticulocytes present in the bone marrow show higher levels of DARC

expression on their membrane (Duchene J, 2017.) This helps in their direct contact with hematopoietic stem cells. Conversely, DARC silenced erythroid alters stem and progenitor cells, which give rise to distinct neutrophils. The neutrophil count is often low in individuals with DARC mutation (Reich D, 2009).

DARC on RBC influences the plasma levels of HIV1 suppressive and proinflammatory chemokines such as CCL5/RANTES. A direct connection between HIV and expression of Duffy antigen has been shown by Weijing He et. al, 2008. Duffy negativity increases the chances of HIV susceptibility. HIV-1 appears to attach erythrocytes via duffy antigen (He W, 2008).

Various studies have shown that there is connection between DARC and inflammatory responses. Voruganti VS, 2012 reported an association between DARC and the levels of monocyte chemoattractant protein-1 (MCP-1). The new variant with amino acid substitution of arginine for a cysteine at position 89 (Arg89Cys) reduces the ability of DARC to bind chemokines (Sidore C, 2015). The DARC on the endothelial cells within the synovium recruits the chemokines CXCL5, which in turns results in the migration of neutrophils in the disease state.

Duffy and Transfusion Medicine:

Duffy antigen is clinically significant because of its role in Haemolytic Transfusion Reaction (HTR) and Haemolytic Disease of Feotus and New Born (HDFN). The reaction is typically mild, but it might sometimes results in serious and fatal transfusion reactions occuring usually 24 hours post transfusion. These reactions are usually caused by anti-Fya or anti-Fyb antibodies.

The Duffy blood group system holds fourth rank, after the ABO, Rhesus and Kell systems with regards to HTR and HDFN. Plasmapheresis and intrauterine exchange transfusion have dramatically improved the prognosis of this disease.

The first case of HDFN due to anti-Fya antibodies in Italy was described by Agosti and Moroni (1981). The maternal alloimmunization was related to a previous blood transfusion. The infant developed a mild haemolytic disease. Similarly, the first case of intrauterine transfusion due to anti-Duffy antibody was reported by Cook SG et. al, 1989.

Anti-Fya has been incriminated in HDFN in a published report by Goodrick A et al., 1997. There is an increasing advocacy that pregnancy in which anti-Fya is detected with titre vaues >64 should be closely monitored. Moreover, if there is a constant rise in antibody titres and the father is homozygous, then foetal genotyping should be offered to help in management of that pregnancy (Goodrick A et al., 1997).

Several other studies have been published indicating severe transfusion complication arising from anti-Fya (Dufour P et al, 1991; Shah VP et. al, 1983; Hughes LH et. al, 2007; Westhoff CM et. al, 2004). Study by Dufour P et al, 1991 reported a case of severe post-transfusion anti-Fya allo-immunization which required a treatment of four intrauterine exchange transfusions. The child was born at 32 weeks of amenorrhoea and he benefited from an exchange transfusion at birth.

Anti-Fya has the potential to lead to significant foetal haemolysis resulting in HDFN. Management guidelines developed for RhD sensitization are appropriate for pregnancies complicated by anti-Fya alloimmunization (Shah VP et. al, 1983; Hughes LH et. al, 2007). The alloantibodies,

which frequently develop and are encountered during compatibility testing, are primarily against antigens related to the Duffy blood group system (Westhoff CM et. al, 2004).

Duffy Antigen and Malaria:

Malaria is *a disease* caused by protozoa of the genus *Plasmodium*. It is transmitted to humans mostly by the mosquitoes while taking a blood meal. Of the four species (P. *falciparum*, *P. vivax*, *P. ovale*, and *P. malariae)* that causes malaria in humans, *P. falciparum* and *P. vivax* are clinically the most important parasite. *P. falciparum* causes the most severe form of malaria and hence is responsible for the high mortality. However, *P. vivax* has the largest geographical coverage which include southern and south-east Asia, northern and central-eastern Africa, countries of the western Pacific and Latin America. Infection by *P. vivax* cause recurrent episodes of malaria which eventually lowers the living conditions of the community. Infection of RBC by the parasite is a multi-intensive process and involves interaction of the parasites apical organelle with that of the receptors on RBC.

Miller et al. 1975 suggested that Fya or Fyb antigenic determinants could be receptor for *P. knowlesi*. He proposed that resistance to the invasion of the merozoite in Blacks was due to the Fy(a-b-) phenotype. The strength of Fy(a-b-) against invasion by *P. knowlesi* merozoites was demonstrated in vitro. Later Barnwell et al. 1989 confirmed this with *P. vivax* suggesting that there was a natural selection of individuals with the *FY* gene who do not produce Fya and Fyb.

P. falciparum uses a series of receptors (EBA175, EBL-1, EBA140, EBA181, PfRh1 to name few) on the surface of human RBCs to invade them, while *P. vivax* and *P. knowlesi* depend on an interaction with the Fya or Fyb antigens of the Duffy Blood Group System. In Africa, where the Fy(a-b-) phenotype has achieved stability in different ethnic groups, the transmission of *P. vivax* is uncommon. In the Papua New Guinea population, carriers of the Fy(a-b+) or Fy(a+b-) express half the level of Duffy antigens on red blood cells compared to wild type homozygotes and exhibit reduced susceptibility to blood stage infection by *P. vivax*. These observations suggest that total or partial restriction to access Duffy antigen reduces the ability of the parasite to invade new red blood cells and thus this might inhibit parasitemia by *P. vivax*. *P. vivax* Duffy binding protein (PvDBP) is functionally important in the invasion process of parasites in human Duffy/DARC positive RBCs.

In regards to merozoite invasion of RBCs, Miller et al. observed by electron microscopy that the apical portion of the merozoite makes the first contact with the RBC creating a small depression in the membrane. This area begins to thicken and binds with the membrane of the merozoite which then enters the red blood cell by invagination. After the Plasmodium enters, this access closes. This binding is crucial for parasite invasion. The presence of one member of a large family of proteins bound to RBCs, referred to as Duffy Binding Protein (DBP), is required to mediate with the DARC as this antigen was identified as a chemokine receptor. This critical binding occurs in the cysteine-rich domain between the amino acids at positions 291 and 460 of region II. According to several observations, region II of the DBP (DBPII) can be a critical target for protective immunity of the host. Firstly, certain DBPII are highly polymorphic and appear to be maintained by immune selection. Secondly, antibodies against DBPII found in populations of endemic areas for *P. vivax* inhibit binding of COS-7 cells expressing the DBPII ligand to DARC-positive

RBCs. Thirdly, the high antibody levels against region II of *P. knowlesi*, molecule that is 70% homologous to *P. vivax* DBP, also mediates infections of human DARC-dependent RBCs and may inhibit invasion of human RBCs by *P. knowlesi*. *P. vivax* DBP is a 140-kDa protein that belongs to a family homologous to Duffy (Erythrocyte Binding Protein) and is located inside organelles of merozoites of Plasmodium. The binding functional domains of DBL-EBP lie in region II, which for *P. vivax* is DBP 330-amino acid and the critical residues of map linking a central region of 170- amino acid including cysteines 5-8. The gene encoding the *P. vivax* DBPII is highly polymorphic and this diversity varies geographically from region to region. This polymorphism is consistent with a high pressure on DBP selection and suggests that an allelic variation functions as an immune evasion mechanism.

