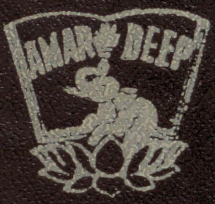

CANADA 60
POSTAGE / POSTES
4
POSTAGE
POSTES
CANADA



NOTE-BOOK

NUMBER FIVE

बे.का.मु.–वि. (ओ) १७७९

Spl. HI/Ly–136

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BOMBAY–12

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Signature of the Reader

2

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in every year.

Renewal-

Year	Signature	Date
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1988-89	Ghatate.	14.3.89.
1989-90	Ghatate.	28.3.90
1990-91	Ghatate.	17.5.'91

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when you come to the Library.

"It is work that gives flavour to life"

Swiss Poet + Philosopher

Henri Frederic

Amiel

Philips Germany 12V 6W (12814)
Linsen HG estimation

Ulnagar - Sindhi appr. 4 lakhs. in 1990
No. of birth per year 10,396 (Jan to Dec 1989)
no. children born at hosp - 9300
~~& hosp.~~ home - 1636.
No. of mat. home + — 25 mat
no. obstetricians homes & Hosp.
obtained from Dr. Wadhwa

ARMS - Amplification Rep of Mutation System

Biotechnics
Eaton Publishing Co.
154 E Central Str.
NATICK. MA. 07160-5414

Biotechnology Letters.
SCI Tech letters
12 Clarence Road
New Surrey TW9 3NL
U.K.

Koya Dora. α 142 → 15 added.
Between HbA2 and Origin in Starch
gel pH 8.5. DEAE Cellulose 0.003-0.01M
Tris-Phosphate buffer pH 8.6 Multi-
plicity of bands. % of Variant 0.5 to 2
Rampa α 95 Pro-Ser (G2) Cellulose-pH 8.9
Sl. faster than S to anode - Paper & Starch
gel pH 8.5. On agar at pH 6 - No separation
CM 30 Cellulose - No separation
DE 52 Cellulose - TRIS/PO4 pH 8.5 NaCl gradient
0.01M. Separates from A. Chain separates
in 8M Urea - No separation from A.
Hybridion c̄ Canine - Abnormal ch - Chain
DE 52 TRIS/PO4 pH 8.5 Separates from HbA.
CM. 30 Cellulose - No separation
Ref. Human Hbs + its variants. H.L &
P A M. Kynoch. North Holland Publishing
Co. 1976

Desai et al Ind. Ped (1973) 10 : 511
511
Bajaj et al HbM

JParis. L12 (A10) Ala-Asp

NOTE BOOK

NO. 5

TABLE OF DAILY WAGES

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85	3 04	2 93	2 83	2 74
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PERSONAL NOTES

Name _____

Address _____

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Telephone No. { Residence _____
Office _____

Motor Car No. _____

Driving Licence No. _____

Life Insurance Policy No. _____ Expire _____

Bank Pass Book No. _____

Season Ticket No. _____

Blood Group _____

Passport No. _____

Club Membership No. _____

In Emergency Notify _____

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INSURANCE POLICY

Life Policy No.	AMOUNT	Premium Due on

CALENDAR 1989

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LIST OF DELIVERY POST OFFICES IN GREATER BOMBAY WITH THE PIN CODE NUMBERS ASSIGNED TO THEM

Post Office	Zonal No.	Pin Code No.	Post Office	Zonal No.	Pin Code No.
Aarey Milk Colony	65	400 065	Khar	52	400 052
Andheri (East)	69	400 069	Kurla	70	400 070
Andheri (west)	58	400 058	Malad	64	400 064
Aug. Kranti Mrg.	36	400 036	M.G. Camp	35	400 035
Bandra	50	400 050	Mahim	16	400 016
Bandra (East)	51	400 051	Malabar Hill	6	400 006
BARC	85	400 085	Mandvi HO	3	400 003
Ballard Estate	38	400 038	Marine Lines	20	400 020
Barve Nagar	84	400 084	Matunga	19	400 019
Bhandup	78	400 078	Mazgaon	10	400 010
Bhavani Shankar	28	400 028	Mulund	80	400 080
Bombay Air Port	29	400 029	Mulund Colony	82	400 082
Bombay Central	8	400 008	Mulund (East)	81	400 081
Bombay GPO	1	400 001	Nehru Nagar	24	400 024
Borivli (East)	66	400 066	Nitie	87	400 087
Borivli (West)	92	400 092	Pant Nagar	75	400 075
Chakala M.I.D.C.	93	400 093	Parel	12	400 012
Chembur	71	400 071	P.M.G. s. Office	30	400 030
Ch. Bunder	9	400 009	Prabhadevi	25	400 025
Colaba	5	400 005	Raja Wadi	77	400 077
Council Hall	39	400 039	Sachivalaya	32	400 032
Cumballa Hill	26	400 026	Saki Naka	72	400 072
Dadar	14	400 014	Santa Cruz (E)	55	400 055
Dahisar	68	400 068	Santa Cruz (West)	54	400 054
Delisle Road	13	400 013	Sewri	15	400 015
Deonar	88	400 088	Sion	22	400 022
Dharavi	17	400 017	Tank Road	33	400 033
Fartilizar Corpn.	74	400 074	Tilak Nagar	89	400 089
Ghatkopar West	86	400 086	Tulsiwadi	34	400 034
Girgaon HO	4	400 004	Tagore Nagar	83	400 083
Goregaon	62	400 062	Vashi (New Bombay)		413 503
Goregaon (East)	63	400 063	Versova	61	400 061
Grant Road	7	400 007	Victoria Gardens	27	400 027
I.I.T. Powai, BY	76	400 076	Vikhroli	79	400 079
Jacob Circle	11	400 011	Vile Parle (E)	57	400 057
J B Nagar	99	400 099	Vile Parle (W)	56	400 056
Jogeshwari	60	400 060	Wadala	31	400 031
Kalbadevi HO	2	400 002	Worli	18	400 018
Kandivli (West)	67	400 067	Yogakshema	21	400 021

GREETING TELEGRAMS

1. Heartiest Diwali Greetings
2. Id Mubarak
3. Heartiest Bijoya Greetings
4. A Happy New Year to you
5. Many Happy Returns of the Day
6. Hearty Congratulations on the New Arrival
7. Congratulations on the Distinction Conferred on you
8. Best Wishes for a Long and Happy Married Life
9. A Marry Christmas to you
10. Hearty Congratulations on Your Success in Exam.
11. Best Wishes for a Safe and Pleasant Journey
12. Hearty Congratulations on Success in Election
13. Many thanks for Your Good Wishes which I/We Reciprocate Most Hearty
14. Congratulations
15. Loving Greetings
16. May Heaven's choicest Blessings be showered on the Young Couple
17. Wish you both a Happy and Prosperous Wedded Life
18. Kind Remembrances and all Good Wishes for the Independence Day
19. Sincere Greetings for the Republic Day. Long Live the Republic
20. My Heartiest Holi Greetings to You
21. Wishing the Function Every Success
22. Many Thanks for Your Kind Message of Greetings
23. Best Wishes for Your Success in the Examination
24. Best Wishes for Your Success in the Election
25. Convey our blessings to the newly married couple
26. Heartiest Pongal Greetings
27. Heartiest Gurpurb Greetings

POSTAL RIN CODE

Name of the Post Office	Delivery Dist. No.	Name of the Post Office	Delivery Dist. No.
A.K. Marg	400 036	Malad	400 064
Antop Hill	400 037	Malabar Hill	400 006
Aarey Milk Colony	400 065	Mandvi	400 003
Andheri East	400 069	Mantralaya	400 032
Andheri West	400 058	Marine Lines	400 020
Anushakti Nagar	400 094	Matunga	400 019
B.A.R.C.	400 085	Mazgaon	400 010
Ballard Estate	400 038	Mulund	400 080
Bandra	400 050	Mulund Colony	400 082
Bandra East	400 051	Mulund East	400 081
Barve Nagar	400 084	M.I.T.I.E.	400 087
Bangur Nagar	400 090	Nehru Nagar	400 077
Bhandup	400 078	Pant Nagar	400 075
Bhavani Shankar Road	400 028	Parel	400 012
Bombay Aerodrome	400 029	Prabhadevi	400 025
Bombay Central	400 008	Rajawadi	400 077
Bombay G.P.O.	400 001	Rajbhavan	400 035
Borivli East	400 066	Saki Naka	400 072
Borivli West	400 092	Santacruz East	400 055
Chakala M.I.D.C.	400 093	Santacruz West	400 054
Chembur	400 071	Seepz	400 096
Chinch Bunder	400 009	Sewri	400 015
Colaba	400 005	Sion	400 022
Council Hall	400 039	Tagore Nagar	400 093
Cumballa Hill	400 026	Tank Road	400 036
Dadar	400 014	Telecom Factory Deonar	400 088
Dahisar	400 068	Tilak Nagar	400 089
Delisle Road	400 013	Tulsiwadi	400 034
Dharavi	400 017	Veer Jeejamata B. Udyan	400 027
F.C.I. Bombay	400 074	Vesava	400 061
Ghatkopar West	400 086	Vidyanagari	400 098
Girgaon	400 004	Vikhroli	400 079
Goregaon East	400 063	Vile Parle East	400 057
Goregaon West	400 062	Vile Parle West	400 056
Grant Road	400 007	Wadala	400 031
Hutatma Chowk	400 023	Worli	400 018
I.I.T. Bombay	400 076	Yogakshema	400 021
J.B. Nagar	400 059		
Jacob Circle	400 011	**Navi Mumbai**	
Jogeshwari	400 060	Belapur	400 614
Juhu	400 049	Mumbra	400 612
Kalbadevi	400 002	Nerul	400 613
Kandivli	400 067	Konkan Bhavan	400 614
Khar	400 052	Ghansoli	400 701
Kharodi	400 095	Airoli	400 708
Kurla	400 070	Vashi	400 703
Mahim	400 016	M.A.C. Kamothe	400 704

Haff Int 4129395
~ 9320

4128750. Kam Bank

STD CODES

Station	Dialling Code	Station	Dialling Code	Station	Dialling Code
Adilabad	08732	Ernakulam	0484	Nagpur	0712
Adoor	04754	Erode	0424	Nandyal	08514
Agra	0562	Faridabad	011-81	Nasik	923
Ahmedabad	0272	Ferozpur	01632	Nellore	0861
Ahmednagar	0241	Gadag	08372	Ongole	08592
Ajmer	0145	Gandhi Nagar	0271	Ooty	0423
Akola	0724	Gangatok	0359	Palghat	0491
Allahabad	0532	Ghaziabad	011-85	Panipet	01742
Alleppey	0477	Gorakhpur	0551	Panjim	0832
Alwar	01442	Gudiwada	08674	Patiala	0175
Alwaye	04854	Gunthakal	08552	Patna	0612
Ambala	0171	Guntur	0863	Pondicherry	0413
Ambernath	929-82	Harihar	08197	Pune	0212
Amravati	925	Hassan	08172	Puttur	08251
Amritsar	0183	Hubli	0836	Quilon	0474
Anakapalli	08924	Hyderabad	0842	Raibareilly	05362
Anantpur	08554	Idukki	04853	Raipur	0771
Asansol	0341	Indore	0731	Rajahmundry	0883
Attur	04282	Jabalpur	0761	Rajapalayam	04563
Aurangabad	02432	Jaipur	0141	Rajkot	0281
Bagalkot	08354	Jalgaon	926	Ranchi	0651
Bangalore	0812	Jammu	0191	Ratnagiri	932
Baroda	0265	Jamnagar	0288	Rohtak	01262
Bareilly	0581	Jamshedpur	0657	Rourkela	0661
Bhatinda	0164	Jullundur	0181	Sangli	927
Bhavnagar	0278	Kakinada	0884	Shahajahanpur	05842
Belgaum	0831	Kalimpong	03552	Shillong	0364
Bellary	08392	Kalwa	922	Shimoga	08182
Bharatpur	08644	Kalyan	929	Sholapur	0217
Bhadravati	08182	Kanpur	0512	Siliguri	0353
Bhiwandi	924	Karnal	0184	Simla	0177
Bhopal	0755	Karwar	08382	Srinagar	0194
Bhubaneshwar	0674	Katihar	06452	Surat	0261
Bilaspur	07752	Khammam	08742	Thakurli (MIDC)	929-86
Bombay	022	Khandwa	0733	Tiruchirapally	0431
Belsar	0263	Kolhapur	0231	Tirunelveli	0462
Calcutta	033	Kota	0744	Tirupati	08574
Calicut	0495	Kottayam	0481	Tiruvalla	047811
Chandigarh	0172	Kovil Patti	04632	Trichur	0487
Chidambaram	04144	Kurnool	08518	Trivandrum	0471
Chikalthana	024328	Ludhiana	0161	Tuticorin	0461
Cochin	0484	Lucknow	0522	Udipi	08252
Coimbatore	0422	Machilipatnam	08672	Ujjain	0734
Coonoor	04264	Madurai	0452	Varanasi	0542
Cuddapah	08562	Madras	044	Vasco	920
Cuttack	0671	Malappuram	04935	Vashi	922
Darbhanga	06272	Mangalore	9284	Vellore	0416
Darjeeling	0354	Meerut	0121	Vijayawada	0366
Davangere	08192	Mehsana	0276	Virudhunagar	04562
Delhi	011	Miraj	972-82	Visakhapatnam	0891
Dhanbad	0326	Mirzapur	05442	Vizianagaram	08922
Dharwad	0836	Muzaffarnagar	01312	Warangal	08712
Dombivli	929-81	Muzaffarpur	0621	Wardha	07152
Durgapur	0343	Mysore	0821	Yamunanagar	01732
Eluru	08812	Nadiad	0268	Yeotmal	07232

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High frequency of α-thal are result of natural
(Thein et al) selection by Malaria Nature (1986) 321: 741
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Gene Deletions located on the same
Chromosome, S.K. Bakkas & Y.J. Fei; TH J Jf.
Brit. J Haemat (1989) 73: 429
[S.K. Bakkas Thomas Jefferson Univ
Jefferson Med College, CARDEZA FDN,
Dept. of Med., Philadelphia, 19107 USA]
Studies of β-thal Mutations in families
living in three provinces of Southern
China by J.Z. Liu; Q.S. Gao;
Z. Jiang; C.C. Liang; K.G. Yang;
G.Y. Wu; G.F. Long; Q. Li, J. Zhang,
B. Deng & R.X. Wang. Hemoglobin
(1989) 13: 585 [J.Z. Liu, Chinese
Acad Med. Sci, Inst of Basic Med Sci;
BEIJING. 100730. P.R.C]
DNA Sequences Regulating Human
Globin Gene Transcription in Non-
deletional HPFH. S. Ottolenghi;
R. Mantovani; S. Nicolis; A Ronchi
and B. Giglioni. Hemoglobin (1989)
13: 523 [S.O. Uni. Milan; Dept of
Genet & Biol Microorganism, I-20122 Milan
Italy]

Interaction of the ddd-globin gene haplotypes and sickle cell haemoglobin
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Frameshift Mutation Codon 5[FSC-5 (-CT)] Thalassemia; a novel Mutation Detected in a Greek patient Hemoglobin (1989) 13:597 E P. Kollia; J.M. Gonsalez-Redondo, T.A. Stoming, D. Loukopoulos; C. Politis and TH J.H. [P. Kollia, Univ. Athens, LAIKON Hosp. Sch. Med. Dept. Med 1, GR-11527 Athens Greece]

Occular abnormalities in patients with β-thal. Am J. Ophthal (1989) 108; 699 Sotiris Gartaganis; Konstantinos Ismiridis; Ouranis Papageorgiou; Nicholas G. Beratis & Dimitris Papanastasiou. [Gartaganis, S. Univ. Patras, Reg Univ. Hosp. Patras, Sch. of Med. Dept. Ophthal. GR-26110-PATRAS]

Practical Management of Sickle Cell Disease. Arch. of Dis. Child (1989) 64:1748 [Evans.

Second Trimester Placental Biopsy versus Amino Centesis for Prenatal

Two different theta (θ) globin gene deletions observed among Black newborn babies Fei Y.J., Fujita, S., THJH. Brit J. Haemat (1988) 68: 249.

diagnosis of β-thial. New Engl. J. Med (~~1989~~ 1990) 322: 60. Giovanni Monni et al [Monni, G. Osped. Reg Microcitemie 1-09100 Cagliari Italy]

Treatment of Severe β-thial (Patients) with Myelosan Am. J. Haemat (1990) 33: 50 Liu De-Pei et al [Liu, D.P. Univ. Calif. San Francisco, Box 0724, R. No. U-426, San Francisco, Ca. 94143]

β-thial Mutations in Indonesia and their Linkage to β-Haplotypes by L.E. Lie-Injo, et al Am. J. Hum Genet (1989) 45: 971 [Y.W. Kan, Univ Calif. San Francis Dept Med. San Francisco Ca. 94143].

Obituary. Luang Eng Lie-Injo, (1918-89). Y.W. Kan Am. J. Hum. Genet (1989) 45: 976.

Molecular Basis of β-thial in Thailand analysis of β-thial mutations in northern and northeastern Thailand Human Genetics (1989) 84: 41. S. Fucharoen, G. Fucharoen, W. Sriroongrueng, S. Tuchinda; Y Fukumaki [Fucharoen S, Dept. Fukumaki Y, Kyushu Univ

20/3/90 RD 18/4/1

For screening of thial mutations, all samples were amplified ↗

The Gene for θ-globin is transcribed in human fetal erythroid tissues (1987) Nature; 329: 551 Leung S.-O., Porondfood NJ, Whitelaw E

Genet. Informat. Lab. 3-1-1 MAIDASHI, Higashi KU, Fukuoka 812. Japan]

The Spectrum of Beta thal mutations in northern + Northeastern Thailand Human Genet (1989) 84: 47. M. Laig; et al G. Flatz [J. HUNDRIESER, Med. Hsch. Hanover Human-genet. Abt. Postfach. 610180 D-3000 Hannover-61]

Immunological Studies in Sickle Cell-β°-thal Comparison with Sickle Cell anemia. ~~Haemoglobin (1989)~~ Haematologia (1989) 74: 537 E. A. Donadi; R. Pesseto Falcao; [EAD. Univ. Sao Paulo, Dept of Clinical Med. BR-14048. RIBEIRO PRETO. SP. BRAZIL].

Detection of Sickle cell anemia mutation by Colour DNA amplification F. F. CHEHAB. Y. W. Kan. Lancet (1990) 335: 15 [FF Chehab, Cetus Corp. Dept. Human Genet. M4. 1400. 53rd Sha Emeryville. Ca. 94608 USA]

→ in vitro 23/3/90 by PCR using Taq polymerase (Stratgene) + either primers S1+S3 (631 bp) or S1+G8 (1.4 kb). Primer S1: 5' TGTCATCACTTAGACCTCA C3' located at positions -122 to -103 from the Cap site in complementary antisense

[Can product of the θ-gene be a real Stobin. Clegg JB. Nature (1987) 329:465
Why genes in pieces Gilbert, W. Nature (1978) 271:501.
A transgenic mouse model of Sickle Cell disorder by D R Greaves; P. Fraser; M. A. Vidal; M J Hedges; D. Ropers; L. Luzzatto + F. Grosveld. Nature (1990) 343: 183 [DR Greaves Nat Inst. Med Res. Genetic Structure and Express Lab. London NW7 1AA]
Molecular Genetics: Mice; men and Sickle Cells. D J W. Nature (1990) 343: 121 [Uni. Oxford, John Radcliffe Hosp. Inst of Mol. Med. Oxford OX3 9DU]
Bone disease in Children with homozygous β-thal. L. Rioja; R. Girot; M. Garabédian & G. Cournot-Witmer Bone + Mineral (1990) 8:69.
[G. Cournotwitmer, Univ of Paris 05, Hop. Enfants maldes, Dept Hemd. CNRS. URA 583, 149 Rue Sevres F-75743, Paris-15]
Rapid removal of Excessive Iron in Thalassemia by High-dose Intravenous Chelation therapy ~~by J. A. FLICK Jt Ped (1990) 116:156 Johns Hopkins Uni School of Med Dept. Ped. Baltimore, MD 21205 USA]~~

Structure + expression of the human
θ1 - globin gene Nature (1988) 331: 94
Hsu, S.L; Marks J; Shaw JP, Tam M, Higgs DR
Shew C.C, Shew K.J.

by D. SABATINO. [D.S. Nassau
City Med. Ctr. Dept. Ped. Div.
Ped. Hemat. Oncol., E. Meadow
NY. 11554]

Detection of β-thal. mutations
in the Chinese using amplified
DNA from dried blood specimens
by S.-Z. Huang; X.-D. Zhou;
H.-Z. Liu; Z.-R. Ren; Y.-T. Zeng.
Hum. Genet (1990) 84 (2): 129

[S.Z. Huang; Shanghai Children
Hosp. Med. Genet Lab. 380 Kang
Ding Road, Shanghai 200040
P.R.C.]

Molecular Heterogeneity of beta-thal
in the Japanese - Identification of two
Novel Mutations Br J. Haemat (1990)
74: 1015. Fucharoen; T. Katsuba;
G. Fucharoen; H. Sawada; H Oishi;
H. Oishi; M. Katsuno; J. Nishimura;
S. Matsumura; Y. Miura; Y. Fukumaki [Y. F., Y. Kyushu Univ 18,
Gen. Info. Res. Lab. 3-1-1. Maidashi,
Higashi Ku. Fukuoka 812. Jpn.

Fast Track on system available for the isolation of high quality intact messenger RNA directly from cells or tissues in 4 hrs.
Invitrogen Corporation 11588 Sorrento Valley Rd. #20 San Diego. Ca. 92121

Circulating Immune Complexes in Sickle Cell anemia Jl. Lab. ~~[illegible]~~ Clinical and ~~[illegible]~~ Laboratory Immunology (1989) 28: 183 by E. A. Donadi; I. F. Carvalho; R. P. Falcao. [EA. D. Univ. Sao Paulo, Sch. Med. Dept Clin. Med. BR-14048. Rebeirao Preto, Sp. Brazil]

Neuropsychological Impairment in Children with Sickle Cell anemia A. V. Swift; M. J. Cohen; G. W. Hynd; J. M. Wisenbaker; K. M. McKie; G. Makari; V. C. McKie. Pediatrics (1989) 84: 1077 [M. J. Cohen, Med College of Georgia Dept Neurol., Ped Neurol Section, Augusta GA. 30912 USA]

Community Control of β-thal. by Roshan B. Colah Ind. Jl. Med Sci (1990) 44: 44 [Instt. of Immohaematology KEM Hosp. Bl. 12] Sindhi; + Gujarat (Screening high risk Comm.) 178 thal trait out of 1095 Screened School Children

Applied Bio Systems. Second Generation model 373A DNA sequence — System handles single-stranded, double stranded & PCR-generated templates & utilizes labeled primers or labeled dideoxynucleotides.

(11–18 yrs) 5700 examined 152 or (2.6%) β-thal trait. Families informed & 32% responded. Parents & sibs examined. appropriately Counselled. Screening — RBC index, on particle Counter. Hb Electro. on Cellulose acetate. L. MCV (>75 μ³) NESTROFT osmotic fragility. HbF in all Cases to pick up other variant Kan, Golbus & Dozy (1975) New Engl. Jl. Med 29: 1165. Antenatal diagnosis of thal — Lab. diagnosis of thal. Ped. Clin of India (1988) 23: 25–28. (Colah RB). DNA analysis [Saiki RK et al 1988. New Engl. Jl. Med 319: 537–541]. There are 30 Centres in 12 Countries where fetal diagnosis is being undertaken & more than 10,000 Cases have been Studied so far (B.P. Alter 1988. Haemoglobin 12: 763].

