

HAEMOSTASIS

Thrombohaemorrhagic balance in haemophilia - implications for an alternative therapeutic approach.

(Funded by Bayer Health Care, USA – Early career Investigator Award to Dr. Shrimati Shetty)

Year of commencement : 2004.

Year of completion : 2006.

Patients with hemophilia demonstrate quite variable clinical phenotype even in cases with same level of deficient factor or the same molecular abnormality. The modulation of disease severity in haemophilia patients may be related to various interacting factors which may include the congenital or acquired alterations of coagulation inhibitors or abnormality or deficiency of factors in the fibrinolytic pathway. These factors can modulate both clinical expression and severity of haemophilia. The project was undertaken with following objectives.

1. Classification of the severe hemophilia A and B cases as per the clinical severity based on the clinical presentation i.e. number of bleeds per year, transfusion history in the preceding 5 years, age of presentation, joint deformity etc. A well-designed clinical Proforma would facilitate this classification based on the clinical symptoms.
2. Study of common thrombophilia markers – acquired and congenital in cases and controls which are as follows , Protein C, Protein S, Anti thrombin III (ATIII), Activated protein C (APC) resistance, factor V Leiden mutation, PT gene polymorphism, MTHFR, EPCR (23 bp insertion) ,fibrinogen β chain (G 455A), tissue factor pathway inhibitor (TFPI), antiphospholipid antibodies (Lupus anticoagulant/ anticardiolipin antibodies).
3. Study of some factors in the fibrinolytic pathway- Tissue plasminogen activator (tpA),plasminogen activating Inhibitor (PAI – 1),Thrombin activable fibrinolytic inhibitors(TAFI),PAI 4G/5G polymorphism, plasminogen and antiplasmin levels.

During the last 2 years we have studied 26 severe hemophilia cases with mild manifestations and 29 severe hemophilia cases with severe clinical manifestations. The patients were initially screened by thromboelastography (TEG) which provides a continuous profile of the whole blood coagulation and also is a broad screening technique for detecting abnormalities of platelet function, coagulation factors, coagulation factor inhibitors or defects in the fibrinolytic system.

Four distinct patterns of TEG were observed in the study group.

- 7 with hypercoagulable patterns.
- 4 with fibrinolysis with normal clot time.
- 9 with long latent period without initiations of clot formations.
- 7 with varied clot initiation times.

A. Blood Coagulation Inhibitors

Table 1 shows the mean levels of some of the blood coagulation inhibitors in cases and controls.

Table 1. Protein C (PC), Protein S (PS), Antithrombin III (AT III) and Tissue Factor Pathway Inhibitor (TFPI) levels in cases of clinically mild hemophilia cases and their controls.

Parameter	Clinically mild (n =26)		Clinically Severe (n=29)	
	Mean \pm S.D.	Def.	Mean \pm S.D.	Def.
Protein C	73.14 \pm 7.8	3(11.5%)	92.14 \pm 19.35	1(3.4%)
Protein S	82.26 \pm 8.1	2(7.7)	104.21 \pm 18.71	0 (0%)
Antithrombin III	105.4 \pm 20.8	2* (3.8%)	123.13 \pm 34.72	0 (0%)
TFPI	74.6 \pm 16.4	2(7.7%)	94.04 \pm 21.81	0 (0%)

- Two brothers with Antithrombin III def.

The mild hemophilia group showed consistently reduced levels of blood coagulation inhibitors though the difference in the levels were not statistically significant.

Three patients (11.5%) were deficient for protein C and two each (7.7%) for protein S and TFPI. Two brothers with milder manifestation were deficient for Antithrombin III.

Overall 34.6% of these patients were deficient for any of these factors.

B. Fibrinolytic factors

Table 2 shows the mean levels of some of the factors in the fibrinolytic pathway in cases and controls.

Table 2. Fibrinolytic pathway factors in cases of clinically mild and severe hemophilia patients.

Parameter	Clinically Mild (n=26)		Clinically severe(n=29)	
	Mean \pm S.D.	Def	Mean \pm S.D.	Def
TpA	82.16 \pm 21.8	2 (7.7%)	91.29 \pm 16.60	0 (0%)
PAI - 1	160 \pm 14.8	0 (0%)	118.71 \pm 44.4	0 (0%)
TAFI	127.4 \pm 6.8	0 (0%)	99.61 \pm 21.8	0 (0%)
Plasminogen	104.7 \pm 16.8	0 (0%)	109.1 \pm 12.05	0 (0%)
Antiplasmin	89.16 \pm 7.8	1(3.8%)	84.41 \pm 10.17	0 (0%)

Only in case of PAI-1 and TAFI, there was a significant difference in the mean levels of TAFI and PAI-1, while tpA, plasminogen and antiplasmin did not show any difference between the two groups.

Two patients (7.7%) were deficient for tpA. However, PAI-1, TAFI, plasminogen levels were within normal range in cases and controls. One of the patients showed a mild deficiency of antiplasmin the reason for which is not clear. Out of two patients deficient for tpA one was also deficient for PC.

Detection of Factor IX gene mutations in hemophilia B patients by CSGE and DNA sequencing.

Year of Commencement : 2002

Year of Completion : 2006

Hemophilia B families till date are given genetic diagnosis based on linkage analysis using polymorphic markers of the factor IX gene. This technique has several limitations such as families being non-informative for the markers or the index cases being unavailable. Conformation sensitive gel electrophoresis and direct automated DNA sequencing will benefit such non-informative families.

In the current year we have screened 150 patients for all the 8 exons of the factor IX gene. We have detected 40 mutations in 73 patients with 33 patients being mild, 14 moderate and 103 belonging to the severe category (Table 1).