DBP is likely to be exposed during the invasion, therefore, it can become accessible to antibodies. Currently, data on humoral immune response to DBP in the human population is limited, and studies have been mainly restricted to areas where malaria is highly endemic. Research on cytokines and their receptors converged in the investigation of the antigens of the Duffy Blood Group System, showing an important physiological role for the alleles of this glycoprotein. It was also observed that if a mutation occurred in the DARC promoter region of the gene in erythroid precursor cells, this would prevent its expression on RBCs and complete resistance to infection by *P. vivax* would occur.

RBCs from individuals homozygous for the wild type promoter express twice the amount of duffy antigen than those heterozygous for the GATA-1 mutation. Heterozygosity for the -33T mutation in the allele promotes partial protection against *P vivax* infection.

Indian Scenario:

There have been few reports on the distribution of duffy phenotype across Indian blood donors. These studies exclusively include phenotype data of the regular blood donors using commercially available antisera (Table 2). Ishwar C V, 1993 published first report of duffy antigen in malaria endemic area of Madhya Pradesh (Muria Gonds) and Delhi. The frequency of Fya antigen was highest, followed by Fyb and Fya/Fyb. Fy negative (null phenotype) was found in 11.3% of Muria Gonds. A single study involving genotyping of duffy antigen (Anita Chittoria, 2012) in 250 samples from six distinct zones of India (North, Central, West, South, East and North East) did not identify Fy null phenotype.

Few studies showing the frequency of Duffy antigens in different parts of India is shown in Table 2. Studies from India have also identified Fya as the most common antigen. Fy(a-b-) phenotype was very rare or absent in these studies. In a larger study (n=3076) from Delhi (Makroo et al 2013) the frequencies of all Duffy phenotypes in the Indian blood donors differed significantly from Blacks and Chinese. The frequency of Fyb antigen was higher in Caucasians. Fy(a+b-) was the most common phenotype identified (42.1%) in Indians, while Fy (a+b+) in Caucasians (49%), and Fy(a-b-) in Blacks (68%). The frequency of Fy(a-b-) phenotype was mere 0.3%. This phenotype is quite uncommon in the Caucasian and Chinese populations whereas 68% of Blacks have this 'null' phenotype (Tabel 1). The only Indian study by Kahar et al 2014 from Gujarat showed very high frequency of duffy null phenotype (48.69%). This is in contrast to observations made by studies conducted in other part of India.

Phenotype	Thakral et al, 2010 (Chandigarh) n=1240	Agrawal et al 2013 (New Delhi) n=508	Kahar et al 2014 (Gujarat) n=115	NIIH (Mumbai) (n=500)
Frequencies (%)				
Fy(a+b-)	43.85	36.22	37.39	49.4
Fy(a-b+)	13.25	15.36	4.35	15.2
Fy(a+b+)	42.90	48.03	9.57	35.4
Fy(a-b-)	0	0.39	48.69	0

Table 2: Frequency distribution of Duffy phenotypes in different Indian populations

NIIH Experience:

In a collaborative study (National Institute of Immunohaematology, Malaria Research Centre, Nicobar; G.B. Pant Hospital, Port Blair, Andaman & Nicobar Islands), blood samples (n=116) from Jarawa tribe were collected to find the frequency of duffy antigen from malaria endemic region from Andaman. The study result showed 17.24% were Fy (a+b-) and 82.76% Fy(a-b-). None of the samples were positive for Fyb antigen. Blood smears from peripheral blood sample were tested and 27.59% were positive for *P. falciparum* while none showed the presence of malarial parasite *P. vivax*.

In a study from NIIH, 500 blood donors from Mumbai were tested for presence of common duffy antigens. The phenotypic frequencies were comparable with that of Thakral et al. and Agrawal et al (Table 2). In another study, 3217 samples were collected from healthy individuals belonging to different geographical area (Maharashtra, Hyderabad, Surat, North Eastern region), and tribal populations (Oraons, Bhil, Mundas etc) to determine the frequency of duffy antigen by molecular typing. PCR-RFLP technique was used to study four polymorphism namely GATA-1 T>C promoter mutation, G42D, A100T and R89C. Fya antigen had the highest frequency (61.4%), followed by Fy(a+b+) and Fyb.

The Fy(a-b-) negativity was found in only two cases harbouring the homozygous mutation. A sample with heterozygous mutation was also encountered during the study. The pathogenic mutation R89C was observed to be less than 1% in the population. R89C results in reduced levels of protein on RBC resulting in lower antigen expression ability to bind chemokines. This phenotype is often designated as Fy(bwk). The A100T polymorphism resulting in weaker expression of Fyb on RBC was identified in about 4% of individuals tested.

Conclusion:

In conclusion, the results of population studies indicate the role of natural selection in the variable distribution of two different alleles of the Duffy gene (FY*A and FY*B) worldwide. The frequency of the Fy (a-b-)

phenotypes is 68% in American blacks and 88–100% in African blacks. The Fy (a–b–) phenotype is, however, extremely rare in other racial groups. Fy(a+b-) and Fy(a+b+) are the most common phenotypes reported in virtually all Indian studies which is in concordance with that of the white population. However, the frequency of Fy(a-b+) is higher in whites than in the Indians and has been reported to be very low in South East Asian populations.

Malaria is the only infectious disease that has been demonstrated to influence population genetics. It has been established for a long time that a high percentage of African and American blacks are completely resistant to *P. vivax* infection. Subsequently it was shown that the resistance is due to the absence of Duffy blood group antigens (which is also the receptor on the erythrocute surface for *P. vivax* invasion). A high prevalence of Duffy-negative phenotypes has also been reported among Muria Gond tribe from Bastar District in Central India and Jarawas tribes from Andaman islands.

As India is a country with huge population, large data from different Indians states would be valuable to provide information regarding the frequencies of duffy antigens in Indian subcontinent.