ICMR (NM Wadia Cutse). Since 1986 90 Couples were studied. 36 Couples were from Bombay & remaining 54 from various other parts of the

Warrington, U.K. Tel: 0925-825.

Genetic linkage map of human genome
CELL (1987) 51: 319 Donis-Keller et al
→ amplified DNA (containing mutant ~~gene~~ allele) is blotted on to nitrocellulose. These spots are then hybridized ē known complementary DNA sequences (for mutant + normal gene) labelled ē P32 or biotin. Absence or presence of mutant allele results from hybridization as mutant gene hybridizes or fails
County. Written consent obtained
for conducting prenatal diagnosis
17 - 18 Weeks for placental position
in the following week sampling
Charges 250 - 1000/- Medical
& missing home charges Rs 300 - 400
In 13 cases fetal blood could not be
obtained. Normal ~~57 cases~~ or β-thal
57 cases; β-thal major 18 (23.4%)
HGD Trait 1 case, HGD-thal case
The rate of fetal loss 24% in first year, now
it is only 13%
Leukaemia cases linked to father's radiation
dose. Peter Aldhous. Nature (1990)
343: 679.
A review of the molecular genetics of the
human α-globin gene cluster. by
DR Higgs, M.A. Vickers, AOM Wilkie,
I.-M. Pretorius, A.P. Jarman & D.J.W.
Blood (1989) 73: 1081 [Higgs, Mol. Haem.
Unit. Nuff. Dep. Cl. Med. Level 7, John
Radcliffe Hl., Headington, Oxford.
England. Ox 3 9DU]
Major clinical syndromes resulting from
α-thal (HGH disease, HG Bart's hydrops fetalis)

Corresponding DNA. In the second technique mutation affecting Rs. endo. suspicionly can be detected. Many fetal blood methods the common 619 - bloc deletion of the hind. β-thal can be affected

Characterization of β-thal mutations using direct genomic sequencing of amplified Single Copy DNA. by Wong C, Dowling CE; Saiki RK; Higuchi RG; Erlich HA; Kazazian HH. Nature (1987) 330: 384-86.

Alpha-Thal in India: Its interaction with sickle cell disease Blood (1988) 71: 467. Kulozik A; Kar BC; Serjeant BE; Serjeant G. R.; DJW.

27/4

Different hematological phenotypes are associated with leftward ($-\alpha^{4.2}$) and rightward ($-\alpha^{3.7}$) α^+-thal. deletions Jr Cl Inv (1987) Bowden DK; Hill AVS; Higgs DR et al.

are first recognized in mid 1950 & early 1960. Adult carriers of HGH and HG Bart's do not produce large amts of these HG. HGH disease results from α-thal 1 × α thal 2. & HG Bart's hydrops α thal 1 × α thal 1. Better understanding came up when specific α-, β- and γ-cDNA were isolated & α-thal resulted from the inheritance of 3, 2, 1 or 0 α-globin genes. These findings explained the genetics of α-thal in SE Asia but did not explain the findings of high frequency of α-thal as judged from cord blood/~~[illegible]~~ surveys for HG Bart's in many regions of Africa & middle east, where HGH disease is relatively uncommon & HG Bart's hydrops fetalis

The molecular Basis of thal major + thal intermedia in Asian Ind.: application to prenatal diagnosis Thein S.L. et al. Brit J Haemat (1988) 70: 225-231

Syndrome does not occur. Thus we ultimately explained by understanding of structural organization of α-globin genes revealed by blot hybridization analysis. Individuals have two α genes on each chromosome 16 (αα/αα) & carriers of α-thal have either one gene (-α/αα) or two genes (--/αα) & most frequently encountered genotype of HbH disease (--/-α) and Barts fetalis (--/--). In some regions only -α determinant is frequent: although α-thal is common (-α/αα and -α/-α) neither HbH disease nor Barts hydrops syndrome occur. By 1980 molecular genetics of α-thal was understood in outline (Table-1). H.

Simple and rapid Enzyme-linked Immunosorbent Assay for the detection of HbC [$\alpha_2\beta_2^6(A_3)$Glu-Lys] in Cord blood using a monoclonal antibody by M. Shyamala, Charles R. Kiefer, Hugo Moscoso & Fred A. Garver Am J. Haemat (1990) 33: 198 [M. Shyamala, Ph.D. Dept of Cell & Mol Biol., MCG. Augusta, Ga. 30912-2100]

An improved method for prenatal diagnosis of genetic diseases by analysis of amplified DNA sequences: Application to hemophilia A. New Engl. J. Med. (1985) 313: 985-990.

See also. Adult Hb levels in newborn babies from different countries and in babies with some significant haemoglobinopathies. Kutlar F., Kutlar A., Gu Y.C., Huisman T.H.J. Acta Haemat (1987) 78: 28.

* we have generated a murine hybridoma that secretes a monoclonal antibody (mAb) that is highly specific for HbC and shows no cross reactivity with HbA, HbA_2, HbF, HbS, HbE or Hb-O Arab. Using this antibody we have developed a sensitive and rapid enzyme linked immunosorbant assay (ELISA) technique for detection of HbC in lysates of adult and fetal (cord) blood. With as little as 10 µl/well of whole blood or 5 µg Hb/well of haemolysate and with dilutions of the antibody up to 10^{-15} we were able to detect HbC unequivocally in cord samples. The ELISA procedure could detect HbC in proportions as low as 0.01% for mass screening 96 samples can be done in less than 45 minutes. Furthermore, this assay

Dideoxy sequencing method using denatured plasmid templates Hattori M; Sakaki Y. An. in Biochem (1986) 152: 232-238.

When employed in conjunction with an mAb specific for β6 Glu of HbA, allows the discrimination between Hb homo-heterozygous and normals (also ff. of Lab Clin. med. 1988, 112: 760 - Kiefer CR; Moscoso H; Shyamala M; Garver, F.A) (Hybridoma procedure as Taggart RT; Samloff I.M. Science 1983: 219: 1228)
BALB/cBy J mice (Jackson Lab, Bar Harbor, ME) were administered I/P injection of an emulsion containing 1mgm of isolated HbC in complete Freund's adjuvant (Sigma St. Louis MO). Second I/P injection of 100μg of the same antigen in incomplete Freund's ~~adjuv~~ adjuvant was administered 2 weeks later. Serum samples were obtained by retroorbital puncture + tested for anti-HbC antibodies using ELISA rest see original)
The molecular basis of thal in India
Its interaction - sickle cell gene
Blood (in press) Kulozik; AE; Kar BC
Serjeant G.R. Serjeant BE, Weatherall D.S

V.R. Kalgaonkar M.Sc. M.D
Chairman & Managing Director.
Bharat Immunologicals & Biologicals
Corporation Ltd.
V-13, Green Park Extn. New Delhi
Telex: (31-73244) 110016.

Treatment of Severe β-Thal (patients) with Myleran by Liu De-pei; Liang Chih-Chuan; Ao Zao-hui; Jia Pei-Chen; Chen Song-sen; Wang Rong-xui; Liu Li-juan; Jui He-qian; Zha Dan-yu and Huang Yon-wen Am J. Haemat (1990) 33: 50 [Dr. Liu-Depei, Box 0724, Room U-426, Univ. of California, San Francisco Ca-94143] Jomdrugs 5-azacytidine (AzaC); hydroxyurea (HU), Cytosine Arabinoside (Ara C) and vinblastin (VIB) have been found to increase γ-globin synthesis of anemic primates. Three of them (AzaC, HU & AraC) have been administered in patients of β-thal and/or Sickle Cell anemia. HbF% & Hb Concentration were found to ↑ transiently. As to the mechanism of action of these drugs, two hypothesis have been proposed. hypomethylation of DNA near a gene is related to the gene's expression and perturbation in erythroid marrow progenitor cell pool play

Characterization of β-thal mutations using direct genomic sequencing of amplified single copy DNA Nature (1987) 330: 384 Wong CD; Dowling CE Saiki RK; Higuchi RG. Erlich HA, Kazazian H.H. Jr.

a role in the augmentation of γ-globin gene expression (See Blood 1984; 63: 201). We found that myleran, a cell cycle non-specific drug can stimulate γ-globin gene expression in adult anemic monkeys (Liang CC et al 1987), both γ-globin RNA and globin chains increased. Two pts with severe β-thal were R/ myleran. Both patients recd an initial course of therapy, constants of myleran at a dosage of 0.2 mg/kg/d for 9 days followed by 0.15 mg/kg/d for next 11 days. One patient recd an additional 20 days course of myleran at a dose of 0.2 mg/kg/d begining 44 days after completion of first course. No ~~ill~~ severe ill effects related to the drug were ~~obsd~~ observed during or after drug administration. After 20 days of myleran R/, levels of HbF & retics ↑ in both cases and ~~bre~~ level of F cells ↑ in patient 1, In patient 1, Hb concentration rose from 42 g/L (9 days

Fucharoen SP; Fucharoen G; Fucharoen P. Fukumaki Y (1989). A novel ochre mutation in the β-thal gene amplified using polymerase chain reaction of a Thai: identification by direct cloning of the entire β-globin gene amplified using polymerase chain reaction J. Biol. Chem (1989) 264: 7780

after transfusion) before treatment to a maxim of 65 g/L afterward; in pt 2, it rose from 74 g/L to a maximum of 106 g/L. A value of 15 g/L above baseline lasted for about 5 months in both patients. Hypomethylation of BM DNA near the γ-globin gene was demonstrated in patient 1. Patient received myleran orally 3 times a day. The total dose was 0.2 mg/kg/day for next 11 days. Blood was weekly checked up for Serum immoglobulins, indirect bilirubin & % HGF and F cells. BM aspirate was done before, during & after myleran administration. DNA methylation analysis was carried out as described by Ley et al (New Engl. J. Med. 1982; 307: 1469.) DNA was prepared from whole BM cells. Fifteen mgm of DNA was first digested ± EcoR I, then ~~cleaved~~ cleaved ± excess of Hpa II. The double digested DNA fragments electrophoresed on 0.8% agarose gel, Southern blotting to nitrocellulose, &

backed. The filter was hybridized
to ^{32}P-labeled γ-globin probe
encompassing the second intron
washed under standard conditions
(Van der ploeg et al CELL, 1980,
19: 947) The dried filter was
autoradiographed & each band
was scanned at 500 μm. Similar
Chromatid exchange was done
after mylera R/ according to Latt's
method see also Sharma AK "Chro-
some techniques" 1984. The whole BM
cell DNA samples, digested with EcoRI
release a fragment of 7.2 kb containing
the 5' end of Gγ and one of 2.7 kb
containing the 5' end of the Aγ gene. Same
both fragment contain a HpaII recognition
site (CCGG) located near 5' end of
these genes, digestion will be
blocked if the internal C (CC*GG)
or both C (C*C*GG) residues are
methylated. Cutting these sites
by HpaII release a 1.5 kb HpaII-
EcoRI fragment. The γ IVS-II probe

hybridizes with all the 7.2 kb, 2.7 kb and 1.5 kb fragments. Cross hybridizes 5.2 kb EcoRI fragment containing the 5' end of human γ-globin gene, which contains no Hpa II site

Evaluation of retics counts by flow-Cytometry in routine laboratory

David F. Fergusson, Sion-Fong Lee and Philip A. Gordon. Haematol (1990) 33:13 [Dr. P. A. Gordon, Univ. of Alberta Hospitals, 8440-112 Street, Edmonton, Alberta T6G 2S7. Canada]. Uses FACScan, Becton, Dickinson, Mountain View, Ca].

Localization of the human α-globin gene Cluster:- The α- and β-globin gene clusters are localized to Chr. 16 and 11 respectively, by observing the specific pattern of c DNA-DNA hybridization to human x mouse somatic cell hybrids. Subsequently, localization of the α-locus has been refined to the distal segment (p13.1-pter) of the short arm of Chr. 16

Recently, two independent studies of individuals with unbalanced karyotypes have unequivocally located the α-locus to the Giemsa negative band 16p13.3 at the very tip of Chr. 16 (Buckle V.J.; Higgs DR et al. J Med Genet (1988) 25: 847) although its orientation in the Chr. is not yet known.

The α-globin cluster includes duplicated α genes (α_2 and α_1), an embryonic α-like gene (ζ_2), three pseudogenes ($\psi\zeta_1$, $\psi\alpha_2$, $\psi\alpha_1$) and a gene of undetermined function ($\theta 1$) arranged in the order 5'-ζ_2-$\psi\zeta_1$-$\psi\alpha_2$-$\psi\alpha_1$-α_2-α_1-θ_1-3'. Recently a pseudogene for the family of small Cytoplasmic RNAs was identified downstream of α_1 and a truncated, processed copy of the θ gene family ($\psi\theta_2$) was found on Chr. 22 (Shaw J-P, Marks J, Mohandas T et al "The adult α-globin gene loci from monkey to man: The θ-globin subfamily and the α-globin duplication in old world monkeys" (1987) p65. Developmental Controls of Globin gene expression, New York: Liss, Stamatoyannopoulos + Nienhuis.

†

Analysis of 5' Flanking Regions of the Gamma-globin Genes from major African Haplotype Backgrounds associated with Sickle Cell disease SR Mouth; R.W. Wood; P.T. Trifillis; P.J. Orchowski; S. Surrey; E. Schwartz; B. Sharon; S.K. Bakas. Jl. Cl. Inv. (1990) 85: 364 [E. Schwartz, Childrens Hosp. Philadelphia, Dr. of Haemat. 34th St & Civic Centre Blvd., Philadelphia Pa. 19104 USA.]

Subarachnoid Hemorrhage in Sickle Cell Disease. J. Carey; Y. Numaguchi; J. Nadell. Child's Nervous System (1990) 6: 47. [Numaguchi Y, Tulane Univ. School Med. Dept. Radiol. 1430 Tulane Avenue, New Orleans L.A. 70112]

Immunological Effects of Zinc Deficiency Sickle Cell ~~disease~~ anemia (SCA). A.S. Prasad; J. Kaplan; G.J. Brewer, M. Davidenne. REDCELL 7th Ann Arbor Conference. Progress in Clinical & Biological Research. Vol 319 1988 Ed. G.J. Brewer Alan R. Liss Inc. (New York) 1989 686 Pages. $160.00

Identification of multiple β-thal mutations by denaturing gradient Gel. Electrophoresis Jl Cl. Inv. (1990) 85: 550 Q. S. P. Cai & Y W Kan [Y W Kan,]

Bone Marrow Transplantation in Pts with thalassemia Guido Lucarelli et al. New Engl. Jl. Med. (1990) 322: 417 [Osped. Pesaro Div. Ematol. 1-61100, Pesaro. Italy]. 23/3/90

• HBV and HIV Seropositivity in Multi-transfused Haemophilics and thalassemics in Eastern India IJMR (1990) 90: 63 M. De; D. Banerjee; S. Chandra; Dr. Bhattacharya [Dr. Bhattacharya, Soc. Res. Haemat. & BW transfns, Gate 4, Flat 20F. Corp. Pl. Calcutta - 700087.]

Assessment of Care of Children c̄ Sickle Cell disease implications for neonatal screening programmes by

• R. I. G. Milne. Brit. Med Jl (1990) 300: 371 [Aylesbury Vale Hlth Author, Dept Publ. Hlth med. Aylesbury Vale HP 19 30X Bucks. England]

Molecular mechanisms associated with increased fetal Hb Gγ-type in a part - Aboriginal family with beta-thal. Pathology (1989) 21: 189.
P.I. Motum; A. Lammi; R. J. Trent.
[P.I. Motum, Univ. of Sydney Clin. Imm. Res. Centr. Mol. Biol. Lab. Sydney. NSW 2006. Australia]

Immological Evaluation of Children with homozygous β-thal. treated with desferrioxamine Ch. P. Speer; M. Gahr; P. Schuff-Werner; W. Schröter. Acta Haemat (1990) 83: 76 [Ch. P.S. Univ. of Gottingen, Depot. Paed. Humboldtalle 38, D-3400 Gottingen. FRG]

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Red Cell allo-antibodies in Patients ō Thalassemia ~~Spanos~~ Spanos Th; Karageorga M; Iadis M; Peristeri J; Hatziliani A; Kattamis Ch. [T. Spanos, Aghia Sophia Paed. Hosp. Centr. Blood GR-11527 GOUDI. Greece].

Sequence analysis of the γ-globin gene locus from a Patient with the deletion form of HPFH.
Catherine Stolle; Laura A. Penny;

Sharlene Ivory; Bernard G. Forget & Edward J Benz Jr. Blood (1990) 75: 499 [E. J. Benz Jr. MD. Dept of Internal Med., Yale Univ. School of Med., 333 Cedar St. New Haven CT. 06510 USA] Pks.

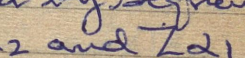
Magnetic Resonance Imaging of Bone Marrow in Sickle Cell Disease: Clinical Hematologic & Pathologic Correlation Vipul N. Mankad; J. Powell Williams; M. D. Harpen; Elizabeth Manci, et al [V. N. Mankad, M.D., 2451 Fillingim St., Mobile, AL 36617 USA]

Clinical features and molecular analysis of acquired HbH disease Higgs DR et al Am. Jl. Med (1983) 75: 181

α/β synthesis ratio extremely low 0.05

Acute lymphoblastic leukemia with brilliant Cresyl blue erythrocyte inclusions — acquired HbH? KUEH, Y. K. New Engl Jl. Med (1982) 307: 193

α-Thalassemia in India: Its interaction with Sickle Cell disease A. Kulozik; B.C. Kar; B.E. Serjeant; G.R. Serjeant + Dyw. Blood (1988) 71: 467.

$-\alpha^{3.5}$ – A further α+ deletion that removes the entire α1 gene and its flanking DNA ($-\alpha^{3.5}$). The break points of this deletion have not yet been examined in detail. Thus Indian have $-\alpha^{3.7}$ rightward deletion that cause α-thal and others with three α genes ($\alpha\alpha\alpha^{anti-3.7}$) These events can be further subdivided depending on exactly where the Z box of the crossing over took place into $-\alpha^{3.7\,I}$, $-\alpha^{3.7\,II}$; $-\alpha^{3.7\,III}$. These regions are defined by sequence differences in the Zα2 and Zα1 boxes that can be detected Southern Blot hybridization. (

) Recombination between homologous X boxes, which are 4.2 Kb apart, also give rise to and-thal determinant ($-\alpha^{4.2}$) and an $\alpha\alpha\alpha^{anti\,4.2}$ Chromosome

Lauer J et al CELL (1980) 20: 119. The Chromosomal arrangement of human α-like globin genes: Sequence homology of α-globin gene deletions
Nicholls, R. Det al CELL (1987) 49: 369

A further α^+ deletion that removes the entire α_1 gene and its flanking DNA1 ($-\alpha^{3.5}$) has been described in two Asian Indian patients in this paper Although the relative expression of the α_2 and α_1 genes on normal Chromosome is approximately 3:1 there are several lines of evidence that indicate that the α gene in the various $-\alpha$ Chromosomes behave like neither α_1 nor α_2 e.g. homozygous $-\alpha^{4.2}$ determinants who essentially have two α_1 genes ($-\alpha^{4.2}/-\alpha^{4.2}$) appear to express more α globin than the predicted 25% of normal (See Higgs et al Blood 1989 Clinical Invs 1989: 79: 39) + homozygote for $\alpha^{3.7}$ III with equivalent of two α_2 genes ($-\alpha^{3.7}/-\alpha^{3.7}$) express less than the predicted 75% of normal

α^0 - that due to deletion - with the exception of $(\alpha\alpha)^{RA}$ mutant (a deletion from the α-Cluster in which both α-gene remains in tact), all of the deletions described in this section either Completely or partially

α-Thal in British People. Br. MJ (1985) 290: 1303
Higgs D Retal.

[$-(\alpha)^{5.2}$ and $-(\alpha)^{20.5}$] delete both α-globin genes, & therefore no α chain Synthesis is directed by this Chr. in vivo Homozygotes for such Chromosome have HB Bart's hydrops fetalis Syndrome. Several deletions remove both α genes and the ζ globin genes; although heterozygote for these variants survive and appear to develop normally (Winichagoon; Higgs and also Wasi P. EMBO 1984: 3: 1813). It is unlikely that homozygotes could survive even the early stages of gestation since neither embryonic ($\zeta_2\gamma_2$; $\zeta_2\varepsilon_2$; $\alpha_2\varepsilon_2$) nor fetal ($\alpha_2\gamma_2$) HB could be made.

Effect of hCG + FSH treatments in young Thai patients with hypogonadotropic hypogonadism R. Balducci, V. Toscano; et al. Jl. of Endocrinological Investigation (1990) 13: 1

[V. Toscano, Univ. of Rome La Sapienza, Ist. Clin. Med. 5, Policlin Umberto 1, 1-00161 Rome]

Hattori, M and Sakaki Y (1986) Dideoxy sequencing method using denatured plasmid templates. Anal Broch 152: 232

Molecular basis of β-thal in Turkey: detection of rare mutations by direct sequencing C. Aulehla-Scholz; S. Basaran; L. Agaoglu; A. Arcasoy W. Holzgrave, P. Miny; F. Ridolfi; J Horst. Hum. Genet (1990) 84: 195 [J. Horst, Univ. Munster, Inst Hum Genetics, Vesaliusweg 12-14; D4400 Munster FRD]

A new Modification of the "Pink Test" for the Δ of here sphero by A. Sureda BALARI, J. Villarrubia Espinosa & J. Fernandez Fuertes. Acta Haemat (1989) 82: 813 [A. Sarada MD, Reina Mercedes, 20 B, E-28020 Madrid. (Spain) See Vettore L. et al Acta Haemat (1984) 72: 258.

The DNA deletions in an Indian δβ-thal begins one kb from Aγ globe gene & ends in an LT repetitive sequence N. Mishima; H. Landman T H Jhusina & J.G. Gilman Brit J Haemat (1989) 73: 375 We have

Wong CD; Dowling CE; Saiki RK; Higuchi RG; Winichagoon P; Fucharoen S (1980) Thal in Thailand. Ann NY Acad Sci 344: 352

Cloned DNA spanning the deletion breakpoint for a new Indian δβ-thal associated with wild anemia. The 5' breakpoint is at 42151 of GenBank file HUMHBB, which is about 1 kb 3' of the Aγ globin gene poly A site at 41003. On the 3' side of the breakpoint, the seq is homologous to LI (KpnI) repetitive DNA located 3.6–10 kb 3' of the globin gene. Indian δβ-thal DNA is 74% homologous to the inverted complement of HUMHBB from 69849 to 70020 followed by a region 78% homologous to the direct direct sequence of HUMHBB from 70534 to 71010.

Summary of restriction endonuclease data for normal and Indian δβ thal DNA following hybridization to γ IVS II probe, see next page

	Normal DNA	Indian S Pb Mo
Enzyme		
Bam H I	4·9, 15·4	4·9; 15·4
~~Bcl~~ Bcl I	17·2	15, 17·2
Bgl II	12·9	10·5; 12·9
Eco R I	2·6; 7·0	2·6; 7·0
Hind H I	3·3; 6·8	3·3, 6·8
Pst I	0·8, 4·0, 4·9	0·8, 4·0, 4·9
Xba I	3·2, 4·9, 7·4	2·4; 3·2; 4·9; 7·4
Xmn I	7·0	7·0

A gene deletion ending within complex array of repeated sequences 3' to the human β-globin gene cluster Henthorn P. S. Mager, D. L; Huisman T H J & Smithies O. PNAS (1986) 83: 5194-98

Lack of Red Cell sensitization in regularly transfused Patients with Thal major C. TSAKONA; E. R. Huehns. Abs. Brit Soc. Haemat Wembley Conf. Cent. London. 10-11-1989.