Table 1 . Mutations detected in haemophilia B patients

Patient ID	Community	Factor IX level (% of NPP)	Nucleotide change	Amino acid change
02	Maharashtrian	3.50 %	G-A G30864A	Arginine-Glutamine
03	Maharashtrian	1.90 %	G-A G30864A	Arginine-Glutamine
04	Maharashtrian	<1 %	G-A G30864A	Arginine-Glutamine
05	Gujarati	8 %	G-A G10430A	Glycine-Serine
06	Gujarati	3 %	G-A G10430A	Glycine-Serine
07	Gujarati	10 %	G-A G10430A	Glycine-Serine
08	Gujarati	3 %	G-A G10430A	Glycine-Serine
09	Gujarati	22 %	G-A G10430A	Glycine-Serine
10	Gujarati	10 %	G-A G10430A	Glycine-Serine
12	Gujarati	11 %	G-A G10430A	Glycine-Serine
13	Gujarati	15 %	G-A G10430A	Glycine-Serine
15	Maharashtrian	<1 %	C-T C31133T	Arginine-Stop
16	Maharashtrian	<1 %	C-T C31133T	Arginine-Stop
17	Rajasthani	2.90 %	G-A G31280A	Glutamic acid- Lysine
19	Bengali	4.60 %	T-C T31313C	Tyrosine-Histidine
20	Punjabi	< 1%	A-G A116G	Threonine- Threonine (Donor splice)

21	Punjabi	< 1%	A-G A116G	Threonine- Threonine (Donor splice)
30	Punjabi	< 1%	C-T C30863T	Arginine-Stop
33	Sindhi	< 1%	T-G T95G	Tyrosine-Stop
36	Maharashtrian	< 1%	G-C G31050C	Tryptophan-Serine
37	Maharashtrian	< 1%	G-A G6365A	Arginine-Glutamine
39	Maharashtrian	< 1%	A-T A31212T	Aspartic acid - Valine
41	Gujarati	< 1 %	C- G C30919G	Tyrosine-Stop
44	Gujarati	12 %	G-A G10430A	Glycine-Serine
47	Punjabi	< 1%	C-T C31118T	Arginine-Stop
48	Gujarati	15 %	G-A G10430A	Glycine-Serine
51	Maharashtrian	<1 %	C-T C30875T	Arginine-Stop
52	Maharashtrian	<1 %	T-G T30939	Leucine-Arginine
54	Maharashtrian	<1 %	G-A G31275A	Tryptophan-Stop
55	Gujarati	< 1 %	G-A G31119A	Arginine-Glutamine
56	Gujarati	< 1 %	G-A G31119A	Arginine-Glutamine
57	Maharashtrian	< 1%	G-T G17756T	Glycine-Valine
58	Maharashtrian	< 1%	C-T C30114T	Histidine-Tyrosine
59	Maharashtrian	< 1%	C-T C30848T	Histidine-Tyrosine
60	Maharashtrian	< 1%	C-T C30848T	Histidine-Tyrosine
66	Bihari	< 1 %	T-A T31041A	Valine-Glycine
67	Muslim	< 1%	T-G T17677G	Cysteine-Glycine
68	Muslim	< 1 %	T-G T17677G	Cysteine-Glycine
70	Gujarati	11 %	G-A G10430A	Glycine-Serine
73	Christian	< 1 %	T-C T20560C	Tryptophan-Arginine
74	Muslim	< 1%	T-G T17677G	Cysteine-Glycine
77	South Indian	< 1%	C-T C17764T	Leucine-Phenylalanine
78	Muslim	< 1%	T-C T30096C	Tryptophan-Arginine
86	Christian	< 1%	G-A G31208A	Glycine- Arginine
87	Gujarati	< 1 %	G-T G30974T	Valine-Phenylalanine
90	Maharashtrian	< 1 %	AT 2bp del 10448,10449	
91	Rajasthani	5.50%	C-G C10400G	Glutamine-Glutamic acid
92	Gujarati	5.40 %	G-A G10430A	Glycine-Serine
97	Gujarati	< 1%	C-G C17700G	Cysteine -Tryptophan
98	Muslim	< 1 %	T-G T17677G	Cysteine-Glycine

100	Muslim	< 1 %	G-A G31119A	Arginine-Glutamine
101	Maharashtrian	< 1 %	C-A C31223A	Proline- Threonine
102	Maharashtrian	< 1 %	C-T C6364T	Arginine-Tryptophan
106	Maharashtrian	< 1 %	G-A G6365A	Arginine- Glutamine
107	Hindu	< 1%	A31355C	Threonine-Proline
109	Muslim	< 1 %	Extra A intronic sequence (polymorphism) 30802-03insA	
111	Maharashtrian	< 1 %	G-A G6436A	Glutamine- Lysine
112	Maharashtrian	< 1 %	G-T G17741T	Cysteine- Phenylalanine
116	Gujarati	20 %	G-A G10430A	Glycine-Serine
117	Gujarati	23 %	G-A G10430A	Glycine-Serine
118	Bihari	< 1 %	T-C T31340C	Tryptophan-Arginine
121	Maharashtrian	< 1 %	G17756T	Glycine-Valine
122	Muslim	< 1%	T-C T30096C	Tryptophan-Arginine
123	Gujarati	12 %	G-A G10430A	Glycine-Serine
124	Gujarati	27 %	G-A G10430A	Glycine-Serine
126	Muslim	10.20%	G-A G31119A	Arginine- Glutamine
127	Hindu	< 1%	A31355C	Threonine-Proline
128	Gujarati	50 %	G-A G10430A	Glycine-Serine
129	Gujarati	37 %	G-A G10430A	Glycine-Serine
130	Gujarati	20 %	G-A G10430A	Glycine-Serine
131	Gujarati	10 %	G-A G10430A	Glycine-Serine
132	Gujarati	19 %	G-A G10430A	Glycine-Serine
136	Maharashtrian	7.20 %	C-T C30106T	Threonine-Isoleucine
141	Muslim	< 1%	T-C T30096C	Tryptophan-Arginine

Figure 1: TEG representative patterns in severe Hemophilia patients.