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सारांश

डफी ॲन्टिजेन रिसेप्टर (DARC): रचना और कार्य

मनिषा पटवर्धन

डफी प्रतिजन (Antigen) लाल कोशिका के उपरी सतह पर स्थित होता है। इसे अन्य नामों से भी जाना जाता है, जैसे CD234 या Fy ग्लयकोप्रोटीन। मनुष्य के शरीर मे DARC जीन, इस प्रोटीन को बनाने के लिए जिन्मेदार होती है। प्लाज्मोडिअम वायवॅक्स (*Plasmodium vivax*, मलेरिया उत्पन्न करनेवाला एककोशी जीव) और प्लाजमोडियम नोलेस्की (*P. knowleski*) इस प्रतिजन का उपयोग रक्त कोशिका के सतह से जुडने के लिए करते है।

डफी प्रतिजन उत्पन्न करनेवाली जीन, गुणसूत्र 1 q 22-23 पर स्थित है। इससे 'डफी' रक्तवर्ग प्रणाली (Blood Group) बनाती हैं। कुबुश और साथिओं ने डफी रक्तवर्ग प्रणाली स्थापित की। फ्यावास नामक वैज्ञानिक ने 43 साल के 'डफी' नामक रुग्ण में इस प्रतिजन की खोज की। (डफी को कई बार खून चढाया जा चुका था।) डफी प्रतिजन के कई रुप अध्ययनों में देखे गये। (जैसे Fy3, Fy4, Fy5 और Fy6)। इनमें से Fy3 को अहम माना जाता हैं। Fy3 चिकित्सिय रुप से महत्वपूर्ण (Clinically Significant) भूमिका अदा करता है। DARC जीन गुणसुत्र 1 पर स्थित है। जीन में 2 कोडिंग एक्झान (Coding exon) होते हैं। एक्झान 1 पहले 7 अमिनो ॲसिड के लिए कोड (Code) करता हैं, जिससे 'डफी' ग्लायकोप्रोटीन बनने की प्रक्रिया आरंभ होती हैं। एक्झान 2 में बदलाव स्वरुप Fya / Fyb पॉलिमार्फिज्म (Fya / Fyb Polymorphism) होता हैं। Fya, Gly 42 अमिनो ॲसिड और Fyb, Asp 42 अमिनो ॲसिड के लिए जिम्मेदार हैं। इसमें बदलाव Fy (a- b-, T >C) के कारण व्यक्ति की लाल कोशिका पर Fy प्रतिजन नहीं बन पाता।

डफी प्रतिजन 336 अमिनो ॲसिड से बनता हैं। इसका आण्विक द्रव्यमान (Molecular mass) 36-46 kDa हैं। लाल कोशिका के अलावा शरीर में बडी आँत, थायराईड, ग्रंथी थायमस, तिल्ली (Spleen) पर भी डफी प्रतिजन पाया जाता हैं।

डफी प्रणाली में पॉलिमॉर्फिजम

डफी प्रणाली में तीन अलील (allele) Fy* A, Fy* B और Fy* O होते है। Fy* A और Fy* B के रहते Fy* a और Fy* b प्रतिजन बनते हैं। Fy* O की मौजुदगी में लाल कोशिका पर डफी प्रतिजन नदारद (absent) होता है, जिसे डफी नल फिनोटाईप (Duffy null phenotype) कहते हैं। आफ्रिका के बाशिंदों में ज्यादा मात्रा में डफी नल फिनोटाईप पाया जाता हैं। दुनिया के विविध प्रजातिओं में इसकी मात्रा अलग अलग हैं। डफी प्रतिजन मलेरिया पॅरासाईट के शरीर में दाखिल होने के लिए बेहद जरूरी हैं। इस प्रतिजन के ना होने पर मलेरिया प्रादुर्भाव की संभावना कम हो जाती हैं।

DARC T- 46C पॉलिमॉर्फिजम का संबंध ॲलर्जी पैदा करनेवाली शरीर प्रवृत्ती (allergic diathesis) आय. जी. ई. (IgE) एन्टीबाडी में वृद्धि, अस्थमा (आफ्रिका के प्रजातिओं में) खून की अनियमित जमावट से प्रस्थापित हो चुका हैं।

रक्त उत्पन्न करनेवाली कोशिका, रेटिक्युलोसाइट (reticulocytes) पर भारी मात्रा में DARC प्रतिजन मौजूद होता हैं। DARC के बदलाव से न्युट्रोफिल (neutrophil) कोशिका की संख्या कम हो सकती हैं। DARC प्रतिजन और एच. आय. व्ही (HIV) का संबंध वैज्ञानिक विजींग हे और साथिओंने प्रस्थापित किया। डफी प्रतिजन के गैरमौजुदगी से एच. आय. व्ही. (HIV) बाधा का खतरा बढता हैं। डफी प्रतिजन, रक्त आधान (blood transfusion) से जुडा हुआ हैं। हालांकी, इस के कारण उत्पन्न होनेवाली प्रतिक्रिया काफी सौम्य रहती हैं।

डफी प्रतिजन और मलेरिया

मलेरिया की प्रजाति, प्लाज्मोडियम वायवॅक्स (*P. vivax*) डफी प्रतिजन की मदद से लाल कोशिका में दाखिल होती हैं। प्लाज्मोडियम एककोशी जीवगण (protozoa) हैं। मानवीय शरीर से रक्त चुसने के समय मच्छर प्लाज्मोडियम को शरीर में छोडते हैं। प्लाज्मोडियम फाल्सिपॅरस (*P. falciparum*) गंभीर रुप की बिमारी हैं। मलेरिया से होनेवाली ज्यादातर मौतों के लिए प्ला. फाल्सिपॅरम जिम्मेदार होती हैं।

आशिया और आफ्रिका उपखंड में प्लाज्मोडियम वायवक्स (*P. vivax*) से ज्यादा मात्रा में मलेरिया फैलता हैं। इस प्रजाति से एक से ज्यादा बार संक्रमण का खतरा भी बना रहता हैं।

मलेरिया फैलानेवाले प्लाज्मोडियम लाल कोशिका पर मौजूद प्रापकोंद्वारा (receptor) संक्रमित हो जाते है। डफी प्रतिजन प्लाज्मोडियम वायवॅक्स (*P. vivax*) को चिपककर उन्हें कोशिकों में दाखिल होने के लिए सहाय्य करती है। मिलर और उनके सहायक वैज्ञानिकों ने डफी प्रतिजन और मलेरिया पर अनुसंधान किया। उन्होंने प्रमाणित किया की डफी प्रतिजन की अनुपस्थिती में मलेरिया से संक्रमण की संभावनाएँ घटती हैं। बार्नवेल और साथियों ने साबित किया, की मलेरिया के जीवाणू को लाल कोशिका में प्रवेश पाने के लिए डफी प्रतिजन की जरुरत होती हैं। इन में *P. vivax* के कारण मलेरिया संक्रमण का अनुपात भी कम है।

भारत में डफी प्रतिजन पर अनुसंधान

ईश्वर और सहयोगीओंने (1993) में डफी प्रतिजन के, भारतीय उपखंडो में अनुपात का विवरण (report) किया। मध्यप्रदेश के मुरीय गोंड प्रजाती, (आदिवासी) और दिल्ली के बाशिंदों में डफी प्रतिजन (Fy antigen) का अध्ययन किया गया। मुरिया गोंड (आदिवासी) प्रजाती में 11.3% लोंगो में डफी प्रतिजन नही पाया गया। शहरी आबादी की तुलना में आदिवासी मूल के लोगों में डफी प्रतिजन **कम** संख्या में मौजुद हैं।