In sickle cell patients irregularly transfused the no. of antibodies found similar to that of other workers. In patients c̄ thal intermedia also regularly transfused ~~since about the lack of~~

~~of the 1st~~ of life 6 out of 19 pts
a variety of blood group antibodies.
However, in 30 pts of that major,
all regularly transfused since about
later half of the 1st year of life,
only one such antibody has been
detected. This was a weak example
of "Wka" which is sometimes
naturally occurring while White
Cell sensitisation occurred frequently
13 out of 16 pts. tested were positive
in that major group while 4/7
were +ve in that intermediate group
Developmental regulation of human fetal-
to-adult globin gene switching to trans
genic mice by Tariq Enver; Natacha
Raich; Allen J Ebens; Thalia Papayan-
nuponlou; Frank Constantini; George
Stamatoyannopoulos Nature (1990)
Vol 344 March 22, 1990 p. 309 [Dn.
of Med Genet, Dept of Med; Univ. of Washington
Seattle, Washington 98195 USA]
A novel mutation in the TATA box
in a Japanese patient with β^+ that
Takihara Y; Nakamura T; Yamada H;

JBC (1989) 264: 7780-7783 (Incharoensp et al)
A novel ochre mutation in the β-thal gene in Thailand
Identification by direct cloning of the entire β-globin
gene amplified using polymerase chain reaction.

Takagi Y; Fukumaki Y. Blood (1986)
67: 547-550 (DNA)
Analysis of Proto-Oncogenes in
Acute Myeloid Leukemia: Loss of
Heterozygosity for the Ha-ras gene
Blood (1990) 75: 819-822 H. G. Ahuja
A. Foti; D. J. Zhou; M. J. Chine
[HGA; MD, UCLA School of Medicine
Hemat/Onco; 11-151 Factor Building
Los Angeles, CA 90024-1678]
A new HPFH deletion has the break-
points within the 3' β-globin gene
Enhancer Clara CAMASCHELLA;
Anna Serra; Enrico Gottardi; Alda
Alfarano; Donatella Ravello;
Umberto Mazza & Giuseppe Saglio -
Blood (1990) 75: 1000-1005 A new
deletion of the β-globin gene Cluster
has been characterized in 2 Italian
brothers who are heterozygous
Carriers of a Gγ Aγ HPFH HbF% 20
and 16; MCV 81 & 80; MCH 25 & 26
HGA_2% 2.1 & 2.0 α/non α (cpm) 1.5
in one [Clara Camaschella, MD,
Clinica Medica A, Via Genova 3, 10126 TORINO
Italy

Previous papers ① The 3' end of Spanish δβ-thal and Black HPFH 1' and 2 lies within 17 kilobases Blood (1987) 70: 593 ② Molecular Characterization & hematological phenotype of Sicilian δβ thal. Haematologica (1986) 71: 287 The Homozygous State of G to A -177 Ar HPFH Blood (1989) 73: 1999. & Italian Type of deletional HPFH Blood (1986) 68: 646.

Molecular Characterization of a high A_2 β-thal by direct sequencing of a single strand enriched amplified Genomic DNA. Thein SL; Hasketh C; Brown J.M; Anstey Ar. Weatherall. Blood (1989) 73 924.

δ-and θ1-globin gene deletions located on the same Chromosomes. Blood (1989) 73: 429 (Correspondence) S.K. Ballas; Y.J. Fei & THJH also see Two different theta (θ) globin gene deletions observed among black newborn babies Brit Jr. Haemat (1988) 68: 249.

Detection of Sickle Cell anemia mutation by Colony DNA amplification Faried F. Chehab + YW Kan Lancet (1990) Jan 6, V 2 335 #15-17. [F F. Chehab Applied Biosystems Inc. Foster City California - & F F. Dept of Human Genetics M4, Cetus Corporate 1400 53rd Street Emeryville, CA 94608 USA]
A fluorescence assay to detect the mutation in sickle cell anemia has been developed. Oligonucleotide homologous to the normal & the mutant DNA sequence are labeled = fluorescein & rhodamine, respectively, used to prime the polymerase Chain reaction. Amplified DNA products = normal sequence fluoresce green, where as those with the mutant sequence fluoresce red; the results can be read by eye & the procedure could be automated. Unincorporated primers separate from amplified DNA: Green fluorescence is diagnostic of AA genotype; red for SS and yellow for AS.

Clinical Uses of Intracellular Immunization
Samuel A. Berkman; Martin L. Lee; Robert Peter Gale. Seminars in Hematol (1988) 25: pp40-158 [Samuel A. Berkman MD c/o Robert Gale MD PhD. Dept. of Med. Hematol/Onco. UCLA School of Med. Los Angeles CA. 90024]

Myb DNA binding inhibited by Phosphorylation at a site deleted during oncogenic activation by Bernhard Lüscher; Erik Christenson; David W. Litchfield; Edwin G. Krebs & Robert N. Eisenman Nature (1990) 344: 517 [Bernhard Lüscher, Dr. of Basic Sciences, Fred Hutchinson Cancer Res. Center, 1124 Columbia St. at, Seattle, Washington 98104 USA

Polymerase Chain Reaction (PCR) amplification with a Single Specific Primer
Miklos Kalman; Evan T. Kalman & Michael Cashel. Biochem & Biophys Res Comm (1990) 167: 504 [M.K. Laboratory for Mol. Genetics, National Inst of Child Health & Human Development, National Institutes of Health. Bethesda MD 20892

Genotype analysis of amplified DNA by dot blot hybridization: Amplified DNA (5 μl) was denatured c̄ 0.2N NaOH at room temp for 10 mts & then neutralized to 1M ammonium acetate before dot blotting on to Zeta probe nylon membrane (Bio Rad) using Bio-Dot™ apparatus (Bio Rad). After baking at 80°C for 1 hr, the membrane was pre hybridized at 42°C

Effect of hydroxyurea on fetal Hb in myeloproliferative & myelodysplastic syndromes S H Advani, P. Venugopal, B. S. Charak, A. Dees, A. T. Mazumbar, R. Gopal, S. Kundekar, V. Lata & R. J. Dash. 1 JMR (1990) [B] 92: 83

A Spontaneous mutation produced a Novel Elongated β-globin Chain Structural Variant (Hb Agnana) with a thal-like phenotype. M. S. Ristaldi et al Blood (1990) 75: 1379 [MSR, CNR INST. RICERCA Talassemie & Anemie, Mediterranee, Cagliari Italy]

A Hb with two sub-mutations in the β-Chain Y. OHBA; M Ami; K. Imai; K. Komatsu; K. Amatsu. Hemoglob (1989) 13: 753 [Y. OHBA, Yamaguchi Univ. School of Med, Dept. Clin. Lab Sci 1144 KOGUSHI, UBE. Yamaguchi 755, Japan]

Hb Chandigarh or α, β 94 (FGI) Asp-Gly observed in an Indian Family S. Dash; J B Wilson; B B Webber

Hb J Paris α

for 30 mts in 3 X SSPE (0.54 M NaCl; 30 mM ~~Na3H~~ NaH_2PO_4 (pH 7.4) 3 mM EDTA (pH 7.4); 5 X Denhardt's solution (0.1% bovine serum albumin, 0.1% PVP; 0.1% Ficoll); 0.5% SDS and 1 mgm/ml herring sperm DNA. Specific oligo nucleotide probe 5' end labeled c̄ 32P-ATP were then added to the same buffer & hybridization performed at 42°C for 1 hr. The membrane was removed under twice with 2 X SSPE, 0.1% SDS at Room temp & washing once c̄ same buffer at desired temp for 10 mts. Membrane was then exposed to x-ray film, per 3 hrs to 1 mg/wt at -70°C using intensive screens

A. Kutlar & THJH. Hemoglobin (1989) 13: 749

Molecular Characterization of β-thal in 174 Greek Patients c̄ Thal major Brit J Haemat (1990) 74: 342. C. Kattamis, H. Hu, G. Cheng; A. L. Reese, J.M. Gonzalez-redondo; A. Kutlar, F. Kutlar. THJH. ~~Brit J Haemat (199~~ (THJH)

The Role of Membrane Skeletal-Associated-globin in the Pathophysiology of Beta-thal. S. Sorensen; E. Rubin; H. Polster; N. Mohandas; S. Schrier. Blood (1990) 75: 1333 [S. SCHREIR, Stanford Univ. Med. Centre Dr. Haemat. S-161, 300 Pasteur Dr.; Stanford CA. 94305, USA.

The Prevalence of Avascular Necrosis in Sickle Marrow: Correlation with α-thal S.K. Ballas CA. Talacki. V.M. Rao, R.M. Steiner. Euro Biopt Jr. (1990) 18: 649 [SKB. Thomas Jefferson Univ., Cardeza Foundation, Hemat Res. Dept. Med. Philadelphia. Pa-19107 USA.

→ for strand separation, before annealing for 2 mts at room temperature. Five units of Taq polymers (Stratagene) were added & preparation incubated at 72°C for 2 mts. The cycle of denaturation at 94°C for 1 mte, annealing at 55°C for 1 mte & extension at 72°C for 2 mts was carried out on DNA Thermal Cycler (Per. El. Cor.) for 30 cycles. When using PCR & Primers G9 & G10, ten units of Taq pl.

Comparison of homozygous Sickle Cell disease in Northern Greece & Jamaica

J. Christakis; N. Vavasti; H. Hassapopoulos; M. Papadopoulou; K. Mandravchi; D. Loukopoulos; J.S. Morris; B.E. Serjeant; G.R. Serjeant. Lancet (1990) 335: 637 [Christakis J. ARISTOTELIAN Univ. Salonika, Ahepa Gen. Hosp. Dept. Med-1, Div. Haemat. SALONIKA Greece]

Zinc and some Zinc dependent Enzymes in Sickle cell anemia A. I. ALAYASH, International J. for Vit. & Nutrition Research (1989) 59: 388 [Duke Univ. Ctr. Marine Biomed. Beaufort. NC-28516 USA]

Latent Cytomegalovirus (CMV) infection affect immunoglobulin levels in pts with thal major. A. Germenis; C. Politis. Haematologica (1990) 75: 42 [A. Germenis Hellen Red Cross Thal Unit, 4. Alkiviadou Str. GR-10439 Athens Greece]

Abn. von Willebrand Factor in pts with HGE - β° thal Peter Benson, Loann. C. Peterson; et al. Ann. J. Cl. Path. (1990) 93: 395

Thermal Cycler (Perkin-Elmer-Cetus Instruments). Genomic DNA (1 μgm) mixed with 50 pmoles of primer; 200 μM each of dNTP in 100 μl reaction ~~mixture~~ volume containing 10 mM TRIS/HCl (pH 8.3); 50 mM KCl; 1.5 mM $MgCl_2$ and 0.01% gelatin. This mixture is incubated at 94°C for 7 min →

The types of Hb and globin Chains in Hydrops Fetalis: F. Kutlar; A. L. Reese; Y. E. Hsia; K. M. Kleman & T. H. J. Ti. Hemoglobin (1989) 13: 671

L. Thalassemia S. A. LIEBHABER. Intro Review Hemoglobin (1990) 13: 685 [Univ. Pennsylvania School of Med. Howard Hughes Med Inst. Philadelphia. Pa. 19104 USA]

Iron deficiency anemia: Adverse effects on Infant Psychomotor Development by Torres Walter; Isidora De Andraca, Patricia Chadud; Carmen G. Perales. Pediatrics (1989) Pediatrics Ind. Edn. [T. W. Instituto de Nutricion y Tecnologia de los Alimentos, CASILLA 15138. Santiago-11, Chile]

Hematology was done by Coulter model IBI (Coulter Electronics) free erythrocyte protoporphyrin, in a hematofluorimeter (Aviv Biomedical Inc. Lakewood NJ)

Serum Iron + Iron Binding by Colorimeter (Fisher D.S.; Price DC, A simple Serum Iron method using the new sensitive Chromogen Tripyridil-s-triazine. Cl. Chem (1964) 10-12) Serum Ferritin by radio-

4% NuSieve TM GTG agarose gel (FMC Bio. Products) in 12% polyacrylamide

in vivo assay.

A mouse model for Sickle Cell disease by A. Kahn. M/S - médicine Sciences (1990) 6: 314 [No address]

Thalassemia Pts are at high Risk for transmission-transmitted Cytomegalovirus A. GERMENIS & C. Politis Acta Haemat (1989) 82: 57 [A. GERMANIS, MD; Thalassemia Unit, Hellenic Red Cross, 4 ALKIVIADOU STR, GR-10439 Athens Greece] Eg Prevalence of Cytomegalovirus (CMV) antibodies in regular blood donors in Greece (in Press)

Arrangement of d-globin gene Cluster in Taiwan HONG-WEN PENG et al Acta Haemat (1989) 82: 64-68 [Dept of Haematology, Dept of Med. Veterans General Hc, TAIPEI Taiwan (Rep. of China)]

In a gene mapping study of 217 newborn babies in Taiwan a d- and ζ-globin positive we have observed 4 cases (1.84%) of d-thal-2 heterozygotes (ζζ-2/ζζdd) with concomitant increased levels of Hb Barts in the cord blood. Eleven subjects (5.07%) were found to have the SEA d-thal haplotype (ζζ-^{SEA}/ζζdd) with increased Barts levels ranging from 2.2-9%

One case of the Bart's level was 14% in the cord blood, was found to have genotype of ζζ--SEA/ζζαα^{T} (0.46%). Four heterozygotes (1.84%) were found with a triple α gene anti-rightward arrangement (ζζααα$^{3.7}$/ζζαα). 21 heterozygotes (9.68%) were found to have a triple ζ globin gene arrangement (ζζζαα/ζζαα). A new triple ζ-globin gene variant with Bam HI polymorphism was also observed in this study.

The normal α-globin gene cluster, located on the short arm of Chr. 16, is arranged in an order of ~~5'ζ2~~ 5'ζ2-ψζ1-ψα2-ψα1-α2-α1-θ1-3'. Located within ~~region~~ gene cluster are two hypervariable regions (HVRs) situated between the ζ2 and ψζ1 genes (designated interzeta HVR) and the 3' region to the θ gene (designated 3' HVR). Recombination events involving functional α-globin genes often result in different genotypes of α-thal, triple α and quadruple α globin genes.

Sickle cell haemoglobinopathies in Sicily

G. Schiliro; M. Spera; E. Giamballuca; M. Maggio

Am. J. Haemat (1990) 33: 81-85 [Prof Gino Schiliro, Chairman, Chair of Ped Hemat; Univ. ~~Catania~~ Catania ~~V. le A. Doria~~ Doria 6, 95125 Catania Italy

Measurement of DNA Concentration: Determine abs. at 260 nm assuming that A1 percent 1cm 260 nm = 200 (i.e. 1 gm/100 ml of DNA Conc. is too dilute it can be precipitated with EtOH) or freeze dried + redissolve in smaller vol

Molecular basis of β-thalassemia in Thailand: analysis of β-thalassemia mutations using the polymerase chain reaction. Supan Fucharoen, Goonpan Fucharoen et al & Y. Fukumaki Human Genet (1989) 84: 41-46.

Impact of public health on human genetics B. Modell and A.M. Kulier. Cl. Genetics (1989) 35: 286-298 (Dr. Bernadette Modell, Perinatal Centre, Dept. Obstetrics & Gynaecology, Univ. College + Middlesex School of Medicine, 86-96 Chenies Mews London WC1E 6HX. London England)

Partial Splenectomy in homozygous β-thal M. de Montalembert & R. Girot Arch. Dis in Childhood (1990) 65: 304 [M. de Montalembert — Reprint R. Girot, Hop Necker Enfants Malad. Hematol. Lab. Rue de Sevres, F-75730, Paris — 15 France]

Assessment of Care of Children with Sickle cell disease Carol Barton. Brit med Jl. (1990) 300: 6727 [C. Barton Royal Berkshire Hosp. Haematol, Reading RG1 5AN Berks Engl]

Aetiology of iceric Hepatitis + fulminant Hepatic failure in Children and the possible Predisposition to Hepatic failure by Sickle Cell Disease by Yohannan M.D., Arif M. Raina Acta Paed. Scand (1990) 79 (2) 201 [Rania S. King Saud Univ Coll. Med. Dept of Path. 32, POB 2925, Riyadh. 11461 Saudi Arabia]

Intramuscular Prioxicam Treatment of Painful Articular Attack in Sickle Cell Disease. A. Sangare, J.P. Durbec + J.F. Sauniere Current Therapeutic Research (1990) 47: 597. [Sangare A., COCODY Hosp. Univ. Centre, Dept of Heart. Abidjan, Cote Ivoire].

The Wave of Advance of an Advantageous Gene: The Sickle Cell Gene in Liberia. Human Biol (1959) 61: 823/831 Update Frank B. Livingstone - Update 831 [Frank Livingstone - Univ of Michigan Dept Anthro. Ann Arbor MI. 48109. USA]

HIV associated oesophageal Ulcers treated with thalidomide Paul. R. Georghiou, Richard J. Kemp. Med J. Australia (1990) 152 382.

Maternal serum Screening for Down's Syndrome in early pregnancy Wald N.J., Cuckle H.S., Densem J.W., Nanchahal K., Royston P., Chard T., & Canick J.A. Brit med Journal (1988) 297: 883-87

The Haemoglobinopathies in Europe. Combined report on two WHO meetings (Brussels 14 March '86 and Paris 20-21 March 1987). Off Set publication EUR/ICP/MCH 110. Request to WHO Regional Office for Europe, Copenhagen Denmark.

Community approaches to the Control of Hereditary disease Report of a WHO Advisory Group. WHO Unpublished Document HDP/WG/85.10. Obtainable from the Hereditary Disease Programme, WHO, Geneva, Switz.

Impact of public Health on Human Genetics B. Modell & A.M. Kulkier. Ch. Genet. (1989) 35: 286-298. [B. B. Modell. Perinatal Centre, Dept. Obst & Gyne. Univ. College & Middlesex School of Med. 86-96 Chenies Mews, London WC1E 6HX. London.]

Coffee Consumption and death from Coronary heart disease in middle aged Norwegian

men women Aage Tverdal et al.
B MJ (1990) March 3, p 566. Conclu
Coffee may affect mortality from coro-
nary heart disease over and above
its effect in raising Cholesterol Concen-
trations.

Malaria and the Red Cells. L. Luzzatto.
Recent Adv. in Haematology. Ed: A.V. Hoffbrand
Churchill Livingstone (1985) page 107-226 London. HbAS
Sickle more readily when they are parasitized
than they are not: One likely mechanism is that
the parasite's metabolism lowers intracrythro-
cytic pH which favours sickling. AS cells in-
fected in vitro c̄ P. falciparum is indeed phago-
cytosed by autologous peripheral blood mona-
cytes - parasitized sickled cells becomes an easy
~~prey~~ prey for macrophages. The fact that
malaria can be extremely severe and even lethal
in SS homozygotes, is one argument in favour
of parasite removal being mediated by phago
cytosis, rather than caused directly by sickling.
Indeed, if sickling per se was sufficient,
killing of parasites ought to be more effective
in homozygous red cell, which certainly sickle
more rapidly. On the other hand, if killing

requires macrophage activity we can easily visualize that this may be inadequate in SS homozygotes where removal of parasitized cells will compete with that of ordinary irreversibly sickled cells, & the spleen is often functionally impaired. The fact that patients with Sickle Cell disease – as opposed to AS heterozygotes – are not malaria resistant must always be remembered as an important point in clinical practice.

Protection against malaria in heterozygotes (HbCC) has been suggested. In vitro, there is reduced growth of P. falciparum in HbCC red cells, but not from AC heterozygotes. (Pasvol & Wilson 1982). For HbE in vitro culture studies have been conflicting. Since it is now clear, from pathophysiological and molecular point of view, that HbE is a form of β^+ thalassaemia (Orkin 1982 Nature 300:768-69). Although that is a genetic disorder for which the notion of a malaria-related genetic polymorphism was first postulated (Haldane 1949 – Disease & Evolution Ricerca Scientifica 19(Suppl. 1):3-13) Studies of in vitro cultures have so far been of limited value in explaining the mechanism. However, the case for malaria selection has been greatly strengthened, in the writer's opinion, by the impressive extent of heterogeneity of the polymorphic

thalassaemia genes that has become apparent in the last 20 years. It is inconceivable that so many independently arisen mutant genes, each one causing severe or total loss of reproductive fitness in the homozygous state, could have increased the frequency in many populations in the absence of a selective agent; and from geographical considerations malaria remains the one and only obvious candidate (this also becomes evident with high frequency of α-thal genes in Papua New Guinea. Oppenheimer et al 1984 Lancet i: 424)

G6PD deficiency, the single genetic abnormality of red cells has the highest prevalence on a World-wide scale. Evidence for malaria selection (Luzzatto & Battistuzzi 1984 G6PD In: Harris H, Hirschhorn K (Ed) Advances in Human Genetics vol 14 pp 217-329. Luzzatto L. (1979) Genetic of red cells & susceptibility to malaria Blood 54: 961) is based on geographical and microgeographical evidence on field studies of clinical malaria; on the distribution of parasites in normal and G6PD-deficient red cells of heterozygote females who are genetic mosaics

Fucharoen S.P. Fucharoen G; Fucharoen P. Fukumaki Y (1989) A novel Ochre mutation in the β-thal gene of a Thai: Identification by direct Cloning of the entire β-gene amplified using polymerase C.R. JBC (1989) 264: 7780-83 as a result of X-Chromosome inactivation and our results of in vitro Culture Studies (Friedman 1979 Nature 280: 245). The mechanism whereby the G6PD deficient cell environment affects adversely the parasite might be related to glutathione metabolism (see Eckman & Eaton 1979 Nature 278 753-754 and Clark & Hunt (1983) Infection & Immunity 39: 1-6). Finding that isouramil, a substance contained glycosylated form of fava beans inhibit P. falciparum under experimental conditions (Golan-Sever et al 1983 Blood 61: 507). However two important points deserve attention. It is necessary to rule out possible artifacts consequent on storage lesions of G6PD-deficient red cells in vitro. Secondly, poor development of P. fal. in G6PD deficient cells cannot per se explain balanced polymorphism since in vivo protection is a prerogative of (G6PD) def. heterozygote females and not of G6PD def hemizygote males (Brinzle et al, U. cited -- Luzzatto 1981 Ann. of Epidemiology 10: 9-15) It was shown recently that compared to normal the G6PD-def red cells become just as competent to support growth of parasite if the latter has already carried out several schizonic cycles in them (Luzzatto et al 1983 CIBA foundation Symposium New No. 94 Pitman, London p 159-173)

This finding is elementals Storage lesions. At the same time it demonstrates an adaptive phenomenon taking place in the parasite which suggests a possible explanation of paradox whereby in vivo heterozygotes are relative resistant against P. falciparum malaria whereas hemizygotes are not. Indeed, in a hemizygote male (or in a homozygote female) with only G6PD-defined cells adaptation with regards take place and parasitemia may reach dangerous levels just as in normal person i.e. there is no protection. On the other hand in a heterozygote female in whom normal and G6PD-defined cells co-exist, a parasite finds itself having, at each new invasion event, approximately even chance to enter a normal or deficient cell, thus decreasing the chance of adaptation. In summary G6PD-deficient heterozygotes have been shown to have a measure of protection against life-threatening levels of P. falciparum parasitaemia. The impairment of growth in G6PD-deficient erythrocytes must be related to the metabolic characteristics of these cells. However, the mechanism of resistance must include some other factor, which is prerogative of heterozygotes. This factor is likely to be their unique mosaicism which interfere with adaptation of the parasite to the G6PD

~~GGPD~~ Report of WHO working group on GGPD def (GGPD) genes. 3-4 Sept 1985. Document WHO/HDP/WG GGPD/85.9 annex 2, p. 37

Rec'd 2/10/90

deficient intracerythrocytic environment. It is clear from the data ~~summarized~~ summarised, that malaria, through its tremendous selective force has contributed a great deal to shape the genetic structure of many human populations. On the other hand, it is equally important to remember that no single gene or combination of genes has yet been found to confer absolute resistance to P. falciparum. (By contrast, Duffy-negative red cells do have total resistance to P. vivax - Miller et al 1975 Science 189, 561) In view of a situation in which resistance to chloroquine + other drugs is ~~gra~~ and combination is becoming popular. But it is obvious that the hope of a vaccine (Cohen 1982 Brit Med Bull 38; 161-65) now stands as an imperative priority. Advances have been ~~dra~~ drawing from the available recombinant DNA technology. A genomic library of P. falciparum in lambda phage was first constructed (Gorman et al 1982 Mol. Biochem. Parasitology 5; 391-400) + can be used for searching for any plasmodial gene. A P. fal. cDNA library from parasites grown in vitro was constructed in the expression vector λgt11 (Kemp et al 1983 PNAS 80; 3787-91)

(See also Mazzer et al 1984 Nature 307: 367. Enact al 1984 Science 225: 628-630 Konen et al 1984 Nature 311: 382-384; Th Coppola et al Nature 1984 310: 789-792).