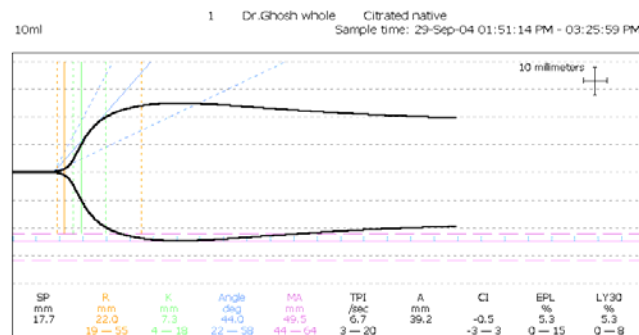
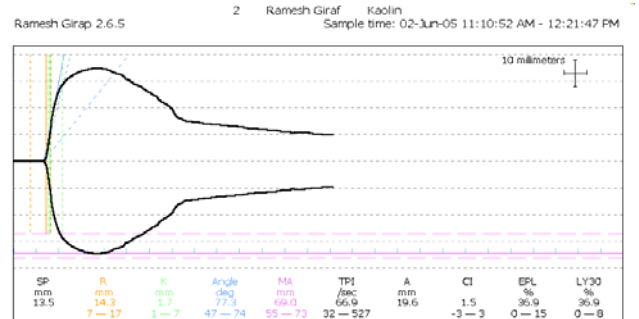
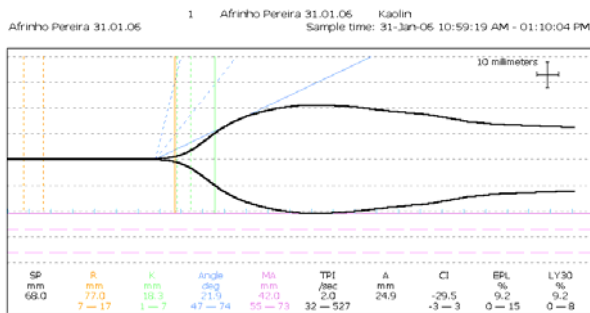
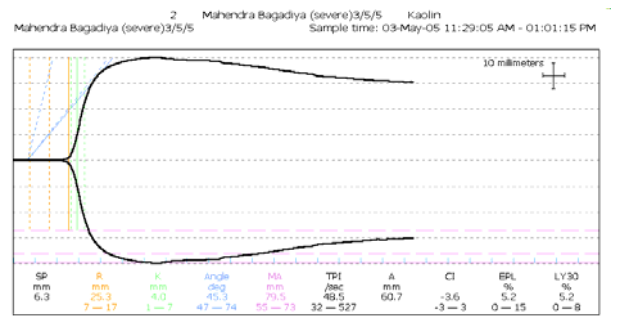
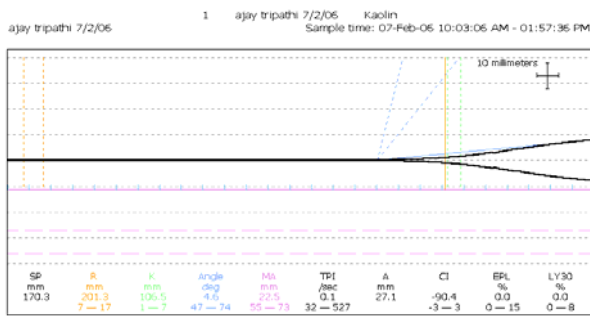
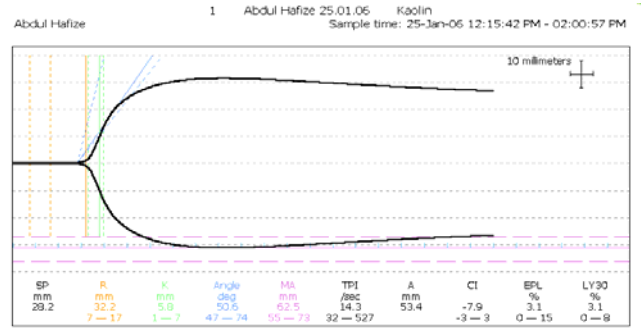
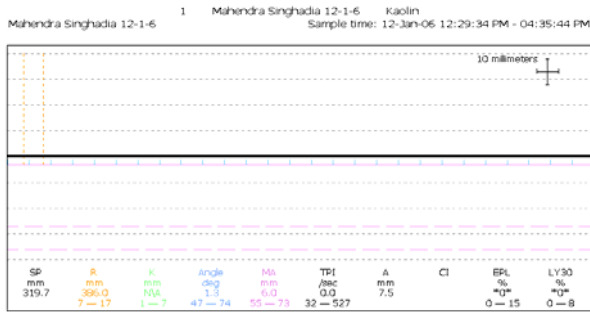


Fig: 2 Altered CSGE patterns in haemophilia B patients

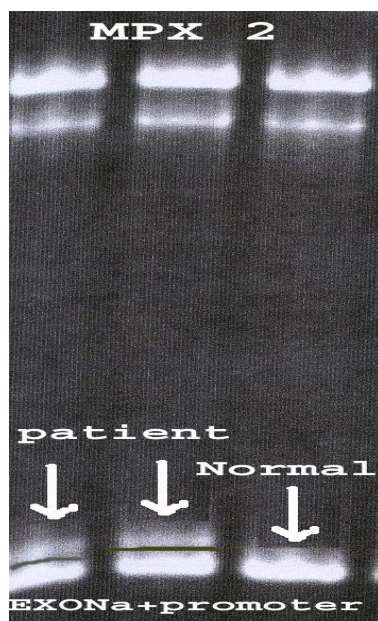
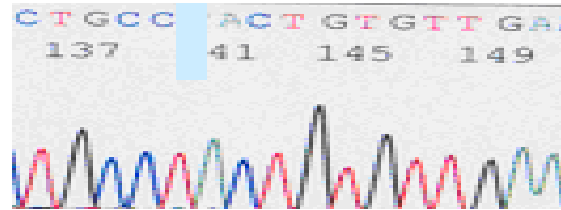


Fig. 3 Electrophoregrams of some of the novel mutations: -

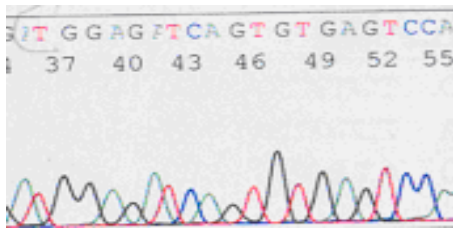
Exon g normal



Exon g mutated sequence
C30114T



Exon d normal



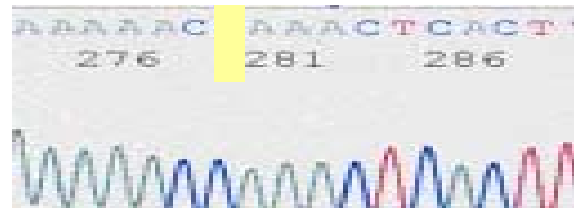
Exon d mutated sequence C10400G



Exon h2 normal sequence



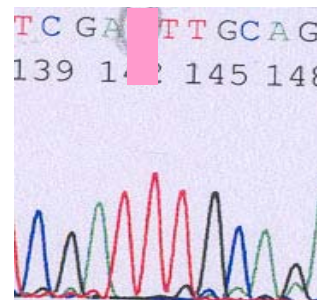
Exon h2 mutated sequence A31355C



Exon e normal sequence



Exon e mutated sequence C17764T



A total of 15 novel mutations were detected in the above patients, 12 are associated to severe and 3 with mild Hemophilia B phenotype.