एन. आय. आय. एच. में डफी प्रतिजन का अध्ययन

3217 रक्त नमुनों को डफी प्रतिजन जीनोटाईपिंग (genotyping) के लिए जाँचा गया। PCR-RFLP तकनीक का उपयोग इस अध्ययन में किया गया। भारत के बिभिन्न भागों के (महाराष्ट्र, हैदराबाद, सुरत, इशान्य भारत) नमुने जाँचे गये। अध्ययन में चार डफी प्रतिजन पॉलिमॉर्फिज्म (Polymorphism) को शामिल किया गया। (rs2814778 T>C promotor mutation, rs 12075 G42D, rs13962 A100T और rs34599082 R89C) निरिक्षण में Fya प्रतिजन का अनुपात अधिकतम, Fya / Fyb का मध्यतम और Fyb का सबसे कम पाया गया। Fy प्रतिजन सिर्फ 2 नमुनों में अनुपस्थित पाया गया। विकृतीजन्य उत्परिवर्तन (Pathogenic mutation) R89C मात्र 1% लोगों में पाया गया।

Award Winning Abstracts

1. Dr. J.C. Patel Best Oral Paper Award at 41st Annual conference of Mumbai Haematology Group held at Mumbai from 16th to 18th March 2018

siRNA mediated silencing of protein C gene (PROC) : a novel therapeutic approach for haemophilia.

Darshana Mirgal, Vaishali Pujari, Kanjaksha Ghosh, Shrimati Shetty. ICMR-National Institute of Immunohaematology

Introduction: Haemophilia A and B are congenital bleeding disorders caused by insufficient thrombin generation due to deficiency in factor VIII and IX, respectively. In the presence of normal levels of endogenous anticoagulants, like protein C, protein S and AT III deficiency of factor VIII or IX results in an imbalance of the haemostatic system towards a bleeding phenotype. Deficiencies of any of the anticoagulant proteins or hypofibrinolytic factors in the anticoagulant pathways can result in increased thrombin generation which in turn results in the amelioration of clinical symptoms in severe haemophilia patients. The present report is a step forward in this line showing partial silencing of PROC improves overall haemostasis and might serve as an important therapeutic approach for haemophilia.

Aim: To improve haemostatic balance in haemophilia by reducing the levels of activated protein C using RNAi mediated partial silencing of protein C (PROC).

Materials and Methods: Human HepG2 hepatic carcinoma cells were cultured. In presence of transfecting reagent i.e lipofectamine, cultured cells were reverse transfected using various sequenced specific siRNAs. Various concentrations and combinations of different sequenced specific siRNAs as well as transfected reagent were tried, to check the efficiency of the particular siRNA to down regulate the PROC expression. After successful transfections of particular siRNA, RNA was isolated from the transfected cells and was converted to cDNA using reverse transcriptase followed by PCR amplification using real time PCR. PROC down regulation was determined using qRT-PCR by calculating fold change. Cells were also check for its viability using trypan blue staining. After optimizing the concentration (1.5pmol-3pmol) of siRNA in vitro, we selected combination of two siRNAs for in vivo studies.

For in vivo study groups of normal as well as hemophilic mouse aged, 10 - 14 weeks were subcutaneously injected with optimized concentration of siRNA (10ml/kg - calculated according to weight of the mouse) with various concentrations of different transfection agents like lipofectamine and GalNAc conjugated siRNA. GalNAc labeled siRNA which is more specific towards hepatocytes were used to increase the bioavailability and specificity of siRNA in the system.

Blood sample from retro-orbital vein was collected before siRNA injection and 1,3,5,7,11,17,25,28,30,38 days after injection. Plasma Protein C, Protein S levels were checked by mouse specific ELISA into injected mice at various time intervals. The liver tissue expression of protein C checked by qRT-PCR using taqman assay and the percent silencing were calculated using comparative $\Delta\Delta CT$ of liver tissue expression (PROC) between siRNA treated & untreated samples against endogenous control(18S).

Liver function test were performed using ELISA. Thrombin generation assay were performed for the comparison of thrombin generation in siRNA treated and untreated samples

Results: In in-vitro study, three different siRNA individually gives silencing effect at very high concentrations (i.e.at >6pmol) which also causes cell death. Combinations of two different siRNAs effectively silence the protein C gene at lower concentrations. (i.e. 1pmol-2pmol) 2 siRNAs at concentration of 1.5 - 3pmol was found to be the best combination with optimum silencing effect at 34% to 58%.

In-vivo, at minimal concentration (1pmol-2pmol) of lipofectamine there were no silencing effect seen, but the increase concentration (i.e. 3pmol) of transfection reagent showed, unstable silencing of PROC. At 6pmol siRNA concentration with 3pmol lipofectamine, the results obtained were not stable. Results showed decrease in protein C levels at 24 hrs but again rise in levels at 72 hrs at single dose and after repeated dose random stable decrease in levels of protein C were seen, and again rise in protein C levels after 4-5 days. GalNAc conjugated siRNA effectively showed stable decrease in Protein C levels for longer duration in normal as well as in hemophilic mice. The specific siRNA against exon 9 of protein C gene showed 62% of gene silencing at day 26. The other siRNA against exon 7 of PROC showed 62% of gene silencing at day 17 and complete silencing by 30th day of injection. And combination of these two GalNAc labeled siRNA showed 74% silencing of PROC by 25th day of injection. Increased Thrombin Generation (TGA) is observed with the decrease levels of protein C in hemophilic mice. Decrease in protein C levels and Normal levels of other vitamin 'K' dependent factors i.e. protein S / factor IX /factor VII levels showed no altered body function and specificity of siRNA towards protein C. Complete hemogram mainly leukocyte count, IL6, TNF – α were within normal range suggest no inflammation

Discussion: Unstable silencing of PROC may be due to the lower specific transfection with lipofectamine of siRNA and early degradation of siRNA. As per the study by Alnylam Pharmaceuticals(1), ALN-AT3 GalNAc conjugated siRNA which is more specific towards hepatocytes as GalNAc is the ligand for the asialglycoprotein receptor (ASGPR) that is expressed on hepatocytes. Similarly this conjugation of GalNAc with specific targeted PROC might increasing the bioavailability and specificity of siRNA obtaining effective PROC silencing. Thus reduction of protein C levels increase the Thrombin generation and improve overall haemostasis in haemopilic mice

Conclusion: These invitro as well as invivo findings shows the feasibility of silencing PROC by RNA interference based approach as an alternate therapeutic approach for hemophilia.