Treatment of pain in Adults with Sickle Cell disease by Samir K. Ballas Am. J. Haemat (1990) 34: 49 [S.K.B, Thomas Jefferson Univ. Jefferson Med College, Cardeza Fdn. Hemat. Res. Dept of Med. 1015 Walnut Street, Philadelphia Pa. 19107 USA]

Gene Transfer into Hematopoietic Cells E. Bayever Blood (1990) 75: 1587 [Univ. Penn. Childrens Hc, Philadelphia, Dept. Ped. Dn. of oncology, Philadelphia Pa. 19104 USA]

— Response: P.F.D. Hughes, C. J. Eaves; D.E. Hogge, R.K. Humphreys. Blood (1990) 75: p. 1587 [P.F.D.H. British Columbia Can. Res. Cntre. Vancouver B.C. Canada V5Z 1L3]

High incidence of Cholelithiasis in older patients with Homozygous β-thal Goldfarb A - E.A. Rachmilewitz E. Acta Haemat (1990) 83: 120 [B.E.A. Rachmilewitz E. Hadassah Univ. Hosp. Dept. Hemat. PO B 12000, IL-91120 Jerusalem. Israel]

Selenium & Glutathione ~~[illegible]~~ Peroxidase levels in Sickle Cell Anemia by Natta CL; Chen LC; Chow CK (New York/Lexington UK) Acta Haemat (1990) 83: 130 [CL Natta, Columbia Univ Coll. Phys & Surg. Dept Med 630 W 168th St. New York. NY. 10032 USA]

A common Chinese mutation found in a Japanese family Hum. Genet (1990) 84: 480 by Y. Naritomi, H. Nakashima, M. Kagiyuoto; Y. Naito; E. Yokota, T. Imamura Hum. Genet (1990) 84: 480. [T. Imamura, National Inst. Genet, IIII YATA, MISHIMA SHIZUOKA 411, Japan]

Retiarlocyte Count by K.G. Badami Nat. Med. Jl. of India (1990) 3: 69-72 [All Ind Inst Med Sc. New Delhi Dept. Haemat]

The CT substitution in the Distal CACCC box of the beta ~~gene~~ globin gene Promoter is a common Cause of Silent β-thal in the Italian Population M.S. Ristaldi; S. Murru; G. Loudianos; L. Casula; S. Porcu; D. Pigheddu; B. Fanni; G.V. Sciarratta; S. Agosti; M.I. Parodi et al Brit J Haemat (1990) 74: 480 [Cao A. Uni Cagliari, 1st Chin & Bio Eta ~~Chin~~ Evolut via Jenner Sn. 1-09100 Cagliari]

Unrestricted growth of Plasmodium Falciparum in Microcytic Erythrocytes in Iron Def and Thal: G. A. Luzzi, M. Torii M. Aikawa; G. Pasvol. Brit J Haemat (1990) 74: 519 [LUZZI, John Radcliffe Hosp. Inst. Mole. Med, Oxford Ox 3 9DU, England]

Deletion Delta-thal – The 7.2 Kb Deletion of Corfu Delta-beta-thal in a Non-beta thalassemia Chromosome M. A. MELIS; A. Podda; M. Monne, L. Perseu; G. Loudianos; A. Cao. M. Piriastu; A. Piga Blood (1990) 75: 1747.

Pulmonary embolism and Splenic Infarction in a fat with Sickcell trait Jeremy Sugarman et al Am J Haemat (1990) 33: 279–281 [Box 3448, Duke Univ Med Centre, Durham NC 27710]

Parkinson's Disease by CD Marsden. Lancet (1990) April 21, p. 948–952 [Univ Dept of Cl. Neurology, The National HC for Nervous Diseases, Queen Square, London WC1N 3BG. (CDM, FRS)]

Use of Gen-Probe and Bactec for Rapid Isolation & Identification of Mycobacteria
Correlation of Probe results with growth index. Barbara A. Body; Nancy G. Mann et al [Dr. Body Roche Biomedical Labs Inc. 1427 York Court, Burlington N.C. 27215
Am. J. Clin. Path. (1990) 93: No. 3: p 415
See also. G.D. Robert et al. J. Clin. micro. (1983) 18: 689
A novel Ochre Mutation in β-thal gene of a Thai, Supan Fucharoen; Goonnappa Fucharoen; Pronee Fucharoen and Yasuyuki Fukumaki J. Biol. Chem (1989) Communicat 264: 7780-7783 Identification by Direct Cloning of entire β-globin amplified using Polymerase Chain Reaction.
Differential Sensitivity of peripheral lymphocytes of untreated Leprosy pts to mitomycin C. Doris D'Souza; B.C. Das; M.V. Thomas. Mutation Res. (1990) 240: 101-107 [Dr. D. D'Souza, Patna Women's College, Avila Convent Bailey Road, Patna 800001 Bihar India] See also Lepr. Review (1988) 59: 121

Mutagenesis (1988) 79: 83-85
Genotoxicity of Chromium Compounds
A review. Silvia de Flora,
Maria Bagnasco, Dominizio Serra,
and Patrizia Zanacchi. Mutation
Res. (1990) 238: 100-164. [Dr. S. De
Flora, Institute of Hygiene & Pre-
ventive Med. Univ. Genoa, 1-16132
GENOA, Italy] (APK)
Identification of Deletion and Triple
Alpha-globin gene haplotypes with
Montreal Beta-Thal Screening Prog-
Implications for Genetic Medicine
B.R. Akerman, T.M. Fujiwara,
G.A. Lancaster, K. Morgan, C.R.
Scriver Am J. Hum Genet (1990)
36: 76-85 [C.R. Scriver, McGill
Univ. Montreal Children's Hospital
Res. Inst. Dept. Pathology, Montreal,
Quebec. Canada H3H 1P3]
The Possibility of HIV ~~Transfusion~~
Transmission via anti-HIV negative
blood in polytransfused Beta Thal
patients in Northern Thailand. by
Chanarat P., Thongkrajai P.;

β-thalassaemia trait in the Punjab (North India) Dash S. Brit J. Haemat (1985) 61: 185

Kulapongs P., Vox Sanguinis (1990) 58: 224 [Chanarat P., Chiang Mai Univ. Fac. Assoc Med. Sci, Dept. of Clinical Microscopy, CHIANMAI 500002, Thailand]

Expression of Cell-Surface transferrin receptor following in vitro stimulation of peripheral blood lymphocytes in patients with β-thal and non-deficiency K. PATTANAPANYASAT. Europ. J. Haemat (1990) 44: 190 [Mahidol Univ. Siriraj HC, Thalassemia Centre, Div. of Hematology, Bangkok 10700]

Clinical Diversity in Sickle Cell anemia Sinasi Ozsoylu. Lancet (1990) 335: 1035 [S. OZSOYLU, Hacettepe Children's Hosp, Med Centre, Dept. of Ped & Hemato. Ankara 06100, Turkey]

Sickle Cell anemia Trial M. H GASTON & J. Verter. Statistics in Med (1990) 9: 45 [M. H. G. CSIC CAJAL INST. Dr. ARCE 37, 6-28002, Madrid, Spain]

Thalassemia in the Maldives Bernadette Modell & others. Lancet (1990) 335: 1169 [Modell B.,

Univ. Coll. & Middlesex Hosp. School of Medicine, Dept. Obs. & Gynec. London. WC-1. Engl.]
~~Pulmonary~~ Pulmonary Syndrome in Patients with Viral-induced Reactive D.V. Desferrioxamine Infusion.
Ann. Fl. Dis of Childre (1990) 144 565. Melvin H. Freedman, MD FRCP Dan Lybisarn MD [M.H.F. Univ. of Toronto, Hosp. Sick Childre, Dept of Pediatrics, Dr. Haenel. Oncol. 555 Univ Ave, Toronto, Ontario Canada M5G 1X8.]
Intradermal Hepatitis B Vaccinal in Thalassemia. S. Leonardi; T. Reggio; A. Sciacca et al. Arch Dis Child (1990) 65: 527 [Musu-Meci, S, Univ. of Catana, Dept of Ped. Viale Andrea Doria 6, 1-95100. Catana, Italy]
Acetabular Protrusion in Thal: A report of 4 Cases. Panagiotis G. Korovessis et al Clinical Orth. and Related Research (1990) 254: 199 [PGK. Haralabi, 65-67 Str.

Southern blotting, 32P labelling and autoradiography account for about 60% of the total ~~cost~~ material cost of approximately £250 for a few Stnd (Old et al 1986) But Taq polymerase (US$0.70/unit) and oligonucleotides (Commercial available at (US$15.00 per base and $260 Unit) must total £10.00 per sample.

G R - 26244, Patras, Greece]

Hemoglobin Birmingham and Hb Galicia: Two unstable β-Chain Variants Characterized by Small Deletion and Insertion

J.B. Wilson; B.B. Webber; H. Hu; A. Kutlar; F. Kutlar; J.F. Cordington; J.T. Prachal; K.M. Hall; J. Mª de Pablos; J. Rodriguez & THJH. Blood (1990) 75: 1883-1887 [THJ]

β-Chain Variant Hb Birmingham found in a black man, is characterized by the loss of - Leu-Ala-~~Lys~~ His; Lys at positions 141, 142, 143 & 144 and their replacement by one Gln residue. These changes are the result of a deletion of nine nucleotides, namely two base pairs (bp) at Codon 141 all of Codons 142 & 143 and one bp of Codon 144. The remaining CAG triplet (C from Codon 141 and AG from Codon 144) Codes for the inserted ~~glut~~ ~~thing~~ glutamine, In the β chain of Hb Galicia from a Spanish patient, His and Val at positions 97 and 98

amplipriment (oligonucleotides) were synthesized, on a "BIOSEARCH CYCLONE DNA SYNTHESIZER" "Control represents Hae III cleaved ΦX174 size master track"

are replaced by one Leu residue. This is due to an ACG deletion in Codons 97 and 98 which causes the removal of one His and one Val residue while the remaining CTG triplet (C from Codon 97 and TG from Cod on 98) Codes for the inserted leucine residue. The two mechanism namely Slipped mispairing in the presence of of short repeats, and misreading by DNA polymerase due to a local distortion of the ~~DNA~~ DNA helix, are considered in explaining the origin of the small deletions

Serum Transferrin Receptor: A Quantitative Measure of Tissue Iron deficiency Barry S. Skikne; Carol H. Flowers, James D. Cook Blood (1990) 75: 1870-76 [B.S.S Dr. Haemat. Kansas Univ. Med Centre 39th and Rainbow, Kansas City K.S. 66103 also Serum transferrin receptor as a new index to erythropoiesis Blood (1987) 70: 1955; Kohgo, Y, Niitsu, Y; Kondo H, Kato J; Tsushima N; Sasaki K. et al

Oligonucleotides synthesized purified by High pressure Liquid Chromatography using AM-314, ODS, YMC packed Cohn (Yamamoto Chemical Laboratory)
Centricon 30 - microconcentrator (Amicon Corp)

Fetal Calf Serum contains Activities That induce Fetal Hb. in adult Erythroid Cell Cultures by P. Constantoulakis; B. Nakomoto; Th. Papayanopoulou; G. Stamatoyannopoulos Blood (1990) 75: 1862-69 [G S-MD. D. Sci, Prof of Medicine, Medical Genetics, RG-25, Univ. of Washington, Seattle. WA 98195]

A novel Ochre Mutation in the β-thal Gene of a Thai: Identification by direct Cloning of the entire β-globin gene amplified using Polymerase Chain Reaction by Supan Fucharoen; Goonappa Fucharoen; Prance Fucharoen & Yasuyuki Fukumaki JBC (1989) 264: 7760-7763 [Y.F.] The β globin genes from a Thai patient Compound heterozygote for β-thal and HbE disease were investigated. The 3.0 kb fragment containing the entire β-globin gene was amplified by PCR, using Taq DNA polymerase followed by direct Cloning of the amplified product into plasmid DNA Sequence

Taq DNA polymerase from Stratagene
Oligonucleotide Synthesis - Applied Biosystems
- model 38A - DNA synthesizer.
Polymerase Chain Reaction - Perkin - Elmer Cetus Instrument

analysis of the Hind gene revealed only
base change, a C-A transversion in the
Codon for an amino acid 35. This new
mutation creates a premature termination
TAA, an ochre Codon, and results in a β°-
thalassaemia phenotype. The same result
was obtained when this mutation was ana-
lyzed using a conventional Cloning technique,
dideoxy-sequencing of the amplified products,
and hybridization with allele specific
Oligonucleotide probes. No mis-incorporation
was detected in the sequence analysis
of the 3.0 Kb insert of five Clones of the
amplified products obtained from genomic
DNA of a normal individual. This approach
is a rapid and accurate method for mole-
cular Cloning of the β-globin gene and
also the gene, the partial nucleotide sequ-
ences of which are unknown.

Oligonucleotides were synthesized on an
Applied Biosystems model 38A DNA Synthes-
izer and purified by high pressure liquid
Chromatography using an AM-314 ODS
YMC packed Column (Yamamoto Chemical
Laboratories Co.). Deoxyribonucleotides, dideoxy

ribonucleotides, [α-^{32}P] ATP and [γ-^{32}P] ATP were purchased from Amersham Corp, T4 polynucleotide Kinase, T4 DNA ligase and restriction enzymes were from Takara Shuzo Co; Taq DNA polymerase from Stratagene. (Saiki et al Science 1988, 239: 487-491).

Polymerase Chain Reaction: Direct Cloning of the Entire β-globin gene and Direct Sequencing: One μgm of genomic DNA was amplified in vitro by the PCR using Taq DNA polymerase and primers G9 and G10. Primer G9, 5' TCCCCAGTTAACCT CCTATT 3' located at positions -823 to -804 from cap site is complementary to the antisense strand, and a primer G10, 5' AGACTAGCACTG CAGATTCC 3' is complementary to the sense strand of the region from 550 to 569 bp to the poly(A) site of the β-globin gene. The size of the region to be amplified is 2998 bp. The restriction enzyme site for HincII and PstI are located within G9 and G10 respectively.

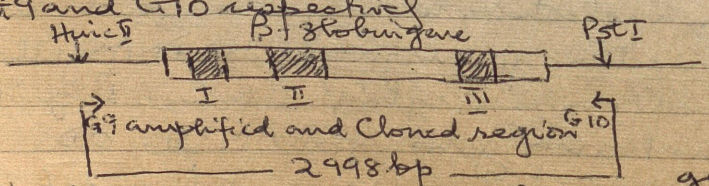
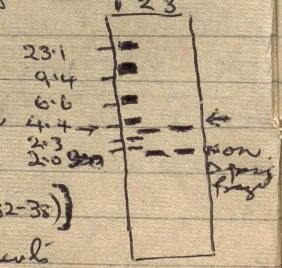


Fig. Schematic diagram representing β-globin gene region and two amplification primers, G9 & G10. Sequence

described above. Horizontal arrows indicate locations and directions of primers. Vertical arrows indicate restriction sites for HincII and PstI. Exons I, II & III respectively.

One of genomic DNA was mixed with 30 pmol of each primer, 200 μ mol of dNTP in a 100 μl reaction containing 10 mM TRIS-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 0.01% gelatin. The mixture is incubated at 94°C for 7 mins for A strand separation before annealing for 2 mins at room temperature. Ten units of Taq DNA polymerase (Stratagene) were added and the preparation incubated at 72°C for 7 mins. The polymerase chain reaction was carried out on a DNA Thermal Cycler (Perkin-Elmer Cetus Instruments). Each cycle of PCR consisted of incubation at 94°C for 1 min, 2 mins of incubation at 54°C and 3 mins of incubation at 72°C. After 30 cycles of PCR, the amplified DNA was desalted and dNTPs eliminated using a Centricon 30 micro-concentrator (Amicon Corporation). An aliquot of the amplified products was then double digested with HincII and PstI. The entire β globin gene fragment of approximately 3.0 Kb was purified from the gel before being ligated to pUC 13. Transformation was done under standard conditions using Escherichia

Coli JM83 as the host (Maniatis et al Lab Manual). Clone for the β-globin gene were easily screened by digestion of the plasmid DNA extracted from the white colony with BamHI which generates the fragments of 1.3 Kb [Agarose gel electrophoresis of amplified products: 3 Cul of amplified DNA was run on 0.7% agarose gel and visualized with Ethidium bromide staining (0.5 μgm/ml). Lambda phage DNA digested with HindIII is used as size reference. Lane 1; Lane 2 and 3 represent PCR-amplified genomic DNA of the normal individual and the pt with β-Thal-HbE. Arrow indicates the 3.0 Kb target fragment containing the entire 3.0 Kb target fragment Cont the entire β-globin gene]



→ Nucleotide sequence of the cloned fragment was determined by the dideoxy (chain termination method [Hattori M, and Sakaki Y Anal Biochem (1986) 152: 232-38)]

Direct sequencing of amplified fragment was performed by as described (Wong C --- Kazazian HH Nature 1987: 330: 384-86)

Conventional β-globin gene Cloning and DNA sequencing:- β-thal and HbE globin genes were cloned with their flanking sequence as a 7.8 Kb HindIII fragment in

bacteriophage Charon 28 (Orkin + Goff. JBC 1981; 256: 9782-9784). Both β-like and HGE globin genes from positive recombinants were subcloned as 4.9kb BglII fragments into pUC13 for DNA sequence analysis (See Hattori M).

Southern blot analysis of Genomic DNA: Restriction site polymorphisms within the β-globin gene cluster were determined by the Southern blot technique using appropriate probes as described (Takihara --- Fukumaki 1986 Blood 67: 547-550 & Southern EM. JMB Biol 1975; 98: 503-517).

Dot blot hybridization with allele specific oligonucleotide probes. Five μl of amplified DNA was dot blotted onto a nylon membrane and membrane was pre-hybridized at 42°C for 1 hr in 3x SSPE (0.54M NaCl, 30 mM NaH_2PO_4 (pH 7.4), 3 mM EDTA (pH 7.4), 5x Denhardt's soln, 0.5% Sod dodecyl Sulfate and 1 mg/ml herring sperm DNA. Each specific oligonucleotide probe, 5' end labelled with [^{32}P]ATP, was then added to the same buffer and hybridization performed at 42°C for 1 hr. The membrane was rinsed with twice with 2x SSPE, 0.1% SDS, and washed once with same solution at 59°C for 10 min before exposure to x-ray film at -70° for 2 hrs. The oligonucleotide probe specific for the mutant allele has the sequence

Sequence analysis of the γ-globin gene locus from a pt with deletion form of HPFH. by Catherine A. Stolle; Laura A. Pennye; Sharlene Ivory; Bernard G. Forget & Edward J. Benz Jr. Blood (1990) 75: 499-504 [E.J.B. Jr. MD; Dept. of Int. Med. Yale Univ. Sch. of Med. 333 Cedar Str. New Haven CT. 06510]

5' GGT GGT CTA ACC TTGG ACC 3' whereas the normal counterpart has the sequence 5' GTC CAA GGG TAG ACC ACCA 3'. The expected band at approximately 3.0 Kb was clearly seen (in the electrophoretic pattern of the amplified products from normal individuals & the β-thal/HGE patient after 30 cycles of PCR using primer G9 and G10) with a trace of non specific fragment which might indicate a non specific amplification of the non-β-globin region.

Direct cloning and DNA Sequencing of the amplified DNA - The 3.0 Kb amplified fragments from both normal individual & the patient were excised from the gel after digestion with Hinc II and Pst I & further cloned into (appropriate vector in this case) pUC13 plasmid at an efficiency of about 10^5 transformants/ng of DNA (Maniatis et al). Clones carrying the β-globin gene ~~were~~ were easily selected by determining the presence of the 1.3 Kb BamHI fragment spanning the BamHI site in pUC13 & the BamHI site in exon 2 of the β-globin gene. The entire inserts of five clones of the normal β-globin gene selected at random were sequence-analysed. No mechanical change was evident when the sequence was compared for the published sequence for human β-globin ⇒

All you need to know about thalassaemia is available in several languages from UK Thalassaemia Society, 107, Nightingale Lane, London. N8 7QY, U.K.

Thalassaemia in the Maladives: B. Modell
The Lancet 12-5-90 (p. 1169-70)
Abdul Razzak (Central HC, Male, Maladives)
Nicholas Hindley (Whittington HC, London N-10)

Island has a population of about 143 000, which probably of Indonesian/Sri Lankan origin, with more recent admixture from Arabia & East Africa. Until recently the islands were highly malarial. β-thal, HbS and HbE are all known to occur but there have been no population surveys. Islands are now malaria free, and clean water, extended immunization and oral rehydration policies are conscientiously implemented. Infant mortality was 60 per 1000 in 1985. It is at this stage in a country's development that inherited diseases may emerge as a health issue. By 1987 it was clear that thal was important in the Maldives one of us (BM) visited the main island at the advice of WHO in 1988. We estimate that 1400 babies born in Male in a year about 6 are affected giving a heterozygote frequency of at least 13%. Osmotic fragility (thal test) was +ve in 15.5% of the 477 samples & equivocal in 3.5%. As screening & counselling become available, attitudes to prenatal diagnosis will evolve, and at that time backup from expert centres in countries such as India or Thailand will be needed.