One mutation each was detected in the exon a (Tyr-Stop, 116) which leads to a truncated protein, exon b (Arg- Glu, 6365) which encodes the propeptide, exon d (AT a 2 bp del, 10422,10423), (Glu-Gln, 10400) which encodes the first epidermal growth factor (EGF)-like domain and which also possesses a high affinity calcium-binding site, exon f (Trp-Arg, 20560) which encodes a region that includes, in addition to flanking sequences, an activation peptide, exon g (His- Tyr,30114), Three mutations were detected in the exon h1 one (Val-Phe 30974), (His- Tyr, 30848) and one at (Leu-Arg 30939). Six mutations were detected in the exon h2 (Try-Stop, 31275), (Glu-Lys, 31280) (Thr-Pro, 31355; Lys- Asp, 31360), (Arg- Glu, 31119) and (Try-His, 31313). The exons g, h1 and h2 encode the catalytic domain and the long 3' untranslated region in the factor IX gene.

Last year we had an interesting observation that 90% of the mild hemophilia B patients (factor levels 5-50%) belonging to the Gujarati community showed a common mutation in the exon 4 at nucleotide position 10430 of the factor IX gene (G 10430A). This mutation results in the substitution of the highly conserved Gly 60 residue to Ser in the EGF domain of the factor IX protein. The factor IX gene haplotype analysis was carried out using the following markers Hha1(-), Mnl1(-), Salt (+) and Dde1 (369bp), Mse1⁽⁻⁾. Interestingly it shows that all of them have a common haplotype background thereby ascribing to a founder effect. The haplotype of 30 normal Gujarati males was also constructed and on analysing the data by fisher's test the difference was found to be statistically significant (p value → 0.0002).

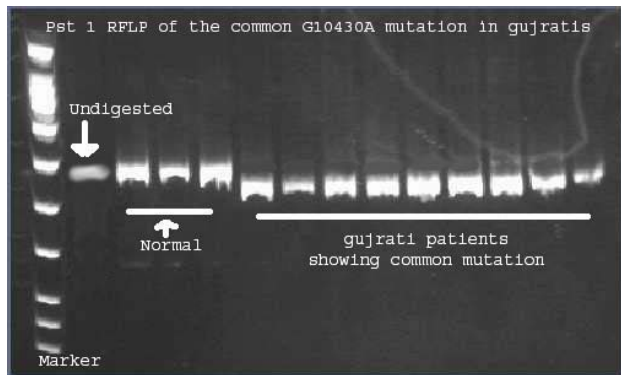
The above finding further simplifies the approach of genetic diagnosis in these families.

Also since we detected a common mutation in the Gujarati community we thought it would be better to detect these mutations by a simple RFLP technique so that these mutations can also be detected by a simple RFLP – PCR technique.

Mismatch PCR priming technique for the detection of G10430A mutation in haemophilia B patients

A set of primers with two base mismatch to create PstI restriction site when the mutation is present was designed in the laboratory. PCR amplification will result in a 250 bp amplified product which on digestion with PstI restriction enzyme will create 240 bp and 10 bp fragments while in the absence of mutation remains undigested i.e. 250 bp fragment.

Fig: 4_ Mismatch priming PCR showing Gly60ser (G10430A) mutation in factor IX gene



Molecular characterization of factor VII and X deficiency cases from western India

Year of commencement :2004

Year of completion :2007

(ICMR Best Research Paper Award to Dr.Kanjaksha Ghosh for the year 2004)

Factor VII and X deficiencies are the commonest coagulation factor deficiencies amongst the rare coagulation disorders. Factor VII deficiency occurs in approximately 1 in 500,000 and factor X deficiency in 500,000-1000,000 persons worldwide. Both factor VII and X deficiencies are inherited as autosomal recessive traits affecting males and females equally. Both the disorders are common in regions where consanguinity (marriage among close relatives) is common such as Jews, Muslims and some endogamous groups in South India.

There is only a single report about mutation detection in factor VII and factor X deficiency patients from India. Although the deficiencies are very rare, yet due to the high incidence of consanguinity in many parts of India, families are often being referred for antenatal or carrier diagnosis. Currently we are providing antenatal diagnosis by cord blood sampling procedure in the second trimester of pregnancy.

Under this project we have analyzed 12 patients with factor VII and 15 with factor X deficiency.

Table 2 shows the clinical and laboratory data of cases with factor VII & X deficiency.