2. Dr. J.C. Patel Best Oral Paper Award at 41st Annual conference of Mumbai Haematology Group held at Mumbai from 16th to 18th March 2018

First time development of a point of care (POC) technique for diagnosis of Hemophilia A and von Willebrand disease

Priyanka Kasatkar, Shrimati Shetty. Department of Thrombosis and Haemostasis, ICMR-National Institute of Immunohematology

Introduction: von Willebrand factor (VWF) and procoagulant FVIII play major role in haemostasis in response to vessel injury. There are few centers in India which have

the required laboratory facilities for the diagnosis of Hemophilia, von Willebrand disease (VWD) and several other coagulation factors. A rapid and accurate diagnosis is critical in these patients, as early therapy can be lifesaving. The existing methods for the detection of Hemophilia A, VWF:Ag have several limitations i.e. time consuming, needs expert personnel and are expensive. So far, no commercial rapid test kit is available for diagnosis of any of the common bleeding disorders. Lateral flow immunoassay (LFIAs) based Point of care (POC) testing is an emerging option that will assist in quick evaluation for laboratory-assisted diagnostics [Sajid M et al, 2014].

Aim: To develop a rapid, specific, user friendly and cost effective lateral flow immunoassay based technique for the detection of Hemophilia A and VWF:Ag from human plasma samples.

Materials and Methods: Blood sample was collected from 43 Haemophilia A and 22 VWD patients referred from various Municipal and Private hospitals. The existing standard protocols for the detection of FVIII:Ag, FVIII:C and VWF:Ag were assayed accordingly for all the samples. The most reliable, widely used gold nanoparticles (GNPs) were synthesized in our laboratory using different citrate reduction methods [Isaac O et al, 2011]. GNPs were characterized by Visual Inspection, UV-Vis Spectra Analysis, Dynamic Light Scattering Analysis and Transmission Electron Microscopic Measurement. GNP-Ab Conjugation was achieved by passive adsorption, covalent bonding techniques. Different combination of lateral flow membranes were assembled manually in our laboratory according to the GNPs size and mobility on the membrane.

Results: Smaller (15 ± 2nm) and stable GNPs were synthesized using the citrate reduction method (Turkevich method). All possible permutation and combination of different types of membranes were assembled with different combinations of antibodies (primary as well as secondary from different sources) were used for both VWF and FVIII. Sharp bands were seen in test for the VWF polyclonal antibody, whereas faint bands with the monoclonal antibody to VWF. LFIA for FVIII:Ag showed prominent band with polyclonal antibody and good with monoclonal antibody on the test zone. The secondary anti-IgG used on the control zone showed good reaction for both the VWF:Ag and FVIII:Ag. The LFIA developed detects upto 3% VWF and 1% FVIII with respective polyclonal antibodies. Multiplexing of FVIII and VWF on single strip was not feasible with GNPs.

Discussion: The citrate reduction process was a simple and reproducible way to produce monodisperse, quasi-spherical, smaller, stable GNPs. Electron microscopy with high resolution was required for the estimation of shape and exact size of the smaller GNP synthesis. Different LFIA membranes showed differential migration of the sample on the strips. Immediate visualization of signals or bands were generated within 10 minutes after the application of the sample. Sensitivity of the strips was tested on the standard plasma dilutions with known concentration of antigen. LFIA designed for the detection of the VWF:Ag is sensitive, it can detect upto 3% VWF and is 99% specific. For FVIII strip, it needs further validation.

Conclusion: A simple POC semi-quantitative test for diagnosis of Hemophilia A and severe von Willebrand disease established. The working cost per test is less than Rs.50. A quick diagnosis of

Hemophilia A and type 3 VWD, which can be done even without any technical expertise.

3. Second prize for an oral presentation at 4th National conference on Primary Immunodeficiency Diseases organized by Indian Society of Primary Immune deficiency (ISPID) held at Jaipur during 10th-11th March 2018.

Clinical, Immunological, and Molecular Findings in four cases of B cell Expansion with NF-κB and T cell Anergy (BENTA) Disease for the first time from India.

Maya Gupta1, Jahnavi Aluri[1], Mukesh Desai [2], Prasad Taur [2], Michael Lenardo[3], Jenna Bergerson[3], Aparna Dalvi[1], Snehal Mhatre[1], Manasi kulkarni[1], Priyanka Kambli[1], Manisha Madkaikar[1].

1 Department of Pediatric Immunology and Leukocyte Biology, ICMR-National Institute of Immunohaematology, 2 Division of Immunology, Bai Jerbai Wadia Children's Hospital, 3 Molecular Development of the Immune System Section and Clinical Genomics Program, Laboratory of Immune System Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda.

Background: B cell Expansion with NF-κB and T cell Anergy (BENTA) is a rare primary immunodeficiency disorder caused by mutations in the CARD11 gene and results in constitutive NF-κB activation in B and T cells. Affected patients present with polyclonal expansion of B cells at an early age with splenomegaly, lymphadenopathy and mild autoimmunity.

Objectives: Clinical, immunological and molecular characterization of BENTA patients.

Methods: All clinically suspected cases of BENTA disease were immunologically evaluated for polyclonal expansion of B cell with elevated immature transitional (CD10+CD24hiCD38hi) and mature naïve (IgD+) followed by molecularly confirmation of the defect.

Results: We have identified four BENTA cases with previously reported gain of function of mutations (G123S, G123D, and C49Y) in the CARD11 gene. Severe autoimmune manifestations were noted for the first time in all our patients associated, in some cases, with recurrent sinopulmonary infections.

Conclusion: Significant B cell lymphocytosis and polyclonal expansion of both immature transitional (CD10+ CD24hi CD38hi) and mature naïve (IgD+) polyclonal B cells, with normal T cell numbers are important clues for the diagnosis of BENTA disease which can be confirmed by genetic analysis. There exists a clinical overlap between BENTA and Autoimmune Lymphoproliferative syndrome (ALPS) patients hence, patients with persistent lymphocytosis and splenomegaly should be evaluated for both the diseases.

4. First prize for an e-poster presentation at 4th National conference on Primary Immunodeficiency Diseases organized by Indian Society of Primary Immune deficiency (ISPID) held at Jaipur during 10th-11th March and 2018.

Clinical and molecular spectrum leukocyte adhesion deficiency-I: A large patient cohort study

Priyanka Kambli1, Aparna Dalvi[1], Maya Gupta[1], Snehal Shabrish[1], Manasi Kulkarni[1], Jahnavi Aluri[1], Gouri Hule[1], Umair Bargir[1], Ankita Patel[1], Raksha Shinde[1],Shraddha Shelar[1], Prasad Taur[2], Mukesh Desai[2], Manisha Madkaikar[1]. [1]ICMR-National Institute of Immunohaematology, Mumbai, [2]Bai Jerbai Wadia Hospital for children, Mumbai.

Background:- Leukocyte adhesion deficiency-I (LAD-I) is an autosomal recessive disorder clinically characterized by delayed separation of umbilical cord, leukocytosis, neutrophilia, omphalitis, severe recurrent, life threatening bacterial infections. It is characterized by defect in leukocyte adhesion and migration, resulting from mutations in the ITGß2 gene, encoding the beta2 integrin family, which leads to markedly reduced expression of CD18 & CD11 on leukocytes.This study was undertaken with the aim to identify the molecular defects underlying LAD-I in Indian population and to develop a strategy for mutation detection in these patients which may be further utilized for prenatal diagnosis.