Hepatitis Adrian Eddleston. Lancet (1990) May 12, 1990. p. 1142-45 [Liver Unit, King's College School of Medicine, and Dentistry of King's College London, Denmark Hill, LONDON SE5 8RX. UK (Prof. A. L. W. F. Eddleston DM)

Detection of β-thal mutation in Chinese using amplified DNA from dried blood sample Shu-Zhen Huang et al - Yi-Laozeng. Hum Genet (1990) 84: 129-30 [Shu-Zhen Huang. Lab of Med Genetics Shanghai Children Hl. 380 Kang Ding Road, Shanghai 200040. PRC]

⇒ gene except known polymorphic changes (Poncz et al JBC 1983, 258: 11599). For the β-thal HbE patient, both β-globin alleles were selected for the presence of both the presence and absence of the Ava II polymorphic site in the second intron of the β-globin gene (Clones E2D). Sequence analysis of both clones revealed that the HbE mutation (GAG→AAG at codon 26) is is located in Clone E but not in D. Comparison of the nucleotide sequence of Clone D with that of with that of human β globin sequence reveals only one notable nucleotide substitution ... Clone D, the C→A transversion within the codon (TAC) for an amino acid 35 (tyrosine) that produces nonsense codon (TAA). This substitution causes a premature termination of the β-globin chain synthesis and results in a β° thal phenotype. The β-globin gene exhibits polymorphism limited almost exclusively to common sequence types and designated as β-globin gene frameworks (Orkin et al Kazazian Am. Rev. Gen. 1984, 18: 131). According to this definition the Clone E and D sequences were framework 1 and framework 3 Asian, respectively. We analyzed the polymorphic restriction sites in the

~~Gγ-globin gene~~ β-globin gene cluster (the HincII site 5' to the ε-globin gene, the HindIII sites in the Gγ- and Aγ-globin genes, the HincII site in the ψβ globin gene, the HincII site 3' to the ψβ-globin gene, the HgiAI; AvaII sites in the β-globin gene, and the BamHI site 3' to the β globin gene) to determine the haplotype of the Chromosome bearing this Ochre mutation. We found that the haplotype (+,-,-,-,-,-,-,+) was linked to this mutant allele.

Confirmation of Ochre mutation by Conventional DNA Cloning, Direct sequencing and Dot-Blot hybridization. The β-globin of the patient was also cloned conventionally using bacteriophage Charon 28 as a Vector. The 4.9 Kb BglII fragment from positive recombinants was further subcloned at the BamHI site of pUC13 before sequencing. Sequence analysis of the β-globin gene clone that was undisturbed by the lack of an AvaII polymorphic site in its second intron revealed the same C-A transversion at Codon 35 (data not shown).

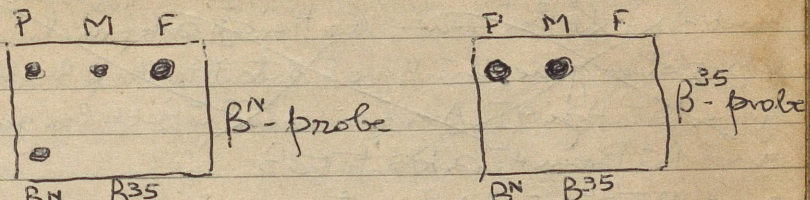


Fig 4. Dot blot hybridization of amplified DNA of the patient (P), his mother (M) and his father (F) with specific oligonucleotide probes designed to detect β^{35} Ochre mutation (see experimental procedure). β^N and β^{35} are the Clone DNA used as control for hybridization. The patient and his mother are heterozygotes for this mutation whereas father does not carry this mutation

Discussion :- A molecular defect of this β-thal was identified by demonstration of the nucleotide sequence of the Clone of the PCR-amplified entire β-globin gene. This patient was a valid candidate for the investigation because extensive screening for known mutations causing β-thalassemia showed negative results. DNA sequence analysis of the β-thal gene revealed a C-A transversion at the codon for amino acid 35. This mutation changes the normal tyrosine codon TAC to TAA, an ochre termination codon. This defect prevents β-globin chain synthesis by a premature termination and

Frequency and molecular types of deletional α-thal in Egypt. Hum. Genet (1989) 81: 211-213

Andrea Nuzzo et al. ~~Dept. Med. Chem.~~ A correlation has been established between high frequency of α-thal and malaria endemicity, possibly due to selective advantage of α-thal carrier against malarial infection (Flint et al 1986 Nature 321: 744 and Aγ-globin genes, the HincII site on the ψβ-globin gene, the HincII site 3' to the ψβ-globin gene, the HgiAI, AvaII sites in the β-globin gene, and the BamHI site 3' to the β

and the β° thal phenotype ensues. This mutation was confirmed by analysis of the β-globin gene, cloned in a conventional manner, dot blot hybridization, and by direct sequencing of the amplified product. Since this mutation has hitherto not been defined in other ethnic groups, this may be the first report of a mutation originating in Africa.

Conventional β-globin gene cloning is time consuming, labor intensive and relatively large amount of genomic DNA is required. To avoid these problems, PCR have been used to generate a part of the β-globin gene fragment in vitro which can be directly sequenced or further cloned into the M13 vector (Scharf et al Science 1986, 233: 1076). At present direct sequencing of the amplified DNA is the rapid method of characterization of unknown mutations. However a cloned DNA fragment of the gene is favorable for analysis for following reasons. At most, 200 bp

In Med. Population α-thal is prevalent and usually results from a 3.7-kb rightward deletion which removes one of the duplicated α genes by unequal crossover between homologous α and ψα genes (Embury et al 1980 N. Eng. J. Med. 303: 1318). According to the point of Xmn I over between ψα genes, 3 types have been described. Type I is most frequent in Med. Pop. Type II is also seen but type III is specific for Melanesian & Polynesian (Hb H) from the sequencing primer can only be resolved by the direct sequencing. Direct sequencing analysis does not usually reveal an association of polymorphic sites with the specific mutation. DNA cloning is required for the in vitro or in vivo expression studies of the mutant allele. When a 110 bp fragment of the β-globin gene was amplified using a Klenow fragment and directly cloned into M13 mp10 vector, only 0.9% of the clones corresponded to the desired fragment (Scharf et al Science 1986, 233: 1072). To develop the rapid entire β-globin gene cloning, we used Taq I DNA polymerase and the primers G9 and G10 to amplify the 3 Kb fragment. This fragment contains the entire β-globin gene, from 823 bp 5' to the Cap site to 569 bp 3' to the poly(A) site of the β-globin gene. The primers G9 and G10 have within their sequences, restriction sites for HincII and PstI respectively. Therefore after diagnosis with both HincII & PstI the 3.0 Kb fragment can be directly cloned into an appropriate vector (e.g. pUC13 in this paper). Gel purification of the 3.0 Kb fragment before cloning makes it more ~~clo~~ specific to the β-gl.

⊗ Less common is 9.4 & 2-kb deletion removing 2 genes (leftward deletion) & several non-deletional mutations

International Conference on Genetics and Human Welfare (Jan. 15-17, 1990)

Inst. of Pharmaceutical Sciences, Dr. ALM Post Graduate Inst. of Basic Med. Sciences, Univ. of Mayo, Taramani, Madras. PM Gopinath Prof. & Head

gene because amplification creates a trace of the non specific fragment, especially with smaller sizes. In our work all 12 clones selected at random had β-globin gene inserts. Therefore, Misincorporations due to the relative lack of fidelity of Taq DNA polymerase might be the only problem with this approach. The misincorporation rate/nucleotide/cycle for Taq DNA polymerase was estimated to be 2×10^{-4} in the case of 239 bp products of 30 cycle PCR amplification. In our experience there can be no misincorporation when the smaller amplified fragments (eg. 0.63 kb) has been cloned and sequence analysed (data not shown). In this paper when as large as 3.0 kb amplified fragment was cloned, complete sequencing of five independent clones (14,990 bp) derived from normal DNA revealed no error, thereby suggesting that the amplified products of the relatively large size are less likely to be misincorporated during DNA synthesis with Taq DNA polymerase. Amplification of the 3.0 kb fragment facilitated ~~Scre~~ screening by dot blot hybridization of all known mutations causing β-thal with only a single PCR reaction. Second, the amplified

Dept. Genetics, Dr. ALM P.G. Inst. of Basic Med Sciences, Taramani Madras 600 113.
" Strategy for detection of β-thal in

DNA can also be sequenced directly to characterize an unknown mutation. Finally the DNA fragment can subsequently be cloned directly when cloned DNA is required. The approach described here is a rapid one for molecular ~~hybridization~~ characterization and molecular cloning for the β-globin gene and presumably for other genes.

Rapid non radioactive prenatal diagnosis of β-thal and sickle cell disease: Application of polymerase Chain Reaction (PCR) - Bil J Haemat (1988) 78: 455-458 E Andreas E. Kulozik; P Lyons; Elizabeth Hohne; Claus R. Bartram & Enno Kleihauer [AEK; Section of Med. Biol. Depot of Pediatrics II, Prittwitzer strasse 43, D-7900 ULM. FRG]. Reprint reco

*Determination of the spectrum of β-thal genes in Spain by use of Dot-blot Analysis of Amplified β-globin DNA Am. J. Hum Genet (1988) 43: 095-100 S. Aselem; V. Nunes; M. Vidaud; X. Estivik; C. Wong; L. d'Auriol; D. Vidaud; F. Gilbert, M. Baiget;

x. PCR technique was used to preferentially amplify β-globin DNA sequences that contain the most frequent β-thal mutations in Med. region and analysed 58 β thal alleles in adult-blood format either by hybridization with allele-specific radiolabeled oligonucleotide probes or by direct sequence analysis of the amplification product. Spanish population carries 7 different

and M. Goossens. [Dr. Michael Goossens, INSERM U91, Hôpital Henri Mondor, 94010, Créteil, France]

Prenatal Diagnosis of β-thalassaemia based on restriction endonuclease of amplified DNA M. Priastu; M.S. Ristaldi & A. Cao.

Molecular basis for dominantly inherited inclusion body β-thal PNAS (1990) 87: 3924-3928 [S.L. Thein, John Radcliffe Hosp. Inst. Mol. Med. MRC, Mol. Haemat Unit, Oxford OX3 9DU Engl] S.L. Thein; C. Hesketh; P. Taylor; I. J. Temperley; R.M. Huchinson; J. Mold; W.G. Wood, J.B. Clegg & D.J.W. Reprint Rec'd. 16/8

Bone & Joint Manifestations in Sickle Cell Anemia OM Bennett; SS Namnyak

Jn. of Bone & Joint Surgery (British Vol) [OMB: 44 Paget Rise, London SE18 3QQ England]

Cerebral Thrombosis in beta thal/HbE disease Stroke (1990) 21: 812 V. Wong; Y.L. Yu, RHS. Liang; WK. Tso; AMC Lu; TK Chan [Y.L. Yu, Univ. of Hongkong: Depr of

β-thal mutations; the non-sense Codon 39 is predominant (64%) whereas the IVS1 position 110 mutation, the most common cause of β-thal in the eastern part of Mediterranean basin, is underrepresented (8.5%) IVS1 mutations at 6 position accounts for 15% + lead to more severe β-thal. Genomic DNA (1 μgm) was amplified by PCR using E. Coli DNA polymerase I (Klenlow Reagent) & Eli primes.

Physiology, Sasson Road, Hong Kong
Hong Kong]

Gerhard Braunitzer (1921-1989)
Obituary by JASMINKA GODOVAC-ZIMMEMANN, John Curtin School of Med. Research, Australian National Univ.
P.O Box 334, Canberra. ACT 2602.
Australia]-

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Glucose-6-Phosphate dehydrogenase deficiency W.H.O. working group updated version March 1989. Meeting 3-4 Sep 1985 ~~AE~~ Dr. E. Beutler USA (Chairman); G. Gaetani (Italy) V. der Kaloustian (Lebanon now in Canada) L. Luzzatto UK (Reporteur) S. Niwa (Japan) V. Pannich (Thailand)

O. Sodeinde (Nigeria) WHO Secretariat
Dr. M. Belsey; A.M. Kulier (Secretary
Now in USSR) B. Modell (Temp. ~~Secretary~~
Advisor) + Dr. P.M. Shah Bulletin
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Acta Ped. Scandinavica (1990) Suppl.
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Hurwitz R.L.; Coleman M & Adams J.G.
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basis for dominantly inherited Inclusion
body β-thal) PNAS (1990) 87: 3924-28
SL Thein et al
is that many exon 3 mutations are
associated with a dominant phenotype
for heterozygous β-thal. They are rare
and are usually found in North
Europeans. Presumably because of their

·x· Update of the Progress of Haemoglobinopathy Control Report of the III & IV annual meetings of the WHO Working Group for Community Control of Hereditary

severity they would not have come under selective pressure as in the case ~~of~~ for the recessive forms of beta thal & hence, unlike the latter, will not have reached high gene frequencies in ~~malaria~~ ~~malaria~~ malarious populations.

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Level and Composition of Fetal Haemoglobin Expression in Normal New Borns are not Dependent on β Cluster DNA Haplotype Jacques Rochette; Catherine Dodé, France Leturcq & Rajagopal Kishnamurthy Am. Jl. Haemat (1990) 34: 223. [Rochette J. CHU Cochin ICGM, 24 Rue Faubourg St. Jacques, F-75014. Paris]

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Variations in the level of Fetal Hbs in $(\delta\beta)^0$-Thalassemia Heterozygotes with different numbers of Globin Genes C. Oner; A. Gurgey C. Altay; F. Kutlar & T H J. H (Th J H) Am. J. Haemat (1990) 34: 230

Exclusion of β-thal by biopsy and DNA amplification in Mouse pre-embryo R. Lindeman; J. Lutjen; C. O'Neill; and R. J. Trent. Prenatal Diagnosis (1990) 10: 295 [R. Lindeman, Univ of Sydney, Clinical Immunology Research Ctr. Molecular Biology Lab, Sydney. NSW 2006. Australia]

Molecular Characterization of high A_2 β thal by Direct Sequencing of Single Strand Enriched Amplified Genomic DNA. S. L. Thein; C. Hesketh; J M Brown; A V Austey and D. Jn. Blood (1989) 73: 924–930.

Reprint Read DNA analysis was by extrasp DNA from peripheral blood leukocytes and analyzed by Southern Blotting. Five micrograms of DNA were digested to completion with various restriction enzymes & the fragment separated by electrophoresis

Community control of hereditary anaemias: Memorandum from a WHO meeting. Bulletin of the World Health Organization 61: 63-80 (1983)

in 0·8% or 1·0% Agarose Gel. Fractionated DNA was transferred to Nylon filters (Hybond-N, Amersham) + hybridized to DNA probes radiolabelled ~~=~~ ^{32}P-dCTP by Random Hexamer Priming method (Feinberg et al. Analy. Biochi. (1983) 132-6). Filters were washed to high Stringency and autoradiographed between intensive Screens at -70°C. Two different probes were used (a) the β IVS-2 is a 0·9kb BamHI / EcoRI fragment obtained from plasmid pβIVS-2 which contains the Second intervening Sequence of the β-globin gene and (b) the 5' probe is a 1·9kb BamHI containing 5' flanking Sequences exon I, IVS-I and exon 2 of the β-globin gene.

DNA amplification and Sequencing: The method DNA amplification was a modification of that described by Saiki et al (Science 230: 1350, 1985) One µgm of genomic DNA was used in each amplification. The reaction primers CHA 5C and CHA 3C were chosen to flank the 5' and 3' end points as defined by deletion restriction endonuclease mapping and were designed to yield an amplified fragment of 1·8kb from normal

Oral iron chelating drugs: coming but not yet ready for clinical use: BMJ (1988) 296: 1081-82
Office of Publications, WHO, 1211 GENEVA 27, Switzerland.
β-allele or a smaller fragment from the mutant allele.

Amplification by PCR was carried out using a DNA thermal cycler (Perkin-Elmer; Cetus) in a 100 μL reaction volume containing 1 μg genomic DNA, 0.2 mmol/L each of dGTP, dATP, dTTP and dCTP, 100 pmol of each primer CHA 5C and CHA 3C and 2.5 Units of Taq DNA polymerase (Cetustorp) in a buffer containing 50 mmol/L KCl, 10 mmol/L Tris-HCl, pH 8.3 at 20°C, 2.5 mmol/L $MgCl_2$ and 0.01% gelatin (Sigma Chemical Co. UK). The mixture was overlaid with 100 μL of liquid paraffin (BPC). After an initial denaturation of two mts at 94°C, annealing of two mts at 55°C and extension of five mts at 70°C, 29 cycles of PCR were performed under the following conditions: 94°C (one mt), 55°C (Two mts) 70°C (five mts), the last extension reaction at 70°C was prolonged to ten mts and the reaction mixture stored at 4°C until analysis. After amplification and removal of the mineral oil, the products were examined in a 1.5% low-melting point agarose

(Bethesda Research Lab, Bethesda MD). Stained with ethidium bromide, the fragment of expected size was localized and isolated by electroelution on a DEAE membrane (NA45, Schleicher and Schuell) followed by two phenol-Chloroform extractions and ethanol precipitation. About 5 ng of the purified fragment was added to a new 100 µL PCR and subjected to another 30 cycles of amplification. The reaction conditions were as described above with the exception of using a different set of primers; one, internal to the previous at 1 µmol/L and the other as previous, at 0.01 µmol/L. It was reasoned that the excess of one primer over the other should lead to a preferential amplification and enrichment of one strand. Thus a second round PCR using 1 µmol/L of CHA 3B and 0.01 µmol/L of CHA 5C should lead to a preferential amplification of the CHA 3B primed single strand (Fig 1). Four identical second PCR amplifications were setup for each set of primers

the reamplification products were pooled, extracted once with Phenol-$CHCl_3$, ~~desalted~~ desalted, and the excess dNTPs removed by Spin-dialysis on a CENTRICON 30 (Amicon, Danvers. MA), ethanol precipitated and resuspended in 20 µL of TE (10 mmol/L Tris-HCl, pH 8, 1.0 mmol/L EDTA). 7 µL of this final product ~~was~~ with 0.5 pmol of the appropriate primer (CHA 5A for CHA 3B enriched Single Strand DNA and CHA 3B for CHA 5A enriched Single strand DNA) was used for sequencing by dideoxy chain termination method (Sanger et al PNAS, 1977: 74: 5463). The primer-template mixture was heated to 95°C for five to eight mts and immediately placed on ice. Reactions were then carried out ~~on~~ using ^{35}S-dATP (Amersham UK), 2 Units of sequenase and the reagents provided in the sequenase sequencing kit of USB Corp.

Results Hematology: Two family member of one family ECQ; RCQ; ACO $\bar{c}$ 8.4%; 8.0% + 8.2% A_2 respectively $\bar{c}$ F 3.5 + 4.0% in two. (Anglo-Saxon + Dutch descent)

Vit B_{12} and Folate deficiency presenting as Leukemia Anne Lennard; IS Dokal; et al BMJ (1990) 301: July 7, 1990 [A. L. Royal Victoria Inf. Dept of Haemat. New Castle upon Tyne & Wear; England.

Another LBO; CBO = A_2 8.2 + 7.8 with F 1.9 + 6.5% respectively consistant = hetero β-thal but were asymptomatic

Restriction endonuclease analysis – deletion in the β-globin gene: DNA from "RC" digested with various res. enzymes + hybridized ~~with~~ to the ~~the~~ β IVS-2 and 5' β probes. In addition to the expected normal fragments, restriction fragments of abnormal sizes were also present with both the probes.

HG South Milwaukee [β105(G7) Leu-Phe] a newly identified HG variant with high Oxygen affinity. George R. Honig; ~~Loyda~~ Loyda N. Vida; Rocco Latorraca and Ajit B. Digvi. Am. J. Hemat (1990) 34: 199 [G. R. H. Univ. of Illinois College of Med Dept Ped. 840 S. Wood Street M.C ~~[illegible]~~ 856 Chicago ~~[illegible]~~ Il. 60612 USA]

Level ~~of~~ and Composition of fetal HG Expression in Normal Babies are not dependent on β Cluster DNA haplotype Jacques Rochette; Catherine Dode; France Leturcq & Rajagopal Krishnamoorthy Am. J. Hemat (1990) 34: 223 [J. Rochette CHU Cochin ICGM. 24 Rue Faubourg St. Jacques F-75014 Paris]

↑ eluted fragments were estimated spectrophotometrically at 415 nM[3] Hb F estimated by Betke.

Galanello et al (Proc. Natl. Acad. Sci. USA 30 Dec. 1988, 85:9158) reported 2 alpha (-/22 or -3.7 -2/-1) had Barts level between 2-7.1%. One gene deletion (-2/22) 0.7 to 2.5%. Double 22 Gen Barts ranged from <1 to 9%. Such low levels in fetal blood, these might also likely to develop HbH disease subsequently. High levels of Barts at birth - human globin gene activation region (LAR) - Role in temporal context by Tim M. Townes, & Richard Behringer. Trends in Genet (1990) 6: 219 [TMT, Univ. of Alabama Sch. of Med. Dept. Biochem. Birmingham, AL 35294 USA]

Detection of alpha thalassemia in Sickle cell trait patients by Hb Barts screening and quantitating HbA and HbS.

Mishra RC; Ind. Jl. Med. Res (1989) 90: 459. 7.7% Barts in cord bloods; (Sickle cell trait 4%. — Jl. Obs. Gyn of India 1988, 38:31; Mishra RC & Padhi K. Haemoglobinopathy and erythrocyte G6PD def in pregnant women of North Western Orissa) Where tribals constitute about 30% of population.

(The paper now received was referred as JHR ref no. 90-0242/5582 & 10th Feb. 90 titled "High prevalence and heterogeneity of thal in Orissa — RC Mishra & others. Costal alal districts of orissa tribals are less than 5% & sickle cell trait rare. The Communicators present the results of investigation on the incidence of α-thal; Hb-CS

Haemolysates were prepared using refrigerated Cuthinge. Nafal Solvent was used while preparing haemolysate. Cellulose acetate membrane (CAM) Malta Modena Stain was used after electrophoresis & stained either with amidoblack or Ponceau S. 20 µl. of haemolysate was applied on CAM + electrophoresis run for 45 min with 4 MA at 250-300 volts in Tris EDTA borate at pH 8.6 (Dacie). Gel cut dried. Compared with JRIS Buffer at 30 mA

and HbA2 see that amongst the people of Costal Orissa:

of 515 CB. 65 (12.6%) revealed Hb Barts distinct fast band beyond HbA using CAM electrophoresis. In some smaller bands were seen after stamp. Eleven (2.1%) showed Hb CS; 5 along with Hb Barts and 6 without any other Hb. Hb CS was seen as faint distinct either one or two bands taking the position of HbA2.

Of the 65 Hb Barts positive samples only 10 of their parents could be traced. Electrophoresis of their Hb revealed CS in 8 and Hb CS with Hb H in one case. Only one case had shown no abnormal and only AA pattern.

Of 450 routine electrophoresis performed in Agarose gel showed Hb CS in 23 cases (5.1%). These samples belonged to hospital patients of various age groups. Quantitation of Barts in 22 samples showed

no Barts 18 (81.8%)
2%-4% 4 (18.2%)
more than 4% (5.6%-1; 6.2%-1; 9.0%-1) 3 (13.6%)

* Haemolysates were prepared using refrigerated Cuthinge. Normal Saline was used while preparing haemolysate. Cellulose acetate membrane (CAM) Malta Modena Stain was used after electrophoresis & stained either with amidoblack or Ponceau S. 20 µl. of haemolysate was applied on CAM + electrophoresis run for 45 min with 4 MA at 250-300 volts in Tris EDTA borate at pH 8.6 (Dacie). Gel cut dried. Compared with JRIS Buffer at 30 mA

Table II. Quantitation of HB Components from 100 Control Subjects. All figures expressed in %.