Table 2. Clinical and laboratory data of the factor VII and X deficiency cases

Case No	Age/ Sex	Cons ang.	Fam. Hist	FVII/ X:C levels (%)	Clinical data				
					Mu-cosal	Soft tissue	Joint Bleed	Cerebral bleed	Menorrhagia
Factor VII Deficiency									
1	10/M	N	N	<1%	Y	N	N	N	NA
2	4/M	Y	Y	<1%	N	N	N	Y	NA
3	9/M	Y	Y	<1%	Y	N	N	N	NA
4	62/F	N	N	5.2%	N	Y	N	N	Y
5	6/M	N	N	8%	Y	N	N	N	NA
6	19/F	N	N	<1%	N	N	N	N	Y
7	62/M	N	N	<1%	N	N	N	N	N
8	1/M	Y	N	<1%	N	Y	Y	Y	NA
9	72/M	N	N	33%	N	N	N	N	NA
10	45/F	N	N	3%	Y	Y	N	N	Y
11	54/M	N	N	6%	N	N	N	N	NA
12	24/F	N	N	<1%	N	N	N	N	Y
Factor X Deficiency									
1	25/F	N	N	1.5%	Y	Y	N	N	Y
2	4/F	Y	Y	2.2%	Y	Y	N	Y	NA
3	10/F	N	N	<1%	Y	Y	Y	N	NA
4	28/F	Y	Y	<1%	Y	Y	N	N	Y
5	30/M	Y	Y	<1%	N	N	N	N	N
6	12/M	N	Y	<1%	Y	Y	Y	N	NA
7	10/M	N	Y	<1%	N	Y	N	N	NA
8	9/F	N	N	17%	N	Y	N	N	NA
9	45/F	N	N	<1%	Y	Y	Y	N	Y
10	3/F	N	N	<1%	Y	Y	Y	N	NA
11	21/F	Y	N	<1%	Y	Y	Y	N	Y
12	50/F	N	N	3.8	Y	N	N	N	Y
13	9/M	Y	N	<1%	Y	Y	N	N	NA
14	14/F	Y	N	<1%	N	N	N	N	Y
15	27/M	Y	Y	<1%	Y	N	N	N	NA

N Negative Y Positive NA Not applicable

There is no direct correlation between the factor levels and the clinical manifestations. This is more prominent in cases of FVII deficiency which is probably due to the requirement of only trace amounts of FVIIa to initiate coagulation in vivo.

Phenotypic Characterization of factor VII and factor X deficiency

Several factor VII and X variants have been reported by their different activities using tissue thromboplastins from different sources. Table 2 and 3 show the varied FVII and FX levels respectively in cases using thromboplastin from different sources.

There was no variation in the levels of factor X: C when thromboplastins from various sources were used. However in factor VII deficiency cases, except one case, low levels of factor VII were detected with the recombinant thromboplastin (Innovin) while the highest levels were detected in rabbit brain thromboplastin. Intermediate levels were observed with Thromborel S.

Mutation Characterization in cases of factor VII and X deficiency

Fig 5 shows some of the altered CSGE patterns in a case of FVII deficiency and factor X deficiency respectively. Five cases of factor VII deficiency and two of factor X deficiency showed altered mobility which were further characterized by direct DNA sequencing.

Table 3 shows the mutations detected in cases of factor VII and X deficiency.

Except patient 5 who is a double heterozygote all the rest of the mutations have affected the catalytic domain of the factor VII gene. Except one, all were novel mutations in both factor VII and X genes

Table 3: Phenotypic characterization of cases with FVII deficiency using Thromboplastin from various sources.

Patient ID	Age/sex	Prothrombin time (PT)			FVII levels		
		Neoplastin	Thromborel S	Innovin	Neoplastin (%)	Thromborel S (%)	Innovin (%)
NPP	-	12.5	12.6	8.2	-	-	-
1	62/M	36.1	29.8	20.8	13	21	13
2	54/M	22.5	23.6	21.8	26	16	4
3	10/M	54.0	107.8	>200	6	>1	>1
4	6/M	42.6	44.1	29.3	16	7.6	2.8
5	9/M	64.0	100.5	178.0	6	1.2	>1
6	24/F	46.3	77.4	19.8	7	3.3	>1
7	19/F	79.6	103.2	170.0	5.4	1.5	>1
8	19/F	23.2	26.5	20.9	26	21	11

Table 4: Phenotypic characterization of cases with FX deficiency using thromboplastin from various sources.

Patient ID	Age /sex	Prothrombin time (PT)			F X levels		
		Neoplastin	Thromborel S	Innovin	Neoplastin (%)	Thromborel S(%)	Innovin (%)
NPP		12.5	12.6	8.2	-	-	-
1	4/F	69.7	90.2	69.3	>1	>1	>1
2	50/F	25.6	26.0	19.0	6	5	5.6
3	12/M	164.0	170.4	148.0	>1	>1	>1
4	10/M	151.1	164.8	124.3	>1	>1	>1
5	9/M	73.2	72.5	47.7	>1	>1	>1
6	28/M	>200	>200	>200	>1	>1	>1
7	30/M	>200	>200	>200	>1	>1	>1
8	21/F	57.5	44.2	34.4	>1	>1	>1

Table 5: Mutations detected in cases of FVII and FX deficiency cases.

F VII deficiency	Factor VII level	Mutation detected	
1	FVII <1%	C(10931)G	Asp338Gly
2	FVII <1%	G(10846)C	Cys310Ser
3	FVII <1%	C(10888)T	Thr324Met
4	FVII <1%	G(8962)A	Arg152Gln
5	FVII <1%	T(3886)C G(9672)C	Cys22Arg Ala191Pro
F X deficiency			
1	FX <1%	G(27049)A	Gly 406 Ser
2	FX <1%	G(670)C	*Gly20Arg

*Reported earlier

Some of the novel mutations detected in cases of factor VII and X deficiency are shown in Fig.6.

Fig: 5 Some of the altered CSGE patterns in a case of FVII deficiency and factor X deficiency respectively.

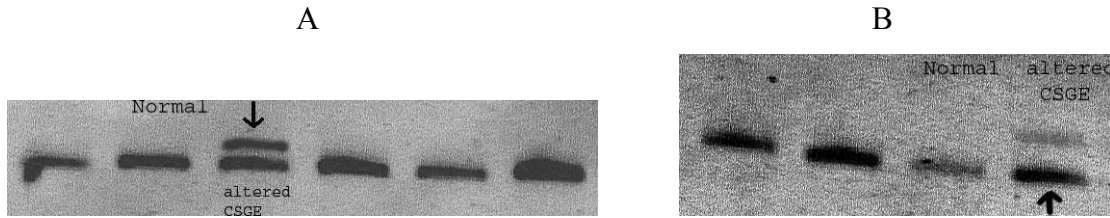
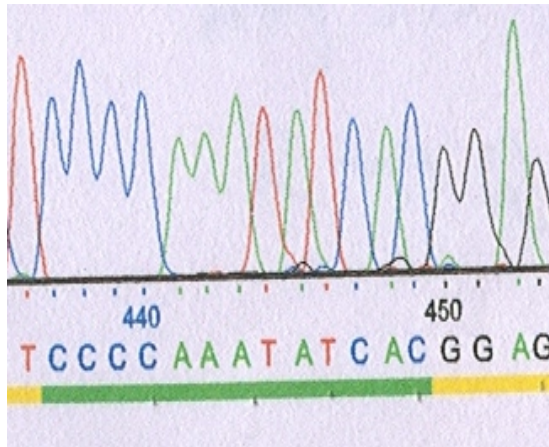