Material & Methods:-On the basis of clinical manifestations64patients were screened for absent or reduced expression of CD18/CD11 on peripheral blood leukocytes by flow cytometry. Genomic DNA of the phenotypically diagnosed individuals with LAD-I defect was used for molecular confirmations.

Results:-The study compiles 10 years data for the clinical, immunological and molecular study of total 64 affected individuals comprising of60% males and 40% females. It was observed 79% individuals presented severe LAD-I0 phenotype(CD18 expression ≤ 3% on neutrophil);5% with LAD-I-phenotype (CD18 expression 2-20% on neutrophil) and 16% with LAD-I+ phenotype (CD18 expression ≥ 20%on neutrophil andreduced mean fluorescence intensity). Clinically LAD-I0 presented delayed umbilical cord separation, omphalitisand several recurrent infections due to organisms included Pseudomonas, Staphylococcus and Klebsiella species. Severely affected patients die at young age. Moderate phenotypepresentedlate onset of disease (Median age–7years) with recurrent skin ulcers mimicking pyodermagangreosum. Molecular characterization revealed distinct types of mutations in ITGß2 gene including frame shift, Missense, Nonsense, Splice site and Indel mutations. Till date 35 variants were detected in 64 patients,of which22 are novel and 13 have previously been reported. Prenatal diagnosis was offered to 14 affected families, of which the fetus was found to be affected in 5 families.

Conclusion:-In this study we present the mutation spectrum for 64 affected cases. It was observed that ITGß2 mutations are spread over the functional domain. However, approximately 40% of our cases had mutations in exons 6 and 7 which may be used for primary screening followed by analysis of other exons. The molecular characterization of ITGß2 gene in the index cases also helped in providing genetic counseling and pre-natal diagnosis in the affected families.

5. 2nd Best Poster Award at 41st Annual conference of Mumbai Haematology Group held at Mumbai from 16th to 18th March 2018

Coagulation disorder - an insight on two rare bleeding cases

Rutuja Deshpande, Nikesh Kawankar, Sharda Shanbag, Anshul Jadli, Shrimati Shetty. ICMR-National Institute of Immunohaematology

Introduction:

This study involves two interesting cases a) combined congenital Glanzmann's thrombasthenia (GT) – platelet aggregation defect and Factor VII (FVII) deficiency. Prevalence of isolated GT and FVII deficiency is very rare approximately 1: 10,00,000 and 1:500,000 respectively.

Therefore its combined occurrence in a single patient makes it interesting.

b) Novel mutation in GGCX gene contributing to congenital Vitamin K deficiency. Vitamin K dependent clotting factor deficiency (VKCFD) is rare autosomal recessive disorder. Only 22 missense mutations are reported in Human Gene Mutation database (HGMD) in GGCX gene.

Case Summary:

a) A 13 year old male was referred with the complaints of recurrent gum bleed and history of echymotic patches. On screening along with PT, platelet function with ADP, Ristocetin and collagen was also deranged. Glanzmann's thrombasthenia was confirmed by flow cytometry. Factor assay was carried out to explain deranged PT and Factor VII was found to be moderately low i.e. 22%.

b) A patient with a known diagnosis of reduced factor VIII was referred to reconfirm the diagnosis. Patient's one sibling died of intra cranial bleed on 8th day of life. On screening both PT and APTT was found to be deranged. On further screening his factor VII, IX and X were found to be reduced while factor VIII level was normal. Screening of the entire GGCX and VKORC1 gene (coding region, promoter and the splice site areas) showed the presence of a novel homozygous mutation p.Pro61Leu in exon 2 of GGCX gene. Thus, patient was diagnosed as congenital case of VKCFD.

Discussion: In the first case, the patient presented with two rare genetic abnormalities i.e. GT and factor VII deficiency. However, interesting aspect in this case is attributed to FVII deficiency due to cumulative impact of polymorphisms i.e. -122T>C (rs561241) in the promoter region, rs6039 in Exon 1a and R353Q (rs6046) in Exon 8 resulting in mild FVII deficiency. In the second case a novel mutation p.Pro61Leu in exon 2 was found in GGCX gene. Mutation was found to be deleterious by prediction softwares (Mutation Taster, SIFT and Provean). Pre-natal diagnosis was done for next child of the family and was found to be unaffected for this mutation.

Conclusion: These two cases were interesting as they were not only rare but also could have been easily misdiagnosed as single isolated factor deficiency or single platelet function defect. In most of the cases after identifying single defect no further assays are performed that might lead to incomplete diagnosis. Accurate phenotypic diagnosis is important not only for therapy, but for an accurate genetic diagnosis as well.

6. Dr. H.M Bhatia and Dr. L.D. Sanghvi best oral paper presentation award (first prize) at 41st Annual conference of Mumbai Hematology Group held from 16-18 March 2018 at Mumbai, ITC , Parel.

New insight into the pathophysiology of Sickle cell disease associated with Ubiquitin proteasome system.

Prashant Warang, Riddhi Pandya, Anuja Sawant, Nikhil Shinde, Debjeet Pandey, Manisha Madkaikar, Malay Mukherjee. Department of Haematogenetics, ICMR-National Institute of Immunohaematology,

Introduction: Oxidative stress and hemolysis induced depletion of nitric oxide (NO) altering the redox equilibrium is one of the key factors that modulate the phenotypic expression of sickle cell diseases (SCD) (1). The ubiquitin-proteasome system (UPS) is an important intracellular proteolytical pathway responsible for the degradation of the damaged protein caused by oxidative stress (2). The present study was undertaken to investigate the

involvement of ubiquitin-proteasome system in SCD patients.

Material and Methods: A total 30 patients with Sickle cell disease (19 patients with hydroxyurea (HU) treatment since last two years and 11 patients without hydroxyurea treatment (WHU) and 45 normal healthy individuals as a control group were studied. The catalytic activities of ß1, ß2 and ß5 subunits of proteasome were measured using fluorogenic proteasome substrates. Western blot was performed using antibodies against polyubiquitin, proteasomal subunits, and other RBC proteins. Detection of oxidized proteins was done using the commercially available OxyblotTM Protein Oxidation Detection kit.

Results: Western blot analysis showed that oxidatively modified proteins as well as polyubiquitinated proteins, were increased in RBCs of SCD- WHU as compared to SCD-HU and normal controls.

It was also observed that the SCD- HU treatment had a significantly higher proteasomal activity (mainly ß1-caspase and ß2- trypsin) as compared to SCD-WHU treatment ($p<0.0001$ and $p<0.0002$); as well as a normal control group. Interestingly, we have also observed two cases of SCD with HU treatment, had increased accumulation of polyubiquitination in their RBCs (one case had leg ulcer with avascular necrosis of the femoral head (AVNF) whereas the other case had AVNF and acute chest syndrome associated with the severe painful crisis). Both cases had moderate anemia (Hb-10. 1 g/dl and 10.4 g/dl) and did not respond well to hydroxyurea treatment.