HB Component	No of Cases	Mean Value	Range
Higher $A_2 > 3.5$	8	4.2 ± 1.55	3.6 ... 5.4
Low $A_2 < 1.8$	3	1.6 ± 0.32	1.2 ... 1.8
Normal 1.8 – 3.5	89	2.7 ± 2.1	1.8 ... 3.5
	100		
HBCS.	15	-1.4 ± 2.33	0.3 ... 5.4

All samples showing HBCS were within the group of 89 subjects showing HB A_2 within normal range.

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B.C. Mehta Oration JAPI (1990) 38: 421-424.

Iron Deficiency: Prevalence & Problems (Part II)
B.C. Mehta (Oration) JAPI (1990) 38: 491-494. Ref 11 - Mehta, Sukumara Patel.

Glucose-6-Phosphate Dehydrogenase Def. W.H.O. Working Group Based on the meeting of WHO Working Group which met in Geneva on 3-4 Sep. 1985. [E. Beutler - USA Chairman; G. Gaetani; V. der Kaloustian; L. Luzzatto - rapporteur, S. Niwa; V. Pannich + O. Sodeinde. WHO Secretariat Dr. M. Belsey & Dr AM. Kulicw (Security)]
(Bull. of WHO 64(8) 601-611)

8-Thal. Page 512. The Thal II. Haemolytic anemia vol: 2; The Hereditary Haemolytic anemia Part 2. Sir John Dacie. Churchill Livingst Sukumar & Co-workers (1983) Nucl. Acids. Res 11: 4635-4643.

Request for Single Copyer of the full report (document WHO/HDP/WG/GBD/85.9) or reprints of this article sent to 'Hereditary Diseases Programme, Dn. of Non-Communicable Diseases. WHO. 1211 Geneva 27. Switzerland)

Primary Health Screening by haemoglobinometry in tropical Community W. L. Johns & S M Lewis Bull WHO 67 (6) 627-633 (1989). [S.M.L. Hamd Dept. Royal Postgraduate Med School, Ducane Rd, London W12 ONN England].

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Diagnosis of Human Immodificiency Virus Infections in Infants & Children Robert N. HUSSON; Anne M. Comeau; Rodney Hoff. Pediatrics Special Article (1990) 86: 1-10 [Robert N. HUSSON, MD Ped. Branch, National Cancer Research Ist Bldg 10; R No. 13 N240, 9000 Rock Vile Pike Bethesda. MD; 20892]

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Detection of α-thal in Sickle Cell trait patients by Hb Bart's Screening and quantitation of HbA and HbS: R. C. Misra IJMR (1989) 90: 459.

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DNA sequences regulating human globin gene ~~an~~ transcription in non-deletional HPFH. Ottolenghi S; Mantovani R., Nicolis S; Ronchi A & Giglion B. Haemoglobin (1989) 13: 523

Paroxymal Nocturnal haemoglobinuria: the biochemical defects and Clinical Syndrome ~~Am. J. Med (1988)~~ Rosse, WF Blood (1989) Rev. 3, 192

Relationship of foetal Hb levels and βs haplotypes in homozygous Sickle Cell disease ~~B~~ A. G. Falusi & A. E. Kulozik. Europ J. Haemat (1990) 45: 1-5 [Falusi A.G. Univ College Hl; College Med, Postgraduate Inst of Med. Res & Training, Ibadan Nigeria

Silent β-thalassemia – Silent Carrier of β-thal
New Engl. J. Med (1969) 281: 1327 – 1333. Normal indices
MCV 80; MCH 80.0 pg. Normal A_2 + HGF level, β:α ratio
0.60 – 0.69. (Ratio Children in the family 0.32 and 0.24
and that of mother (β thal trait) 0.47. See also
Sciarratta et al (1985) Ann N.Y. Acad Sci. 445: 111–118
Sl. reduced MCV; MCH normal A_2 % – one propositus was
homozygous for silent carrier state

A truncated human Chromosome 16
associated with α-thal is stabilized
by addition of telomeric repeat
$(TTAGGG)_n$. Andrew O.M. Wilkie;
Janette Lamb; Peter C. Harris; Roger
Finney & Douglas R. Higgs. Nature
Aug 30, (1990) 346: 868–871 [D R Higgs
MRC Mol. Haemat Unit, Inst of Mol Medicine,
John Radcliffe Ctr, Headington, Oxford.
OX3; 9DU. UK] Here we describe an
α-thal mutation associated a terminal
truncation of the short arm of Chr. 16
(within band 16p13.3) to a site
50kb distal to the α globin gene,
and show that $(TTAGGG)_n$ has been
added ~~directly~~ directly to the site of the
break.

H6 J Paris (L 12 Ala-Ap) Acta Haem
1987/88. [A10]

Treatment of Sickle Cell anemia = Hydroxyurea & Erythropoietin Mark A. Goldberg et al & H. Franklin Bunn. New Engl. J. Med (1990) 323: 366-372 [~~H.A.G.~~ Bunn HF. Brigham & Womens Hospital, Dept. of Med. Dn. of Hemat Thorn. ~~919143~~ 919 75 Francis Str. Boston MA. 02115]

Diarrhea due to Cryptosporidium infection in thal major. Julia A. Gledhill, John Porter. B.M.J (1990) Jul 28, 301: 212 A Single [

Thalassemia can be prevented by A. M. Kulier. World Health Forum (1986) 7: 3, 286-290. [Dr. A. M. K. Head Lab for Control of Hereditary Desis, Inst. Med Genetics MOSKNORECHIE Street 1, Moscow 115478 USSR

How thalassemia was Controlled in Cyprus Michael Angastiniotis, ~~Soph~~ Sophia Kyriakidou, and Minas Hadjiminas. World Health Forum (Public Health Practice). 1986: 7: 3, 291-297 [Dr. Angastiniotis, Pediatrician, Thalassemia Centre,

Hb - Hofu (unstable HC) II associated = β°-thal Arends T et al HC Acta Haemat (1985) 73: 51-54

Archbishop Makarios III Hospital Nicosia Cyprus:]

Table I Worldwide births of infants with Serious Congenital hereditary disorders

Category or disorder	Birth/1000	Birth/yr.	% of total
Acrocephalosyndactylia	0-16	220,000	5
All serious Congenital hereditary & Chr. disorders	26-62	4,590,000	100

An Initiation Codon mutation as a Cause of a β-Thal. L. Jankovic; G.D Efremov; O. Josifoboska; D. Juniak T. A. Stoming; A. Kutlar & F. H. Ht. Hemoglobin (1990) 14: 169 [L. Jankovic; Med. Coll. Georgia, Dept Cell & Mol Biol Augusta, Ga. 30912]

Bº-Thalassemia in a Thai family is Caused by a 3.4 kb deletion including entire beta globin gene T. Sanguansermsri; M. Pape; M. Lang. J. Hundrieser. & L. Haly. Hemoglobin (1990) 14: 157 [T.S. Chiang Mai Univ. Fac. Med. Hemanglobin Unit, Chiang Mai Thailand].

Thai Gγ(Aγδβ)°-Thalassemia and its Interaction with a Single γ-globin Gene on a Chromosome Carrying β-Thalass. P. Winichagoon; S. Fucharoen, V. Thonglairoam & P. Wasi. Hemoglobin (1990) 14: 185 [P.W. Mahidol Univ. Siriraj Hk, Fac. Med. Dept Med. Div. Hemat. Centr. Thal. Bangkok 10700].

Frequencies of Common Beta Thal Alleles Among Among different Populations —

Variability in Severity. (Annotation)
THJH. Brit Jl. Haemat (1990) 75:
454.
Blood Transfusion in the Para Bombay
Phenotype M. Luichey R. E Broadberry
Brit Jl Haemat (1990) 75: 568.

Interferon-γ Modulates Fetal Hemoglobin Synthesis in Sickle Cell anemia and Thalassemia Jl. of Interferon Research (1990) 10: 357: B.A. Miller, M. Oliveri, S.M. Hope, D.V. Faller, S.P. Perrine. [B.A. Miller, Penn. State Univ., Milton S. Hershey Med Ctr. Dr. Ped. Hemat. Oncol. POB 850, HERSHEY PA. 17033 USA]
Desferrioxamine Improves Neutrophil Phagocytosis in Thal. major Hem Jl. Hemat (1990) 35: 13 Brigette Cantinieaux, Chantal Hariga, Alma Fraster, Michèle Toppet, Pierre Fondu [B.C. Univ. Libre, Bruxelles Hop St. Pierre Dept. Hematol, 322 Rue Haute, B-1000 Brussels. Belgium]

Antibody to Hepatitis-C Virus in Multiply Transfused Patients with Thal. major. B. Wonke, A.V. Hoffbrand, D. Brown, G. Dusheiko J. Cl. Path. (1990) 43: 638 [B. Wonke, — Rep. Hoffbrand A.V. Royal Free Hosp. Dept. Haemat. London NW3 2QG. England]

Prevalence of antibodies to HIV and to Human T Cell Leukemia Virus type I in Transfused Sickle Cell patients by Oswaldo Castro et al [O.C. Howard Univ. Coll. Med. Dept of Med: 520 W St. NW. Washington DC. 20059. USA]

Quantification Analysis of 5'-Splice Signal Sequences in RNA Precursors: Mutations in 5'-Splice Signal Sequences of Human β-globin gene and β-thal. I. IDA, Y. J. of Theor Biol (1990) 145: 523 [Y. I., Hokkaido Univ Fac Science, Dept Biol Sci, Hiroshima 730. Japan]

MS Localization Characterization of β-thal gene in Egypt. A. Novelletto; M. Hafez, G. Deidda; A. D. Rienzo; L. Fellicetti

H. El-Tahan, Z. El Morsi, M. El-Zeiny, Y. Al-Tonbary; A. Sitten L. Terrenato. Human Genet (1990) 85: 272 [A. N. Univ. Tor. Vergata. Dept. attments Bio. 1-00173 Rome Hof

Determination of β^s haplotypes with specific oligonucleotide probes.

CIHAN ÖNER and Aleksander J. Dimovski. SPHERE (1990) Sept Vol. 13 No. 4. Complex Origin of the β^s Gene

The β^s gene occurs mainly in association with five distinctly different haplotypes (Characteristic sets of alleles at linked polymorphic sites). These five haplotypes have named for their geographic origin Fig 1 and are numbered (in Africa). Benin (#19) Bantu (Central African Republic or CAR) (#20) Senegal (#3), Cameroon or $A\gamma^T$ (#17) and and Saudi Arabian/Indian (#31). In any one of these five geographic areas, the β^s gene is found mainly in association with only one haplotype which indicates that β^s gene has arisen by mutation on atleast five separate occasions in different geographic areas.

The geographic origin of β^S Chromosomes with distinct haplotypes in Africa. Haplotype #31 found exclusively in Saudi Arabia and India

Variations in gene expression. Although all SS patients have A→T mutation at codon 6 of their β-globin genes and therefore, the Glu→Val substitution in their β-globin chains, the hematologic features + Clinical severity of this disease vary considerably. Sickle cell anemia seems to be less severe when patient also has α-thal or when his HbF is increased. The expression of the characteristic fetal globin genes (Aγ and Gγ) is especially sensitive to mutations in their adjacent 5' flanking regions. Some forms of HPFH are known to be caused by such alterations. The haplotype of a particular β^S Chromosome also seems to be related to its γ gene expression. Typically β^S Chr of haplotype #17, #19 & #20 all express low levels (≤10%) of HbF with more than 60% Aγ chains. The β^S Chromosomes of haplotypes #3 and #31 express higher (>15%) HbF levels with more than 60% Gγ chains.

β^S - Haplotype-Specific Mutant Sites (Fig 2)

It has been thought possible that certain unique mutant sites linked to the β-globin gene cluster might be responsible, at least partly, for differences in the γ-globin gene expression observed among the SS patients with different haplotypes. In a search for such sites, we acquired DNA derived by polymerase Chain Reaction (PCR) from the 5'flanking (promoter) regions of Aγ and Gγ genes of a few selected SS individuals whose haplotypes were previously characterized. In the sequence analysis of this DNA, we observed following three mutant sites on a β^S chromosome of haplotype #19: C→G at -369 (Gγ), a G→T substitution at 657 (Aγ) and A→G at -309 (Gγ); the -309 Gγ site has also been described previously by other investigators.



Position	-1105 to -394	TO -400	-369	-158	-657	-271
Mutation	CG→TT	7BP DEL	C→G	C→T	G→T	C→T
Hap19	−	−	+	−	+	−
Hap20	+	+	−	−	−	+
Hap3	−	−	−	+	−	−
Hap17	−	−	−	−	−	−

Fig 2: Specific sequence differences in the 5'flanking region of the Aγ- and Gγ-globin genes among β^S

Chromosomes with different haplotypes; + or − indicates the presence or absence of a particular mutant site.

The β^S Chromosome with haplotype #20 also had three alterations, namely C→T at position −271 (Ay), CG→TT at position −1105 and −1106 (Gγ) and a 7 bp deletion at position −394 to −400 (Gγ). The β^S Chr. haplotype #17 (Cameroon or AγT) did not show any unique peculiarities in the Aγ and Gγ 5′ flanking regions although it specifically features the synthesis of an abnormal Aγ Chain (Ile→Thr at Codon 75) Factor 5 Chromosomes of haplotype #3 and #31 #31 each had the same C→T change at position −158 (Gγ). In order to test the degree of specificity of these mutant sites for the β^S haplotypes in which they were first found, we used dot-blotting to screen about 400 previously characterized β^S Chromosomes of haplotypes #19, #20, #3, #31 and #17 (Table I) in 55 individuals from the Southeastern USA and Kenya. The appropriate βS-haplotype-specific mutant sites were found in all β^S Chromosomes.

The absolute precision with which these specific mutant sites were correlated with haplotype

indicated that dot-blotting of these βS haplotype specific mutants might be useful as a substitute for haplotyping with restriction enzyms. Haplotype #19 and #20 would be readily distinguished by this technique. The βS Cameroon (#17) haplotype can be detected by a Chromatographic search in Hb for the $A\gamma^T$ variant, which appears to be highly specific for this Chromosome. Haplotypes #3 (Senegal) and #31 (Saudi Arabian/Indian) share C→T Change at -158 of the Gγ promoter region, but are distinguishable by the geographic origin of the particular patient.

TABLE I

Distribution of particular Haplotype Among Different Populations

Country	No. of βS Chr tested	HAPLOTYPES 19	20	3 or 31	17	Rare
Southeastern USA	366	205	71	58	13	19
Kenya	55	1	52	—	—	2
Turkey	179	174	—	—	—	5
Syria	13	13	—	—	—	—
Tunisia	115	109	—	—	—	6
Italy	70	70	—	—	—	—
Suriname	75	41	23	2	—	9
South Africa	5	—	—	5	—	—
Canada	14	10	—	4	—	—
INDIA	(105)	—	—	(94)	—	(11)
Greece	14	13	—	1	—	—
Nigeria (West)	104	97	—	—	3	4

Geographic Survey:- In addition, 694 Chromosomes from Turkey, Syria, Tunisia, Italy, Surinam, South Africa, Canada, India, Greece, and Nigeria were also tested for the presence of each of the six B^s-haplotype-specific features listed in Fig 2. Restriction enzyme haplotyping was not performed. In 659 of these chromosome, the result indicated one of the five common haplotypes as indicated in Table 1. Of the 56 chromosomes characterized as having a rare haplotype, most contain none of these B^s-haplotypes-specific sites, while the remainder had one or more such mutant sites attributable to recombination. In this geographic survey, haplotype #19 was predominant in 55 samples from Mediterranean area. This indicates that B^s gene was introduced into Mediterranean Countries from Central West Africa most probably via North Africa. In Canada, Surinam, and USA, B^s chromosome are characterized by haplotypes #19, #20, #3 and #17 in frequencies reflecting the different contributions from the various original regions in Africa. The B^s Ch. of 55 individuals from Kenya and India were nearly exclusively associated with haplotypes #20 and #31 respectively. This simplified

procedure should facilitate large scale screening programmes designed to estimate frequencies of the β^S haplotypes among selected populations and elucidate the course of flow of the β^S gene. Further studies to clarify which mutant sites might alter the haematylic features and severity of Sickle Cell disease are in progress.

See K.D. Lanclos et al "Sequence Variations in the 5' flanking and IVS-II regions of the Gγ- and Aγ-globin genes of β^S Chrom. with 5 different haplotypes Blood (2) Certain mutations observed in the 5' sequences of Gγ- and Aγ- globin genes of β^S Chromosomes are specific for Chromosome with Major Haplotypes by A. J. Dunowski et al Acta Haemat. in press.

Effect of age at start of iron Chelation therapy on Gonadal Function in β-thal major. New Engl. J. Med (1990) 323: 713-719
Naomi Brons Spiegl - Wenitrobe; Nancy F. Oliveri; B. Taylor; D. F. Andrews M. H. Freedman & F. John Holland [F. J. H. Dn. of Endocrinology, Hosp. for Sick-Children, Room 5114; 555 Univ. Ave, Toronto, ~~Canada~~ ONM5G1X8. Canada]

Microcytosis, iron therapy & thalassemia in preschool children A. Earley; H.B. Valman; D.G. Altman & M.J. Pippard. Arch. Dis. in Childhood (1990) 65: 610-12 [Dr. A. Earley, Wycombe General Hl, Queen Alexandra Road, ~~Hg~~ High Wycombe, Bucks HP11 2TT]

Detection of Sickle cell anemia mutation by Colour DNA amplification Farid F. Chehab. Yuet Wai Kan. Lancet (1990) January 6, vol 335: 15-17. [Dr. F.F. Chehab. Dept. Human Genet. M4, Cetus Corporation, 1400 53rd Street, Emeryville CA 94608 USA] (Y. W. Kan. MD. Univ. of California, U426, San Francisco. Calif 94143-0724)

A fluorescence Assay to detect the mutation in Sickle Cell Anemia has been developed. Oligonucleotides homologous to the normal & the mutant DNA sequence are labelled with fluorescein and rhodamine, respectively and used to prime the polymerase Chain reaction. Amplified DNA products with normal sequence fluoresce green; whereas those with mutant sequence fluoresce red; heterozygotes (AS) DNA fluoresces Yellow. The results can be read by eye and the procedure could be Automated.

The method described is a detection method by which fluorophores are coupled to the primers used in the PCR [See Chehab F F & Kan Y W. Detection of specific DNA sequence by fluorescence amplification: A Colour Complementation assay. PNAS. (1989) 86: 9178-82] We describe the method to diagnose the point mutation in Sickle anemia. DNA samples were obtained from a normal Control; an individual = Sickle cell trait + a subject with Sickle Cell anemia

PCR was done with 1 µg. genomic DNA and 40 p mol. of each of the PCR primers in 10 mmol/l Tris (pH 8.5), 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 0.01 g/dl gelatin and 125 µmol/l each of TTP, dATP, dCTP + dGTP in a vol. of 100 µl. (Mayes RM, Lorimer J, Maniatis T. Detection of a single base substitution by ribonuclease cleavage at mismatches in RNA:DNA duplexes Science (1985) 12: 42-46) Three oligonucleotide primers were used for amplification in each reaction. 2 allele specific primers, each conjugated at their 5' end to a fluorescent dye (Smith LM, Sanders JZ, Kaiser RJ et al Fluorescence detection in automated DNA sequence analysis. Nature 1986; 321: 674-79) were positioned in the region of codon 6 of the β-globin gene, the site of the sickle cell mutation. A fluorescein conjugated 5'-CTTCTCCTCAGGAGT homologous to normal sequence and a rhodamine-conjugated 5'-CTTCTCCACAGGAGT, which differed from the former by one base-pair and was homologous to the sickle mutation. In addition, an oligonucleotide primer corresponding

to the normal sequence. The opposite strand upstream of codon b was used to provide the opposite strand: this primer, which was not conjugated to any fluorophore, had the sequence 5'-GTA CGG CTG TCA TCA CTT-TAG A CCT CA-3'. The mixture was heated to 95°C for 5 min, cooled on ice, and 5 units of Taq polymerase (PerkinElmer Cetus, Norwalk, Connecticut, USA) was added. The reaction was taken through 35 cycles; each cycle consisted of 95°C for ~~30 s~~ 8 30 s (denaturation), 50°C for 30 s (annealing) and 68°C for 30 s (extension). After PCR amplification the products were detected in one of three ways. First the reaction mixture was divided into 2.5 ml & spun in a microfiltration device ('Centricon-100', Amicon, Danvers, Massachusetts) for 10 min at 5000 rpm in a fixed-angle rotor to remove unincorporated fluorescent primers. The amplified DNA remained in the 40-50 µl retentate on top of the filter & was transferred to another tube and examined under long-wavelength ultraviolet light. Secondly, 10 µl of NaOH (1 mol/l) was added to the retentate & the fluorescence of ~~5~~ a 5 µl aliquot measured on a 'LS-5' fluorometer

(Perkin-Elmer) at an excitation-emission wavelength pair of 488-520 nm for fluorescein and 585-605 nm for rhodamine. Thus, 1 µl of the total reaction mixture was loaded on a 2.5% agarose gel, electrophoresed at 100V at 25 mA for 2.5 h, and continuously scanned with actual wavelength laser (See Smith LM et al Nature (1986)). The colorimetric DNA assay is based on the principle that a completely matched primer will be more efficiently used during PCR than one with single base-pair mismatch. When normal DNA is amplified, the primer homologous to normal sequence is extended. Since this primer is labeled with fluorescein the amplified DNA emits a green color under UV light. When DNA from patients with sickle cell anemia is amplified, only rhodamine-conjugated primer homologous to sickle cell mutation is extended & the amplified product fluoresces red. When DNA from heterozygous AS individual is amplified, both fluorescene labeled and rhodamine-labelled primers extended & amplified product is yellow as a result of the ~~complementary product is~~ two dyes as a

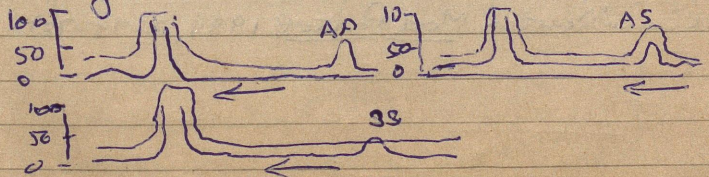
result of complementary fluorescence of the two dyes. After removal of the unincorporated primers by centrifugation ~~the reaction mixture~~ of the reaction mixture on the filter paper core, the colour of the product retained on the paper can be seen & if necessary confirmed by fluorometry.

Relative fluorescence of Amplified DNA

Genotype	Fluorescein (488–520 nm)	Rhodamine (585 — 605 nm)
AA	7·8	0·6
AS	3·8	3·1
SS	0·5	3·3

A relative fluorescence of above 1·0 is positive.

The colour of the amplified product can also be analysed by gel electrophoresis. Thus, when 1 µl of reaction mixture is run on a 2·5% agarose gel, the unincorporated primers are separated from amplified DNA. The colour of the amplified DNA can be determined directly or by real-time laser scanning.