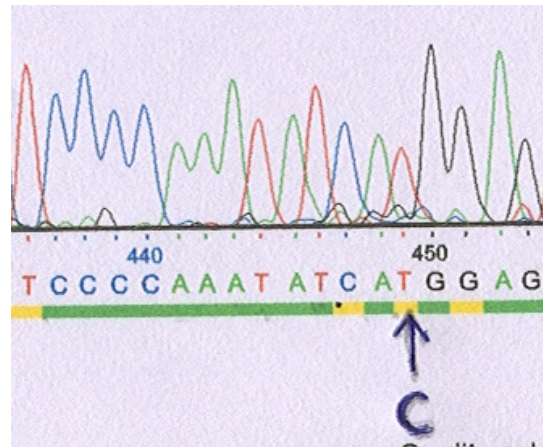
Fig : 6 Shows mutations detected in A. factor VII deficiency B. factor X deficiency patients:

A) Factor VII deficiency

1. Thr324Met ACG → ATG

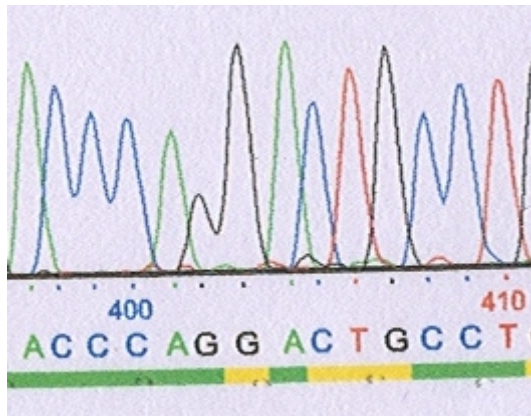


Normal

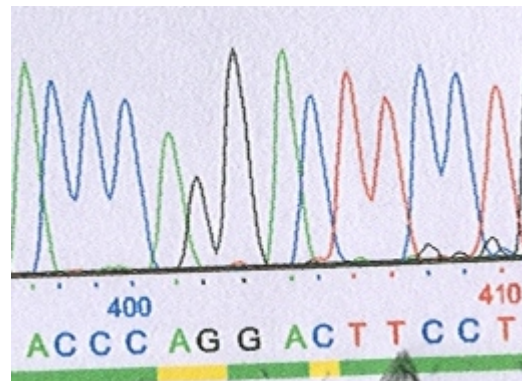


Mutant

2. Cys 310 Ser TGC → TCC

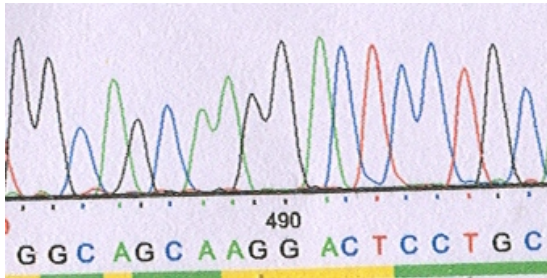


Normal

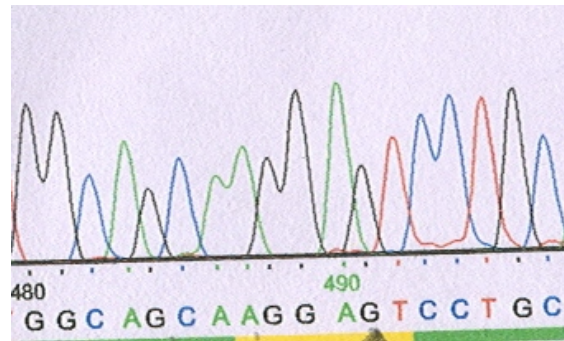


Mutant

3. Asp338Gly GAC → GAG



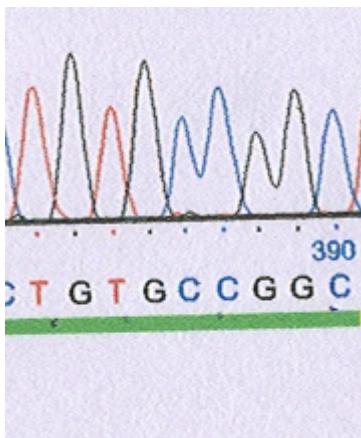
Normal



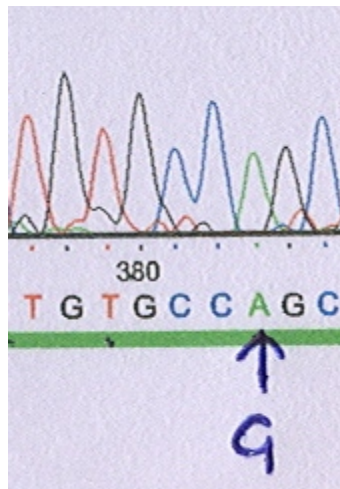
Mutant

B: Factor X deficiency

Gly40Ser



Normal



Mutant

During the period two families i.e. one with factor VII deficiency and other with factor X deficiency were referred for antenatal diagnosis. Cord blood sampling was performed in the second trimester of pregnancy to offer a diagnosis of the fetus.

Family 1:

A 25 year old lady, referred from Indore at 18.5 weeks of pregnancy for antenatal diagnosis of factor X deficiency. She had one daughter with severe factor X deficiency (factor X: C <1%) with severe bleeding manifestations and haemarthrosis, however no other family members were affected. The family was counselled about the possibilities of the second child also having factor X deficiency, the techniques involved and the possibility of misdiagnosis. Subsequently they underwent prenatal diagnosis.

Family 2

A 24-year-old lady reported at 19 weeks of pregnancy for antenatal diagnosis of severe factor VII deficiency. She had two sons with severe factor VII deficiency (Factor VII: C<1%) diagnosed at our center. Both the affected children died of cerebral hemorrhage at the age of 5 weeks and 13 months respectively. The family was counselled for the second trimester antenatal diagnosis and they gave the consent.