Discussion: The proteasome activity is increased even in untreated patients with SCD as compared to healthy controls. However, it is not sufficient enough for the degree of polyubiquitination due to oxidative damage leading to accumulation of this protein resulting in cellular damage and anemia. However, patients with persistent complications inspite of HU therapy did not show such elevation of proteasomal activity resulting in accumulation of polyubiquitinated proteins as compared to the patients with HU response. This is the first attempt to investigate the role of the ubiquitin-proteasome system or the mechanism responsible for how oxidative stress can cause the destruction of RBCs due to the accumulation of damaged protein in sickle cell disease patients.

Conclusion: Our findings suggest that polyubiquitinated protein could be used as a biomarker to evaluate disease progression and the outcomes of sickle cell patients. These findings may also be helpful to understand the pathophysiology of sickle cell diseases and its clinical heterogeneity as well as therapeutic targets for the better management of the patients.

7. Dr. H.M Bhatia and Dr. L.D. Sanghvi best oral paper presentation award (Second prize) at Annual conference of Mumbai Haematology Group held at Mumbai on 17-18th March, 2018.

Molecular mechanisms underlying Rh null phenotype in India: First Report

Harita Gogri[1], Swati Kulkarni[1], K Vasantha[1], Disha Parchure[1], Manisha Madkaikar[1], Claude Ferec[2], Yann Fichou[2]. [1] ICMR-National Institute of Immunohaematology; [2]Etablissement Français du Sang (EFS) - Bretagne, Brest, France.

Background: Rh null phenotype is an extremely rare condition characterized by

absence of Rh antigens at the surface of red blood cells. The syndrome is associated with stomatocytosis, spherocytosis, increased osmotic fragility, altered phospholipid asymmetry, altered cell volume, defective cation fluxes, and elevated Na+/K+ ATPase activity. Rhnull RBCs have shortened in vivo survival and the person may have a mild compensated hemolytic anemia Although rare, genetic bases of this phenotype are well known and include mutations within either the RH (RHD and RHCE) genes or the RHAG gene. Here, we report individuals from two families of Indian origin representing such a rare phenotype.

Study Design and Methods: Red cells of individuals with Rh null phenotype and their family members were tested with anti-D, -C, -c, -E, and -e using serological methods. RHD, RHCE and RHAG genes were analyzed by standard molecular approaches, including Sanger sequencing and quantitative multiplex polymerase chain reaction of short fluorescent fragments (QMPSF).

Results: In propositus of family 1, RHAG gene was found to be deleted at the homozygous state, suggesting Rhnull of the regulator type. In the second family, a homozygous novel splice site variant (c.801+1G>A) in RHCE was found in cis with whole RHD gene deletion. Further functional analysis by minigene splicing assay showed that this variation completely impairs normal splicing, then inactivating the expression of RhCE protein suggesting Rh null of the amorph type.

Conclusion: Overall, we report for the first time the molecular mechanisms responsible for Rhnull phenotype in individuals of Indian origin. This study contributes to extend the molecular spectrum of variations in very rare Rhnull individuals.

8. Dr. H.M Bhatia and Dr. L.D. Sanghvi best oral paper presentation award(third prize) at 41st Annual conference of Mumbai Hematology Group held from 16-18, March 2018 at Mumbai, ITC , Parel

Comprehensive analysis of Fanconi anemia genes: first study from Indian population

Avani P. Solanki, V. Babu Rao. ICMR-National Institute of Immunohaematology

Introduction: Fanconi anemia (FA), a rare recessive autosomal and sex-linked genetic disease with spectrum of clinical features and predisposition to a high risk of developing cancers. However bone marrow failure is one of the important clinical features. FA occurs due to defect in any one of the 21 gene products so far known to be involved in DNA damage repair pathway also called as FA pathway. FA is an ideal model disease to understand DNA damage repair and cancer. Molecular analysis for FA families is important for management of the disease through prenatal genetic diagnosis.

Aim: To identify and characterize molecular pathology underlying FA in the patients and carrying out complementation group wise correlations of somatic and hematologic phenotype.

Materials and Methods: A total of 92 patients with confirm diagnosis of FA (based on FANCD2 immunoblotting results for chromosomal breakage positive patients) was recruited for the study. According to defect in FA pathway, FANC gene screening was carried out using a combination of techniques namely; direct sequencing (DS), Multiplex Ligation dependent Probe Amplification (MLPA), and targeted exome sequencing (TES). Somatic and hematologic phenotype scoring was carried out as described elsewhere [1, 2].

Results: A total of 76 different mutations including 32 novel mutations of FANC gene could be detected in 92 confirmed FA subjects (55 males and 37 females; 1.5:1 ratio) using a combination of advanced (TES) and conventional (MLPA and DS) DNA sequencing techniques. Complementation group is assigned based on FANC gene defect. Distribution of FA complementation group among our study cohort is given in Table 1. Apart from frequently represented complementation groups of FA, about 14.1% of patients belonging to rare complementation groups could be detected with help of TES. Distribution of clinical presentations among FA patients in our study cohort is represented in Table 2.

The median age represented by FA patients in our study group is 8 yrs with range of 2 yrs to 34 yrs. Patients with FANCD2 gene mutations were found to be diagnosed between ages of 2 to 9 yrs whereas age group of patients from FAA group was found to be spread between 2 yrs to 34 yrs. Patients with milder cytopenias could be diagnosed in patients of 5 to 10 yrs (3%) and 25 to 30 yrs age group (33%) and 67% of patients from 25 to 30 yrs age group showed severe cytopenias. Median clinical phenotype score of FAL group patients is significantly low compared to patients of other complementation groups. Frequencies of patients with moderate and severe cytopenias are relatively low in FAL group than that observed in other FA patients. The patients were followed-up after the initial diagnosis and they were enquired about their dependency on treatment and/or transfusion, BMT plans, progression to MDS, leukemia or development of solid tumour. It was observed that those who succumbed to death and patients with progression to MDS, leukemia, or development of solid tumour had a significantly higher phenotype severity score compared to those who were doing well in transfusion or treatment dependent and independent manner.

Discussion and Conclusion: The clinical features of FA patients overlap with that of patients with other genomic instability diseases. Therefore, understanding of molecular pathogenesis underlying chromosomal breakage diseases is important. Literature on molecular diagnosis of Indian FA patients is meagre. The present study represents the very first comprehensive data on molecular diagnosis of FA patients (n=92) from Indian subcontinent with an attempt to genotype-phenotype correlation.