PCR can also be used to detect the Sickle mutation on dried blood spots (Rubin EM, Andrews KA, Kan YW, Newborn Screening by DNA amplification of dried BS Spots

Human Genet (1989); 82: 134-36] Technique could be applied to any genetic disease in which mutation has been defined, even though many mutations may cause the same phenotype. For example, over 70 different point-mutations have been described for β-thal (Kazazian HH Jr., Boehanan CD. Molecular basis and prenatal diagnosis of β-thal Blood 1988; 72: 1107-16) but in any given country, a handful of mutations account for most clinical disease. Appropriate pairs of oligonucleotide primers for normal & mutant alleles could be used for amplification & the genotype determined variably by Automatic Scanning. Fluored primers could also be designed for use in patients with Cancer to detect point mutations and Chromosome translocations and to identify DNA Sequences of infectious agents in patients with Suspected infections (Chehab FF, Xiao X, Kan YW, Yen TSB. Detection of Cytomegalovirus infection in paraffin-embedded tissue specimens with polymerase Chain Reaction Mod Pathol 1989, 2: 75-78)

[Haig H. Kazazian Jr., M.D., The Johns Hopkins Univ. School of Medicine, Dept. of Pediatrics, Center for Med. Genetics, CMSC10-110, 600N. Wolfe St., Baltimore, Maryland 21205]

The Thalassemia Syndromes: Molecular bases and prenatal diagnosis in 1990.
Seminars in Haemat. (1990) 27: 209-228
Kazazian HH Jr. (DNA amplification Methods given).

Molecular bases of thal major & thal intermedia in Asian Indians: Application to prenatal diagnosis. Thein S; Hesketh C; Wallace RB; Weatherall D.J. Brit J. Haemat (1988) 70: 225-231

Ethnic Distribution of β-thal in the Indian Sub Continent Purvish Parikh; Mathew Cyton; Corinne Boehm & Haig H. Kazazian Jr. (Manuscript pending Chitra)

→ DNA amplification was performed as described by Saiki et al (1988) Science 239: 487-491. Briefly, 0.1-0.5 μg of genomic DNA was digested with 10 units of restriction endonuclease in the buffer recommended by the manufacturer (New England Biolabs) for 2 hours at 37°C. Digestion was monitored by electrophoresis of the DNA on a 0.8% "NUSIEVE" agarose gel. Remaining DNA was precipitated with 2 volumes of ethanol and 0.1 volume of 3M sodium acetate, pH 5.2 at -20°C for 30 minutes. The DNA was collected by centrifugation in a microfuge for 10 minutes, washed with 70% ethanol, dried briefly under vacuum and resuspended in 10 μl of water. The DNA was then denatured by heating to 100°C for 10 minutes and quick cooling on ice. The DNA was then amplified in a 100 μl reaction containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 200 μM each of dATP, dCTP, dGTP and dTTP, 2.5 units of Taq polymerase (Perkin-Elmer Cetus) and 1 μM of each of the two oligonucleotide primers. The reaction was overlayed with 100 μl of mineral oil and amplified for 30 cycles in a Perkin-Elmer Cetus thermal cycler. Each cycle consisted of 1 minute at 94°C, 2 minutes at 55°C and 3 minutes at 72°C. The amplified DNA was then denatured by heating to 100°C for 10 minutes and quick cooling on ice. Aliquots of 5 μl were then spotted onto nylon membranes (Biodyne A, Pall) using a Minifold apparatus (Schleicher & Schuell). The membranes were then baked at 80°C for 2 hours and hybridized with 32P-labelled oligonucleotide probes whose sequences were specific for the previously described "Indian" mutations. Subsequently, amplified DNA containing the mutation was sequenced using T7 DNA polymerase (Sequenase)

A total 110 β-thal genes were characterized. To date, 10 of the 91 β-thal mutations known worldwide have been identified in this subcontinent population.

Table: Ethnic distribution of β-thal mutations from the Indian Subcontinent

Mutation	Ethnic group: Sindhi	Punjabi Sikh	Gujarati	Ind. Musl.	Pak. Musl.	Others	Total %
IVS1 n5	1	5	13	4	10	6	39 35.4)
619 bp deletion	7	4	1	5	6	2	25(22.7)
Fr c8/9	2	3	2	4	5	2	18(16.4)
Fr c41/42	3	5	1	0	0	1	10(9.0)
IVS1 n1	3	1	1	0	1	0	6(5.4)
Fr c16	0	0	1	0	4	0	5(4.5)
Non c15	0	0	2	1	0	0	3(2.7)
Cap site (+1)	0	2	0	0	0	0	2(1.8)
−88	0	1	0	0	0	0	1(0.9)
25 bp deletion	0	0	0	0	1	0	1(0.9)
Total	16	21	21	14	27	11	110

Five mutations accounted for the majority (89%) of all alleles. Twenty-nine (48%) families had the the same mutation in both parents indicating a higher incidence of homozygosity for a particular mutation than that seen in other part of the world (Re: Kazazian H H Jr. Seminars in Hemat. 1990. 27: 209–228). This is probably due to the conservative practice practice of marrying within the same ethnic group, still prevalent in India. The Five reported consanguineous marriages (all muslims with mutations at Fr c8/9–2; IVS1 n5–1; 619 bp deletion–1; Fr c16–1) serve to emphasize this observation. We have been able to divide the carriers into 6 broad ethnic groups. Several regions known to have significant incidence of β-thal (eastern India especially West Bengal) still remain uncharacterized because of lack of representation in this selected study. The two most common β-thal mutations are documented in all ethnic groups. However, IVS1 n5 is very common among Gujaratis & Pakistani Muslims. The fact that 13/21 Gujaratis have this mutation makes it the first diagnostic option in any patient from this ethnic group. We have been able to confirm that the 619 base pair deletion is common among Sindhis (7/16). Previously it was reported to be localized in Sindhis and Gujaratis (Thein et al 1983 Br. J. H.)

It is now shown that it is present in all the ethnic group studied. The families from Punjab + Gujarat show almost all the known mutations. However, it is possible that the only two Cap site (+1) mutations appear in the Punjabi Sikh. It is interesting to note the differences between Muslim Indian or Pakistani origin. Larger studies will determine if these differences are significant.

We realize that this study has an important limitation. Assignment of ethnic background is based on the information provided by the family and/or referring Physician. Complete information is available on most but not all families. All those who could not be assigned a definite ethnic group have been tabulated under "Others". Nevertheless, we feel this study is a positive step toward creating a "mutation map" of β-thal in the Indian subcontinent.

The Thalassemia Syndromes: Molecular Basis and Prenatal Diagnosis in 1990 by Hariz H. Kazazian Jr. Seminars in Haematology (1990) Vol 27 IV No 3 (July 1990) [Hariz H. Kazazian Jr. MD Centre for Medical Genetics, Dept of Pediatrics and Medicine, The Johns Hopkins Univ. School of Med. Baltimore MD 21205] Genetics in Haematology II Helen M. Rainey MD. Guest Editor

Structural Haemoglobin Variants That Produce the Phenotype of Thalassemia Junius G. Adams III and Mary B. Coleman. Seminars in Haemat. Genetics in Haemat II. p. 228-238 [Junius G. Adams III MD, Chief Haemoglobin Research Lab. (151), VA Medical Ctr. Jackson MS 39216].

Southeast Asian Immigrants: The New Thalassemias in America Helen M. Anderson & Helen M. Rainey Seminars Haemat. Gen in Haemat II p 239-246 [Helen M. Rainey MD. Veterans Affairs Med Center (11F), 3350, La Jolla Village Dr.

Newton CR; Heptinstall LE, Summers C, et al
Amplification Refractory Mutation System for
Prenatal diagnosis and Carrier assessment
in Cystic fibrosis LANCET (1989) ii: 1481-82.

San Diego. Ca. 92161]
Modek B; Petrou M, Ward RHT. et al
Effect of fetal diagnostic testing on birth
rate of thal major in Britain. Lancet
(1984) 2: 1383-1386
Distribution & Control of Some Genetic
disorders. World Health Stat. Quart.
41: 209-218 (1988) Modell, B &
Bulyzhenkov V.
Review of Control programme & future
trends in the United Kingdom. Birth Defects
Original Article Series (1988) 23: 433-42
Rapid detection and prenatal dia-
gnosis of β-thalassemia: Studies in
Indian and Cyprid population in the UK
J. M. Old, N. V. Varawalla & D. J. W.
Lancet ~~[illegible]~~ 336-834-37, (1990). [Sir David J.
Weatherall, National Haemoglobinopathy
Ref. Laboratory, Inst of Mol. Med.
John Radcliffe Hospital, Oxford OX3 9DU.
UK] The application of the Amplification
refractory mutation system (ARMS) to the
detection of individual β-thal mutations
in heterozygotes parents and "at risk"

The ~~amplification~~ Analysis of any point mutation in DNA. The Amplificat. Refractory mutation system (ARMS) Nucl. Acid Rs (1989) 17: 2503-16. Newton CR, Graham A, Heptinstall, LE et al.

fetuses has been assessed in Indian and Cypriot immigrant populations in the UK, 100 first trimester prenatal diagnoses have been made, entailing the detection 17 different mutations. The method, which allows the determination of the mutations in both parental and fetal DNA on the same day, should have wide application to the carrier detection and prenatal diagnosis of monogenic diseases with heterogeneous molecular defects.

Molecular pathology of single gene disorders is very heterogeneous eg. ~~90~~ over 90 different mutations are known to produce the β-thalassemia phenotype, and studies suggest that Cystic fibrosis, monogenic hypercholesterolemia, Duchenne muscular dystrophy, haemophilia A and B & other genetic diseases have an equally varied molecular basis.

These observations have major implications for screening & prenatal diagnosis programmes aimed at secondary prevention

of common genetic diseases. Prenatal diagnosis of β-thal in the UK offers a good example of the difficulties that will be encountered because of the racial heterogeneity of the immigrant population. In each ethnic group there are a few common mutations & a variable no. of rare ones. The existence of so many different mutations means that fetuses at risk for β-thalassemia may be compound heterozygotes, that is they will have inherited two different β-globin gene mutations. Therefore, to provide a comprehensive prenatal diagnosis programme for β-thal or similarly heterogenious recessive conditions individual mutations in the DNA of parents and fetuses must be identified rapidly. Until recently this entailed the expensive & time consuming process of preparing individual oligonucleotide probes for each mutation, labelling them with ^{32}P, and identifying each mutation by "dot blot" analysis of amplified DNA in decreasing order of frequency for each mutation for a particular racial group (@ Saiki R.K.;

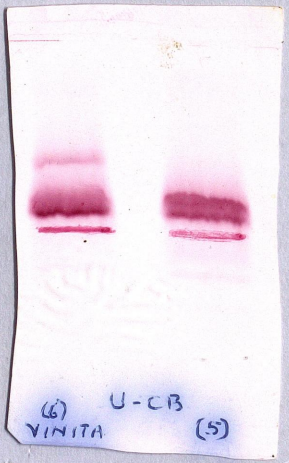
Bougeard TL, Horn GT, Mullis KB, Erlich HA. Analysis of enzymatically amplified β-globin and HLA-DQα DNA with allele specific oligonucleotide probes Nature, 1986; 324: 163-66)
A modification of the polymerase chain reaction (PCR) called the amplification refractory mutation system (ARMS) has been developed, based on a specific mutation, the technique requires two oligonucleotide primers identical in sequence except for the terminal 3' nucleotides, one of which is complementary to the normal DNA sequence & the other to the changed nucleotide in the mutant DNA. For the primer to act as a template for the DNA polymerase enzyme the terminal 3' nucleotide has to be perfectly matched to the target DNA sequence. Under carefully controlled conditions a primer with its terminal 3' nucleotide mismatched (for example the normal primer hybridized to the mutant genomic DNA) will not function properly and no amplification occurs. We have evaluated the method for the prenatal diagnosis of β-thal in populations in the U.K. originally from Cyprus and the Indian Subcontinent

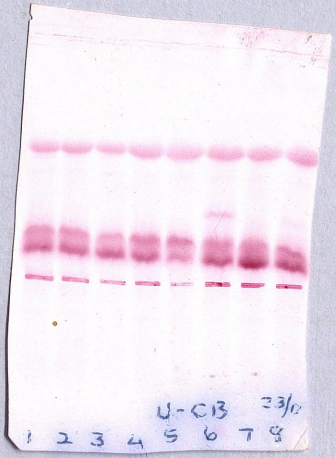
PCR Conditions:- Genomic DNA was isolated from peripheral blood cells and chorionic villus sample (JM Old ed. 'Methods in Hematology vol. 6 The Thalassaemias, 1982. 74-102. Edinburgh, Churchill Livingstone). PCR was done in a mixture of 10 mmol/l ~~Tris~~ "tris" (pH 8.3), 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, and 0.01% gelatin. 0.5 to 1 µg of genomic DNA was added last to the PCR mixture in a total vol of 25 µl containing 0.2 µ mol/l of each of four primers (see below for details) and 0.5 units of 'Amplitaq' enzyme (Cetus). The mixture is overlaid with 25 µl of light paraffin and subjected to 25 cycles of 93°C for 1 min, 65°C for 1 min and 75°C for 1.5 mins with a final extension period of 3 min at 72°C in Techne ~~PHC1~~ PHC1 programmable heating block. 20 µl was then removed, mixed with 5 µl of 15% "Ficoll" and 0.05% bromophenol blue and loaded on a minigel of 1.5% agarose and 1.5% "Nusieve" agarose (FMC Bio Products). After electrophoresis at 100 v for 30 mins the gel was stained with ethidium bromide & photographed under ultraviolet illumination.

Design for ARMS primers:- Oligonucleotide

primers were synthesized by the Oswell DNA Service, at the dept of Chemistry, Edinburgh Univ. and purified by high pressure liquid Chromatography. ARMS primers were designed to detect normal and mutant DNA. The 3' nucleotide of the mutant primer was complementary to the normal DNA sequence. A single mismatched base (the most suitable mismatch and its position found by trial and error) was introduced three or four nucleotides from 3' end of each primer to enhance their specificity. A control pair of primers were included in each assay. The control primers amplify a region of DNA a short distance from that amplified by the ARMS primers; and must produce a fragment of a size distinguishable from any produced by the ARMS primers. Initially, 24-nucleotide primers were used for both the control and ARMS amplification; however, increasing the length to 30 nucleotids permitted a 65°C annealing temperature, thus preventing non-specific bands and improving the specificity of the ARMS primers.

↓Hb – BART'S ↓





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Primers for Indian β-thal mutations :-
Primers* (*Details of oligonucleotide sequences of primers for Indian & Cyprus mutations & RFLP analysis are available from the Lancet) were developed to detect 10 mutations in the Indian population. The Control primers A and B amplify an 861 bp fragment from the 3' end of the β-globin gene. Each primer lies on either side of a 619 bp deletion, which was one of the five most common Indian mutations (Dhar S.L. et al The molecular basis of thal major and thal intermedia in Asian Indians : application to prenatal diagnosis (Br. J. Haemat (1988) 70: 225-31) Therefore they directly detect thalassemia mutations by amplifying a characteristic fragment of 242 bp. The other four mutations, which with the 619 bp deletion comprise 90% of Indian β-thal, are intervening sequence nucleotide numbers 5 (IVS I nt 5) (G to C), frame shift (Fr) 8-9 (+G), IVS 1 nt 1 (G to T) and Fr 41-42 (-CTTT). DNA samples were screened for these mutations using four separate reactions; each containing two control primers, the common primer C (the primer coupled

to either normal or mutant ARMS primers, and one of the mutant ARMS primers for each of the four mutations. After amplification & electrophoresis, the presence of an amplified band in addition to the Control band of 861 bp in one of the reaction designed a particular β-thal mutation. If a band of 242 bp was observed in all four of the tracks, the mutation present was the 619 bp deletion gene. If no band apart from the 861 bp Control was observed in all four reactions, it could be concluded that none of the five Common mutations was present, and the DNA Sample was screened in a similar fashion for the five less frequent Indian mutations.

To diagnose individuals homozygous for a β-thal mutation ARMS primers for normal DNA are required so that homozygous and heterozygous DNA can be distinguished. Initially the normal primer for IVS1 nt1 repeat gave a band with DNA Samples from patients homozygous for the IVS1 nt1 mutation as determined by allele-specific oligonucleotide dot blot ~~mutation~~ analysis. The primer was re-made in the reverse direction with its 5'

and upstream of the mutation site. On re-testing the combination with common primer D it did not produce an amplified fragment with DNA samples homozygous for the IVS1 nt1 mutation (data not shown).

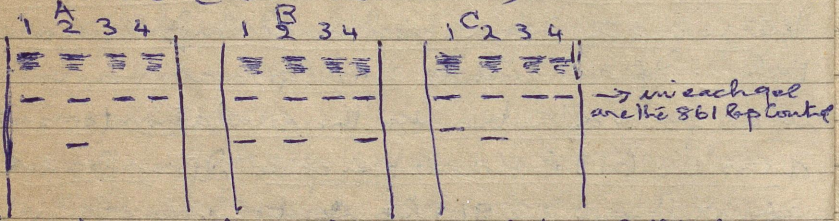


Fig. 1. ARMS analysis of Indian mutations. Gel (A) shows analysis of an unknown DNA sample with the primer for the IVS1 nt 5 mutation (lane 1), IVS1 nt 1 (2) Fr. 31-42 (3) and Fr. 8-9 (4). Gel (B) shows a parental diagnosis for the IVS1 nt 5 mutation: Lane 1 and 2 = maternal & paternal DNA respectively with IVS1 nt 5 mutant primer. Lane 3 = normal DNA with IVS1 nt 5 normal primer, lane 4 = Chorionic villus DNA with IVS1 nt 5 normal primer, lane 5 = Chorionic villus DNA with IVS1 nt 5 mutant primer. Gel (C) shows Chorionic Villus DNA with IVS1 nt 5 mutant primer (lane 1) and Fr 8-9 mutant primer (2) and normal DNA with IVS1 nt 5 mutant primer (3) and Fr. 8-9 mutant primer (4). The upper bands in each gel are the 861 bp control bands.

Primers for Cypriot β-thal mutations:- ARMS primer sequences (~~Details of oligonucleotide primers were developed~~ were developed (Details of oligonucleotide sequences of primers for Indian & Cypriot mutations and RFLP analysis are available from the Lancet) both normal and control to detect each of the seven mutations found in the UK Cypriot population. All

The mutant ARMS primers were used in combination with Common primer C, except for the primer for IVS II nt 745 mutation which is too far away. The Control primer B was used in this case as a Common primer. Primers A and B Could not be used as Controls with mutant IVS II nt 745 primer, so two primers which amplify a 323 bp fragment across the Hind III polymorphic site at Gγ-globin gene were used instead (primers E & F). The IVS I nt 10 mutation is the most Common found, and all parental DNA samples were screened for this mutation first. If it was not found, each DNA was screened for 6 other 6 mutations.

Primers for haplotype analysis:- Primers were used to determine β-globin gene haplotypes. Primers for Hind III Gγ gene restriction fragment length polymorphism (RFLP) & the Hinf I βA gene RFLP are as described previously (Kulozik AE et al Brit J Haem (1988) 70: 355 & Semenza GL, Dowling CE, Kazazian HH Jr. Hinf I polymorphism 3' to the human β-globin gene detected by the PCR Nucleic Acids Res (1989) 17: 2376). The same PCR conditions as described above were used

for 30 cycles, and the products ~~[illegible]~~ visualized on a 3% agarose gel following digests ≥ 10 units of appropriate enzyme. Direct sequencing of the PCR products (see for method Thein S.L. et al Molecular Characterization of a high A_2, β-thal by direct sequencing of single strand enriched amplification genomic DNA Blood (1989) 73: 924-30 ~~[illegible]~~ (Kertz KA, Carson GS, O'Brien JS, Direct sequencing from low-melt agarose with Sequenase Nucl Acids Res (1989) 17: 5864). Every prenatal diagnosis were result by ARMS was checked by RFLP linkage analysis or direct DNA sequencing.

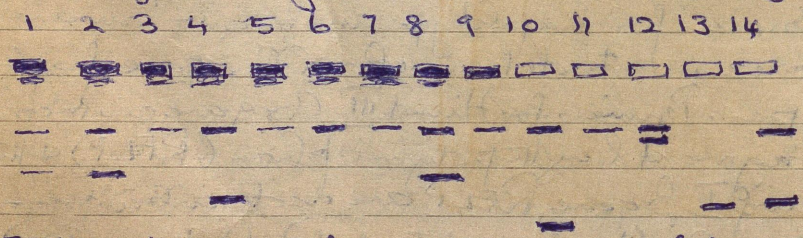


Fig 2 — ARMS analysis of Cypriot mutations. Odd numbered lanes contain an unknown DNA sample even numbered lanes contain a control DNA with the appropriate mutation. Mutant-specific ARMS primers used were IVS1 nt 110 (lane 1&2); IVS1 nt (3,4); IVS1 nt 6 (5,6) Codon 39 (7,8); Codon 6 (9,10), IVS1 nt 1 (11,12) and IVS11 nt 745 (13,14). The upper bands in lanes 1-12 are the 861 bp control bands. The lower bands in the lanes 13,14 are 323 bp control bands from primers E+F while the upper

lo and in lane 14 is produced by the ARMS primer. Fig 1 shows the detection of various Indian mutations. A typical screening for the five most Common mutations is an uncharacterized DNA Sample from a β-thalassemic heterozygote is shown in fig 1A. An amplified fragment was produced by IVS I nt 1 mutation primer in lane 2. No band was observed with the other three ARMS mutation primers, indicating that this individual Carries IVS I nt 1 mutation.

A foetal diagnosis for IVS I nt 5 mutation is shown in fig 1B; lanes 1 and 2 show the presence ~~with both of parents of an amplified band with~~ with both parents of an amplified band with the mutant IVS I nt 5 ARMS primer. Lane 3 shows DNA from their normal Child which did not produce an amplified fragment of the mutant primer. Foetal Chorionic Villus DNA was tested both the normal IVS I nt 5 primer (lane 4) and the mutant primer (lane 5). An amplified fragment was seen only with mutant primer, indicating there was no normal DNA at IVS I nt 5 mutation position and a diagnosis of β thal major was made. This was confirmed by RFLP linkage analysis. For Couple whose Child is at risk from more than one β-thal mutation the normal

ARMS primers are not required. An example of a prenatal diagnosis for a Couple heterozygote of IVS1 nt 5 and Ex 8-9 mutations is shown in fig 1C. First two lanes show an amplified fragment for each mutant primer, indicating an affected fetus. The controls in this case were normal DNA samples tested with same mutant primers. Again the diagnosis was confirmed by RFLP linkage analysis. Fig 2 shows results of an uncharacterised DNA sample from a Cypriot β-thal trait testis with each of the seven ARMS primers (odd numbered lanes), and the results produced by DNA samples from individuals carrying each of these seven mutations (even numbered lanes). The uncharacterised DNA sample produced a band with IVS1 nt110 mutation primer and all the at-risk couples screened, either each partner had the IVS1 nt 110 mutation + the other had one of the other six mutations. Although normal ARMS primers have been synthesized for each of these seven mutations, only the IVS1 nt110 and IVS1 nt1 normal primers have been tested because of lack of DNA from individuals homozygous for the other five mutations. However, the

prenatal diagnosis in 73 cases to date (table) only the normal IVS1 nt 110 primer has been required. In all but one family the result obtained with ARMS primers have confirmed those predicted by RFLP linkage analysis. The exception was a case in which the ARMS primers predicted a normal fetus but RFLP analysis indicated a heterozygote for IVS1 nt 110 mutation. Further testing of the IVS1 nt 110 mutation with ARMS primers showed that two samples had been mislabelled and the wrong haplotypes assigned. This was confirmed by analysing fresh DNA samples and obtaining haplotypes which predicted a normal fetus.