Table shows the coagulation factor levels detected in the fetal blood samples.

Table 6: Coagulation factor levels in fetal blood samples

Disorder	F VIII : C (%)	F IX:C	F VII : C	F X:C
F X deficiency	28	4.5	ND	21
F VII deficiency	19	5.2	22	ND
*Normal Range	15-35	3.6-12	13-29	12-35

* Normal range established in the laboratory

In both the cases the diagnosis of unaffected fetus was made . Both the families have been followed up and the diagnosis has been reconfirmed by factor assays.

Characterization of mutations in the remaining patients is ongoing which would further be followed by phenotypic and genotypic correlation in these patients of rare coagulation factor deficiencies.

Study of some genetic aspects of essential hypertension in North East Region

Year of commencement :2003

Year of completion :2006

Essential hypertension is a polygenic disease whose phenotypic expression is modulated by the environment. It results from the combined and interactive effects of many genetic and environmental factors.

Ethnic variations in blood pressure levels have been reported in several studies. In the north eastern region it has been observed that there are significant differences in the mean systolic and diastolic blood pressures levels in the 2 communities i.e. Tea garden workers and Mizos. Our collaborators have earlier reported the prevalence of hypertension as high as 60% in the tea garden workers as compared to 2% in the Mizos and 3 % in the native Assamese when a cut off point of SBP > 140 or DBP > 90 mm Hg was considered. The project was thus planned with the following objectives.

1. Study of acquired risk factors for hypertension which included salt intake, alcohol, smoking, tobacco consumption etc.
2. Study of biochemical parameters like lipid profile, blood urea, albumin, protein, uric acid, blood glucose.
3. Study of genetic polymorphic markers related to hypertension.
a) ACE I/D b) Angiotensionogen M235T c) ENOS exon 7 Gln / Asp d) ENOS int. 27 bp repeat marker e) Angiotensin II type I receptor (AII 66 C) f) CYB II B2 IC/WT g) Angiotensin II type 1 receptor (AII 66 C) (h) CYB II B2 – SF (i) α abducin
4. Interaction of 1, 2 and 3.

Samples received during the last 3 years.

Tea garden wokers – 147

Assamese – 36

Mizos – 293

Family samples received of tea garden workers – 86 samples from 22 families.

Results : Tables 7 & 8 shows the blood pressure levels and other characteristics of the study population.

Table 7 shows the distribution of blood pressure levels in the study group

Group	Normotensives	Hypertensives
Tea garden workers		
Male (n=63)	24 (38.09)	39 (61.90)
Female (n=84)	18 (21.42)	66 (78.57)
Total (n=147)	42 (28.57)	105(71.42)
Assamese		
Male (n=16)	12(75)	4 (25)
Female (n=20)	15(75)	5 (25)
Total (n=36)	27(75)	9 (25)
Mizo's		
Male (n=141)	137 (97.16)	4 (2.83)
Female (n=152)	144 (94.73)	8 (5.26)
Total (n=293)	281 (95.90)	12 (4.09)

Table 8: Other characteristics of study population

Age Group	Mizo Community(n=293) %	Assamese Community(n=36) %	Teas Garden workers(n=147) %
20-29	8.4	4.6	18.2
30-39	34.2	8.3	38.6
40-49	16.8	16.8	15.2
50-59	24.2	24.6	26.64
60 and above	16.4	45.7	1.36

Table 9-12 shows the prevalence of the various genetic polymorphisms studied in different groups.

Table 9: Prevalence of ACE insertion / deletion and M235 T angiotensinogen polymorphism in the study groups

Polymorphism	Mizo (n=293)		Assamese (n=36)		Tea Garden worker (n=147)	
	NT N=281	HT N=12	NT N=27	HT N=9	NT N=42	HT N=105
1.ACE I/D						
DD	26 9.25%	7 58.33%	1 3.7%	1 11.1%	4 9.5%	61 58.1%
DI	99 35.23%	4 33.33%	7 25.9%	17 77.8%	9 21.4%	29 27.62%
II	156 55.51%	1 8.33%	19 70.4%	1 11.1%	29 69.0%	15 14.29%
2.M235T						
MM	52 18.5%	4 33.3%	2 7.4%	1 11.1%	5 11.9%	41 39.0%
MT	49 17.43%	5 41.7%	9 33.3%	4 44.4%	13 30.95%	45 42.9%
TT	180 64.1%	3 25.0%	16 59.3%	4 44.4%	24 57.1%	19 18.1%

Table 10: Prevalence of IC and AGTRI genotypes in different groups

		WT/IC	n	%	SF	n	%	AGTRI	n	%	
Tea garden worker	total n=119	IC	40	33.6	TT	80	67.2	AA	111	93.3	
		IC/WT	18	15.1	TC	32	26.9	AC	8	6.7	
		WT	61	51.3	CC	7	5.9	CC	0	0	
	hypertensive n=107	IC	34	31.8	TT	75	70	AA	100	93.5	
		IC/WT	16	14.9	TC	25	23.5	AC	7	6.5	
		WT	57	53.3	CC	7	6.5	CC	0	0	
	Non hypertensive n=12	IC	6	50	TT	5	41.6	AA	11	91.7	
		IC/WT	2	16.7	TC	7	58.4	AC	1	8.3	
		WT	4	33.3	CC	0	0	CC	0	0	
	Assamese Population	total n=36	IC	12	33.3	TT	19	52.8	AA	35	97.2
			IC/WT	1	2.7	TC	11	30.6	AC	0	0
			WT	23	63.8	CC	6	16.7	CC	1	2.8
hypertensive n=30		IC	7	23.3	TT	14	46.7	AA	29	96.7	
		IC/WT	1	3.3	TC	10	33.3	AC	0	0	
		WT	22	73.3	CC	6	20	CC	1	3.3	
Non hypertensive n=6		IC	5	83.3	TT	5	83.3	AA	6	100	
		IC/WT	0	0	TC	1	16.7	AC	0	0	
		WT	1	16.7	CC	0	0	CC	0	0	
Mizo Population	total n=83	IC	11	13.3	TT	43	51.8	AA	81	97.6	
		IC/WT	4	4.8	TC	29	34.9	AC	2	2.4	
		WT	68	81.9	CC	11	13.3	CC	0	0	
	hypertensive n=13	IC	2	15.4	TT	4	30.8	AA	12	92.3	
		IC/WT	0	0	TC	6	46.2	AC	1	7.7	
		WT	11	84.6	CC	3	23	CC	0	0	
	Non hypertensive n=70	IC	9	12.9	TT	39	55.7	AA	69	98.6	
		IC/WT	4	5.7	TC	23	32.9	AC	1	1.4	
		WT	57	81.4	CC	8	11.4	CC	0	0	