There is a conspicuous difference in frequency of complementation groups in our study cohort compared to world literature. The highlight of the findings is 10% of FA patients representing FAL group, as FAL is considered to be the rarest complementation group (0.02%) as per world literature. FAG group is found to be represented by 15% of the FA patients in our study cohort, which again raises contradiction to frequency (10%) represented by FAG group patients worldwide. Thus, apart from FANCA gene screening; molecular diagnosis of Indian FA patients should include screening of FANCG and FANCL at 2nd and 3rd most priority. A combination of advanced and conventional molecular techniques should be employed for molecular analysis of FA, as ~14.1% patients with rare frequencies of FANC gene defects (FANCJ, FANCN, FANCM, FANCF, FANCI, etc.) and large deletions of FANCA could be detected using TES and MLPA techniques respectively; which otherwise would have been difficult to diagnose using DS method.

Various correlation studies carried out with respect to BMF (cytopenias) and phenotype severity highlighted the below mentioned facts-

- FA patients with FANCD2 defects could be diagnosed in first decade (2 to 9 yrs) of their life. Thus, it indicates the early onset of haematological complications in FAD2 patients.
- Majority of times the suspicion of FA raises only after the onset of hematologic symptoms, although clinical presentations of FA do exist in the patients. Comparison of somatic phenotypes among patients of different complementation groups revealed significantly lower median score in FAL group patients compared to other FA group patients. Moreover, severity of cytopenias (BMF) is also relatively lower in FAL group patients than frequency of that observed in FAA, FAG and FAD2 group patients. Thus, somatic and cytopenia scoring system is important for their

Table 1 Detection of Complementation groups for FA patients

	Upstream complex defect								Downstream complex defect				
	FA A	FA G	FAL	FAB	FA C	FAE	FAF	FAI	FA M	FA N	FA D2	FAJ	FA D1
N (%)	54 (59%)	14 (15%)	9 (9.8%)	1 (1.1%)	1 (1.1%)	1 (1.1%)	1 (1.1%)	1 (1.1%)	1 (1.1%)	1 (1.1%)	5 (5.4%)	2 (2.2%)	1 (1.1%)

Table 2 Distribution of clinical presentations among FA patients

Clinical presentation	Frequency (%)
Skin pigmentation	68
Skeleton abnormality	50
Short stature	48
Microcephaly/triangular facies	31
Eye abnormality	28
Organ abnormality	24
Bleeding manifestation	18
Ear abnormality	16
High arched palate	14
Cardiopulmonary abnormality	8
Progression to MDS/Leukemia/ development of solid tumor	8
Genital abnormality	4

correlation with FA complementation group.

- Somatic and cytopenia scoring system should be assessed carefully by clinicians to understand prognosis of FA patients, as it revealed significant correlation with follow-up data on clinical condition of FA patients.

9. Dr R S Satoskar best poster award (second prize) in the 11th Annual Conference entitled "Clinical Pharmacology: Contributing to Global Health and Policies " held from 29th April- 2nd May 2018 at Mumbai.

Silver NPs enabled instantaneous, cost effective and multiplexed rare blood group detection system

Chawla S [1], Devarajan P.V[1], Gorakshakar A [2], Ghosh K[2], Madaikar M[2]. [1]Institute Of Chemical Technology, Mumbai, [2]ICMR-National Institute of Immunohaematology, Mumbai.

Background: Some of the clinically significant rare blood groups like Kidd, Kell and Duffy impose severe repercussions if transfused with incompatibility. In present study we developed simple and rapid kit based on silver nps for detection of these rare blood groups.

Methods: AgNps prepared by reduction of AgNO3 with trisodiurncitrate capped with PVD polymer.The capped AgNps were characterized by UV-VlSspectra and PSA. Antisera antibodies AntiFya, Anti-Fyb , Anti-Jka Anti Jkb and Anti K, Anti k are added onto the surface capped AgNps at 1:1 ratio of various titres Neat, 1:2, 1:4, 1:8 using coating buffer and confirmed by FT-1R spectra.These antisera capped surface modified AgNps coated onto the wells using PVD2 film former and freeze dried. ICT (Indirect Coombs testing antisera antibody) is also conjugated at the ratio 1:1 to the surface capped AgNps at titres 1:2. The blood samples are added to the wells and allowed for 15mins, then ICT coated surface capped AgNps at titre 1:2 is added. Observed for results.

Results: Surface modified Ag Nps were stable absorbancemaxima at 431nm, PS 250-500nm. FTIR spectra explained the interaction of antisera antibodies with capped Ag Nps. Rare blood group type positive blood sample showed agglutination in any wells of Anti-Fya, Anti-Fyb , Anti-Jka Anti Jkb and Anti K, Anti k upto titre 1:4 of antisera antibodies. At titres 1:8 antisera coated wells, positive samples lacked agglutination reaction. Minimum titre of 1:4 antisera coated AgNps required for samples analysis.

Conclusion: Rapid Visual results, No processing steps, cost

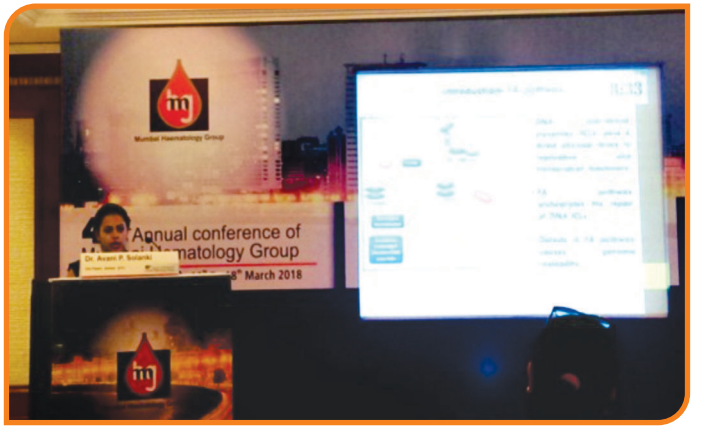
Foundation Day programme (9 Feb, 2018)

Ms. Darshana Mirgal was awarded Dr. J.C. Patel Best Oral Paper Award at 41st Annual conference of Mumbai Haematology Group held from 16th to 18th March, 2018.

Dr. Priyanka Kasatkar was awarded Dr. J.C. Patel Best Oral Paper Award at 41st Annual conference of Mumbai Haematology Group held from 16th to 18th March, 2018.

Dr. Prashant Warang was awarded the Dr. H.M Bhatia and Dr. L.D. Sanghvi best oral paper presentation award at the 41st Annual conference of Mumbai Hematology Group held from 16th to 18th March, 2018.

Ms. Harita Gogri was awarded the Dr. H.M Bhatia and Dr. L.D. Sanghvi second paper best oral presentation at Annual conference of Mumbai Haematology Group held from 16th to 18th March, 2018.



Dr. Avani Solanki was awarded the Dr. H.M Bhatia and Dr. L.D. Sanghvi 3rd best oral paper award at 41st Annual conference of Mumbai Haematology Group held from 16th to 18th March, 2018.

Ms. Rutuja Deshpande was awarded Dr. A.J. Desai and Dr. Hiranandani Second best Poster Award, at 41st Annual conference of Mumbai Haematology Group held from 16th to 18th March, 2018.

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