Table 11: e-NOS-4 and e-NOS-7 gene polymorphisms in the different study groups

		e NOS 4		e NOS 7	
Tea garden worker	total n=119	aa	0(0%)	Glu-Glu	3(2.5%)
		ab	38(32%)	Glu-Asp	30(25.2%)
		bb	81(68%)	Asp-Asp	86(72.3%)
	hypertensive n=107	aa	0(0%)	Glu-Glu	3(2.8%)
		ab	34(32%)	Glu-Asp	24(22.4%)
		bb	73(68%0	Asp-Asp	80(74.7%)
	Non hypertensive n=12	aa	0(0%)	Glu-Glu	0(0%)
		ab	4(33%)	Glu-Asp	6(50%)
		bb	8(64%)	Asp-Asp	6(50%)
Assamese population	total n=36	aa	0(0%)	Glu-Glu	01(2.8%)
		ab	5(14%)	Glu-Asp	06(16.7)
		bb	31(86%)	Asp-Asp	29(80.6%)
	hypertensive n=30	aa	0(0%)	Glu-Glu	01(3.3%)
		ab	5(17%)	Glu-Asp	06(20%)
		bb	25(83%)	Asp-Asp	23(76.7%)
	Non hypertensive n=6	aa	0(0%)	Glu-Glu	0(0%)
		ab	0(0%)	Glu-Asp	0(0%)
		bb	6(100%)	Asp-Asp	6(100%)
Mizo population	total n=83	aa	0(0%)	Glu-Glu	10(12%)
		ab	9(10.8%)	Glu-Asp	13(15.7%)
		bb	74(81.2%)	Asp-Asp	60(72.3%)
	hypertensive n=13	aa	0(0%)	Glu-Glu	2(15.4%)
		ab	01(7.7%)	Glu-Asp	2(15.4%)
		bb	12(92.3%)	Asp-Asp	9(69.2%)
	Non hypertensive n=70	aa	0(0%)	Glu-Glu	8(11.4%)
		ab	8(11.4%)	Glu-Asp	11(15.7%)
		bb	62(88.6%)	Asp-Asp	51(72.8%)

Table 12: Distribution of α -Adducin gene polymorphism in different study groups

α-Adducin	Mizos	Assamese	TGW
Gly/Gly	91 31.1%	11 30.1%	60 40.8%
Gly/Trp	60 20.5%	7 19.4%	38 25.9%
Trp/Trp	142 48.5%	18 50.0%	49 33.35

Conclusion:

1. About 78% of the tea garden workers were hypertensive. The prevalence of hypertension was almost same in both the sexes
2. In contrast out of 2.8% of the mizos were hypertensive in the study group
3. The age group in which there was a prevalence of hypertensives in the tea garden workers group was between 30-39 years
4. About 58% of the hypertensives among the tea garden workers had ACE D/D polymorphism as compared to similar prevalence in the hypertensive group of mezzos. the non hypertensive group of the mezzos showed a very low prevalence of 9.25 5. No significant differences were observed in the prevalence of D/I heterozygotes in the different groups.
5. The angiotensinogen M235T T/T genotypes were less prevalent in the hypertensive tea garden workers group(18%) as compared to the non-hypertensive mezzo group(64%).the M/M homozygotes were increased in the hypertensives in all the 3 groups as compared to non-hypertensives
6. No significant differences were observed in the prevalence of α -Adducin genotypes in the different groups studied
7. The e-Nos-4 and e-Nos 7 genepolymorphisms showed similar prevalence between hypertensives and non-hypertensives.

Studies on the Effect of Dengue Viruses on Hematopoietic Stem Cell Differentiation and Hemostatic Properties of the Vascular Endothelial Cells

Year of commencement : 2005

Year of completion : 2008

Funding: Extramural, Medical Biotechnology program, DBT

Collaborators: Institute of Immunohematology (ICMR) Mumbai.

National Institute of Virology, Pune

The pathophysilologic basis of thrombocytopenia and vascular dysfunctions in dengue infections remain incompletely understood. Several factors like immunopathogenic mechanisms and consumptive coagulopathy have been implicated as primary causative factors but the interactions of the dengue viruses with the platelets and megakaryocytic differentiation is not known. The present study attempts to characterize in-depth the cellular and molecular mechanisms by which dengue viruses might interact with the hematopoietic stem cell differentiation and platelet formation in-vitro.

Objectives

Phase 1 goals: 2005-06

Study the interaction between dengue viruses and human blood platelets

Characterize the effects of dengue viruses on human megakaryopoiesis in-vitro

Achievements

Dengue virus-platelet interactions

In-vitro studies carried out with Dengue 2 virus on human blood platelets from normal donors showed specific loss of collagen induced aggregation of the platelets after exposure to dengue virus .

The ristocetin and ADP induced aggregation were not affected. Interestingly, exposure of latelets with an equivalent dose of Japanese encephalitis virus (JEV) did not show any loss of collagen sensitivity of the platelets .

Transmission electron microscopy of the dengue virus exposed platelets also showed significant changes in the ultrastructure suggestive of activation morphology. Flowcytometric quantitation of the platelet fibrinogen binding and CD62P (P-selectin) expression after dengue 2 virus exposure showed significant increases when compared with JEV and culture supernatants. The collective results suggest activation of platelets by exposure to dengue 2 virus in vitro.

Since similar effects of specific abolition of collagen-induced aggregation of platelets have been reported by proteins present in the venom of hemorrhagic snake venom toxins, we used a bioinformatic approach to compare the envelope glycoprotein of dengue and other flaviviruses with such proteins where the molecular motifs of platelet interactions are well characterized. We could identify zinc binding metalloproteinase motifs in the dengue Egp similar to those present in the snake venom toxins. Further characterization of these motifs are under progress.