Discussion:- The results show that ARMS could make possible a comprehensive prenatal diagnosis programme for β-thalassaemia. The method is extremely quick and samples of DNA samples could be screened for the five common mutations found in the UK Indian population in just 3 hr; and the remaining 10% of the samples could be screened for rare mutations later in the same day. This compares well with other

PCR-based non-radioactive techniques, such as oligonucleotide hybridization (Saiki RK et al New Engl. J. Med 1988; 319: 537-541) or Colour Complementation assays with fluorescent primers (Chehab FF Kan Proc. N. Acad Sci USA 1989; 86: 9178-82).

We have now used ARMS primers for 32 first-trimester prenatal diagnosis of inherited β-thal mutations. In each case, the diagnosis made by the ARMS approach was confirmed either by PCR-based methods of linkage analysis of RFLP or, in one case when no informative markers were available by direct DNA sequencing. i.e. our current strategy for prenatal diagnosis is to screen the parents first to identify the mutations, and to confirm the diagnosis by RFLP linkage analysis whenever possible. Occasionally novel mutation will be encountered, and RFLP linkage or if time permits, direct DNA sequencing provide an alternate approach. This strategy can be adopted for other ethnic groups at risk for β-thal as indeed we have done for UK Cypriot population. Seven ARMS primers

were sufficient to detect the mutation in every case of 73 at risk couples. In couples where both partners carried the IVS1 nt 110 mutation it was possible to screen for mutation in the parents first & then counsel a prenatal diagnosis on Chorionic Villus DNA later the same day. The approach has proved as reliable as haplotype analysis, and in one case more so with the identification of mislabelled samples.

DNA Analysis (Workshop Manual by John Old, Instr. of MD. Med, John Radcliffe Hl, Oxford OX3 9DU-UK)
[Tel: 222449 - Fax 0865-222500]

The application of recombinant technology & Molecular biology techniques have revolutionized the detection of DNA mutations & sequence variations in thalassaemia in particular & haemoglobinopathies (Sickle cell disease) in general. These techniques were based on the digestion of DNA with restriction endonucleases & the detection of specific fragments by hybridization of a

radioactively-labelled gene probe to the digested DNA after it had been subjected to agarose gel electrophoresis & Southern ~~Blotting~~ blot technique

Sickle cell anaemia was the first disease that was studied. This was accomplished by the indirect method of linkage analysis of a MspI Restriction fragment length polymorphism (RFLP) located close to the β-globin gene

Amplification Refractory mutation System for prenatal diagnosis and assessment in Cystic fibrosis CR Newton C Summers M Schwarz A Graham L E Heptinstall M Super R Anwar J C Smith & A F MARKHAM Lancet (1989) ii: Dec 23/30, p: 1481-83 [AMF: ICI Diagnostics, Cadbrook Park, Northwich, Cheshire CW9 7RA UK]:

ARMS have been applied to prenatal diagnosis and carrier detection of Cystic fibrosis. The nucleotide sequence of both alleles of the PstI restriction fragment length polymorphism at KM19 locus, which displays linkage equilibrium with Cystic fibrosis

has been determined. ARMS enables direct analysis of alleles of this polymorphism in DNA isolated from Chorionic Villus biopsy or white blood cells. ARMS is a novel technique that allows rapid, easy and accurate analysis of mutations (Newton CR, et al Nucleic acids Res (1989) 17:2503 ~~Chopra TJ, Hershman J M, et al Ann Int Med (1983) 98~~: 946-57). ARMS does not require DNA of purity needed for conventional restriction fragment length polymorphism analysis by the Southern blot technique: Cruse lysates of epithelial cells from mouthwash or DNA extracted from lynth cards are successfully used for ARMS. ARMS analysis on Chorion biopsy specimen genotype 9 of ~~other~~ alternate sources enables rapid anti-natal diagnosis of genetic disorders in which mutations is known or RFLP have been identified. The PSE I RFLP at the KM19 locus displays linkage disequilibrium for cystic fibrosis and has been used for diagnosis of this disease by restriction digestion of DNA amplified by polymerase chain reaction. The KM19 locus displays

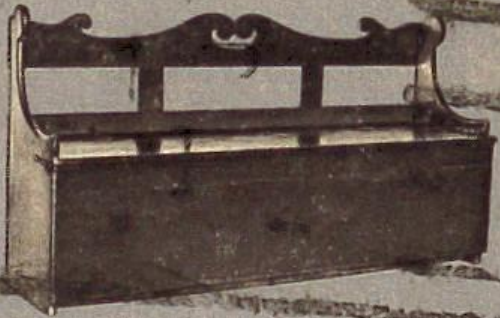
a two allelic polymorphism directed by
by the restriction enzyme PSE I in which
alleles 2 is cleaved by the restriction enzyme
but not allele I was not. We have
used ARMS at this locus for antenatal
diagnosis of Cystic fibrosis in two families
For ARMS analyses 10 ng of DNA purified
from peripheral ~~blood~~ leukocytes or Chorionic
villus sample was used directly with concurrent
amplification of a 220 bp region of Exon V
of human α1-antitrypsin gene.

Liver Histopathologic Finding in Women
with Sickle Cell Disease Given Prophylactic Transfusion During Pregnancy
E. Yeomans; T.W. Lowe; E.H. Eigenbrodt
& F.G. Cunningham, Am. J. Obs. & Gyn.
(1990) 163 (3) 958 [E. Y. Wilford Hall
USAF ~~Surg~~ Med Centre, SGHPG,
Dept. Obs. & Gynec. Lackland AFB, Tx.
78236. USA.

Observations on the levels of HbA_2
in pts with different β-thal mutation
and delta-chain variant.
J.F. Codrington, H.W. Li; F. Kulten
L.H. Gu, M. Ramachandra & T.H.J. H

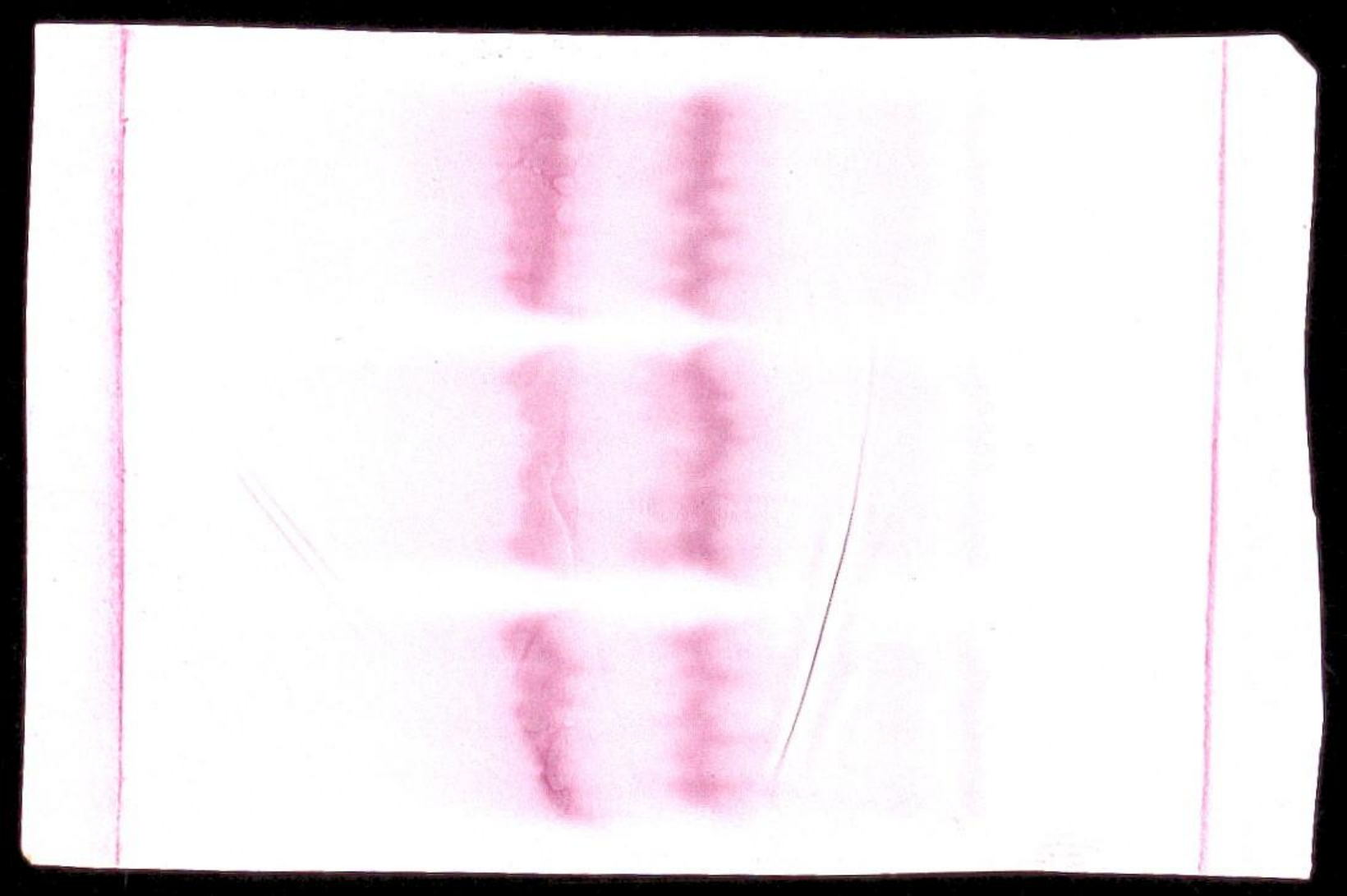
Blood (1990) 76 : 1246 [T.H.J.H].
Iron Stores and iron deficiency anaemia in children heterozygous for β-thal. R. Galanello; M.P. Turco; S. Barella; N. Giagu; C. Dessi; G. Cornacchia; A. Co. Haematologia (1990) 75 : 319 [A. Cao Univ. of Cagliari I Clin. & Bioetà Evolut. 1-09100. Cagliari. Stag]
Pyrimidine 5'-nucleotidase and oxidative damage in red cells transfused to β-thal children by O. David; L. ~~Sacch~~ ~~Saccheti~~ Saccheti; M.G. Vota; L. Comino et al. Haematologia (1990) 75 : 313 (O.D. Univ. Turin, I Clin Pediatr. 1-10126, Turin Stag].

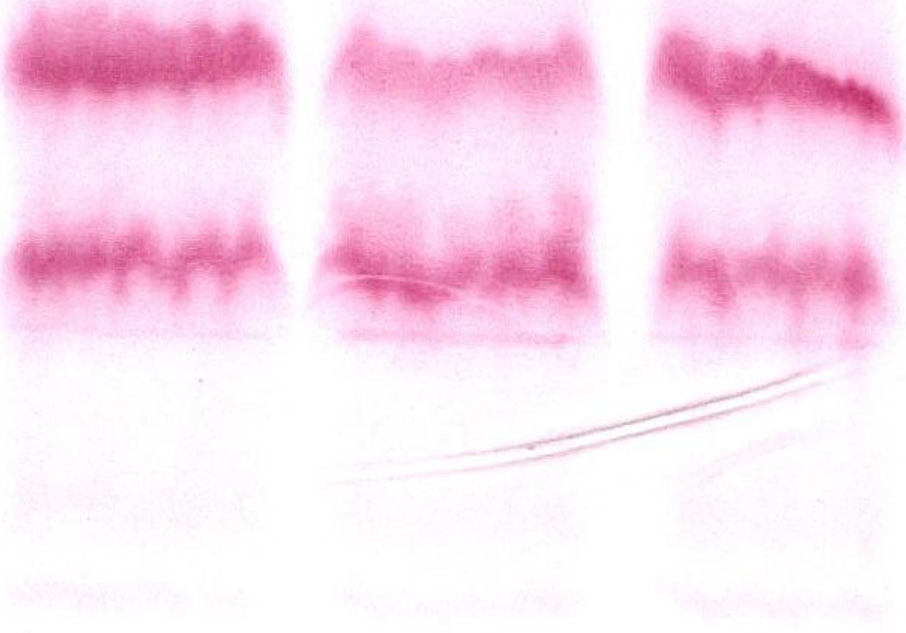
Canada 39



Heritage/Patrimoine

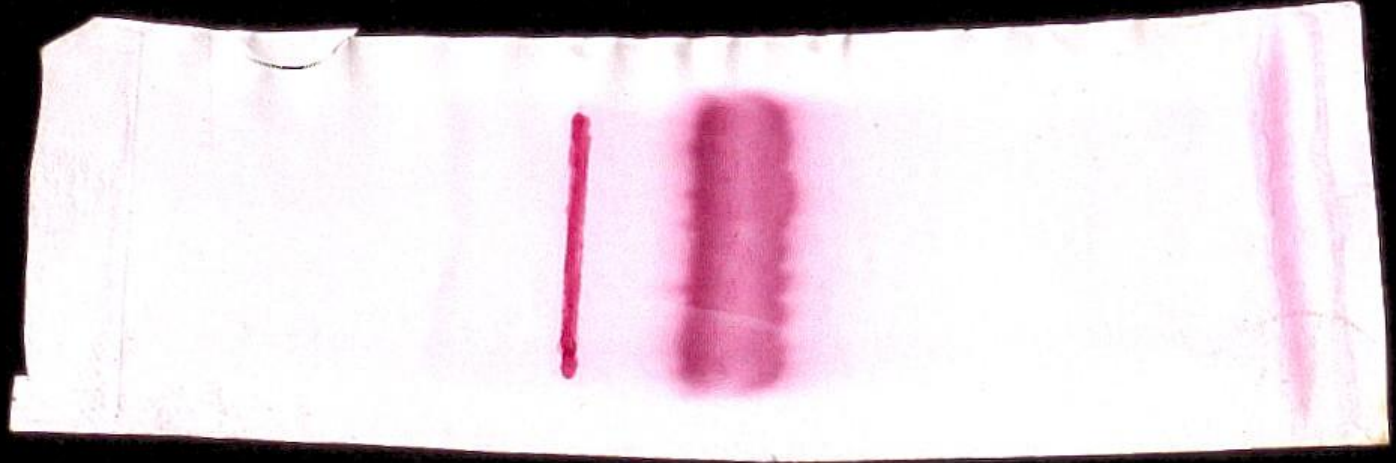
Settle-Bed/Banc-lit





22%40

ASHOK YOGESH SHOBHA



CLEAR P. 30,000/5/87

URGENT

Bai Jerbai Wadia Hospital for Children

Department of Radiology

(Please fill in Block Letters)

Please do not use abreviations. Medical Officer must sign and not initial.

X-Ray No. 11660 Ward No. _____ Cot _____

Hon. Dr. MGY. O. P. D./I. P. No. _____

Patient's Full Name : Dr. SUKUMARAN. Age 70. Sex M

Clinical history and Clinical Hematological, Biochemical & Pathological findings :

Provisional Diagnosis ? # (R) TIBIA.

Radiograms required X-RAY. (R) leg AP. LAT.

10×12

Date 8/11/90

Medical Officer's Signature

(P. T. O.)

X-RAY FINDINGS :-

There is no evid of # noted in tibia-fibula

There is no soft tissue calcification noted.

Cf.

HON. RADIOLOGIST

Dr. Gande Sushensha HC

Res. 4947373

HC : 4552550

for 15.6.90
at 10.00 AM

1981

Ind. Soc. Human Genetics

Ind. Statistical Inst.

Calcutta

Ulhasnagar Rough Statistics (Dr. MS Wathe)

Total Population — 4.5 lakhs.
Swidhi Population — 3.5 lakhs in 1990.
No. of births Jan to Dec 1989 — 10,396.
No. of Children born at Hospitals — 9300
" " Home — 1636 — 10936

10000 That trait would be about 800-900
and about 20-22 new cases will be born.

Meeting with Dr. Wadhwa 21-9-90

1. Prenaarital Studies — voluntary
2. Pregnant mother's carried out for β-thal & if mother, a carrier husband examined, & if found +ve prenatal testing in the foetus.
3.
3.

Epidemiology Studies — High Risk Groups

	Total	Thal Trait	GPD	Other Abn.
Sindhis →	354	31 (8.75)	10	AD-14; AJ 2; AS-1; AQ-3; DD/DT-2
Lohana	285	22 (7.72)	~~8~~	~~AD 13; DT-1~~;
Khojas	512	31 (6.1%)	8	AD 13; DT-1 AQ 1; ↑F-1

Earlier Studies

	Thal
Mixed Sindhis	7.1%
Sindhi Lohana	6.2"
Sindhi Ungar	12.4"
Cutchi Lohana	10.7"
Halai " "	17.2.

From 1983 to 1989.

Thalassemia major — 225 cases — other thal δβ; STh; DTh; QThal δβ/β; HPFH etc.
" Trait — 779 cases besides other haemoglobinopathies

Total Population of India — 750 million — 1981
Incidence of β-thal Trait (10-17%) — 1 to 3%.
Thal. ~~[illegible]~~ carriers will be — 75 to 22 million (1/10 to 1/20 of World Pop)
Thal. Major — 5000 to 6000 per year.

THALASSAEMIAS IN INDIA.

ALPHA - TYPES

Hb H DISEASE
Hb BARTS.

BETA - TYPES

β°
β^{+}
$\delta\beta$
HPFH

SUKUMARAN
PESHKARS, KAYYATH
TELLICHERR

Greetings No. 6

Sukumaran

RAGHAVAN
B-25 GANDHINAGAR
WEST HILL CALICUT-5

Greetings 16

CHARU and K.KRISHNAIA


भारत
INDIA
50
गांधीजी GANDHIJI


19 9/90 PLAIN HB
SULAMAN





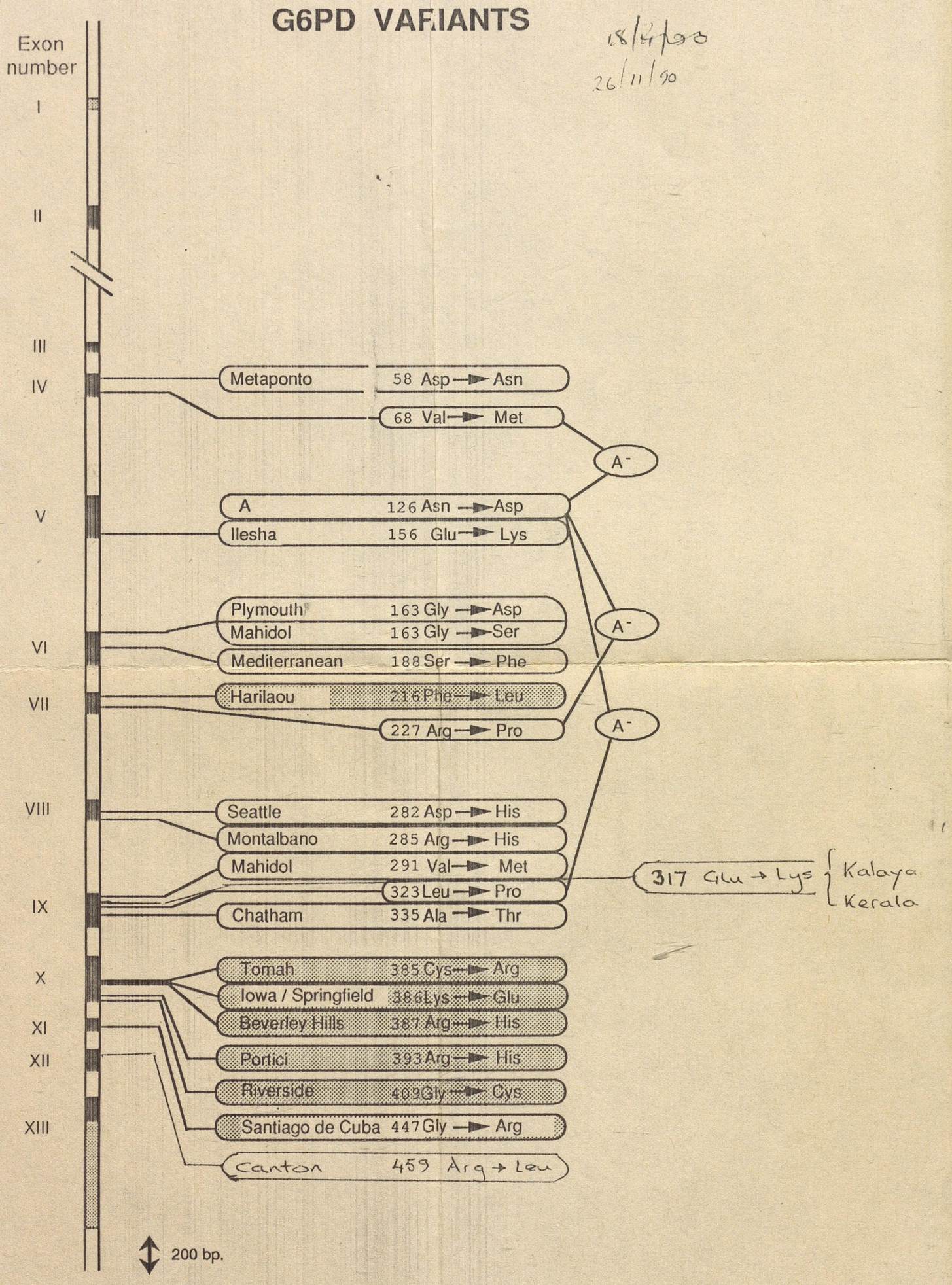
19-9 PLAIN HB
SULAMAN

Dr. Tom Vulliamy

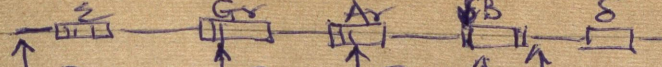
Haematology Dept.
R.P.M.S.
Hammersmith Hospital.
Ducane Rd.
LONDON, W.12 ONN.

G6PD VARIANTS

18/4/90
26/11/90



Apr 1990



	Hinc II	Hind III	Hind III	Hinc II	Ava II	Bam H I
I	+	−	−	− −	+ +	4/4 IVS-1 110
II	−	+	+	− +	+ +	3/3 Nav-Sense Co 3G
III	−	+	−	+ +	+ −	7/7 5' IVS-2 Splice
IV	−	+	−	+ +	− +	1/4 F S Co. 8
V	+	−	−	− −	+ −	1/1 5' IVS-1 Splice
VI	−	+	+	− −	− +	IVS-1 Consensus
VII	+	−	−	− −	− +	5/6 IVS-2 7'45
VIII	−	+	−	+ −	+ −	1/1 .87 Sub.
IX	−	+	−	+ +	+ +	1/1 Non Sense Co 39

In order to label DNA radioactively to make a hybridization probe a technique called Nick translation is used. DNA can serve as a template primer for the enzyme DNA polymerase I if it contains a single stranded break (Nick) with a 3'-OH terminus. Appropriate nick can be introduced into the duplex DNA with various nucleases. The DNA is labelled by incorporating 32P-labelled deoxyribonucleoside-5-triphosphate (dNTPs) at the -3'-OH terminus of the nick by the action of DNA polymerase. In this way it is possible to prepare highly radioactive pure Oligonucleotide Probes. — The gene probes mentioned above are large molecules which identify individual genes or parts of them. For this reason they hybridize with long stretches of complementary sequences but are NOT capable to recognize single base changes or similar small structural alterations. The ability to identify base changes in mutant genes is essential for working out the molecular pathology of human disease. Recently, a new approach to this problem has been developed which makes use of short synthetic DNA fragments called oligonucleotides which are constructed specifically to detect single base changes in DNA

reassociated in vitro by heating & boiling.
It is also possible to form double stranded ~~DNA/RNA~~
DNA/RNA molecules in this way. The reannealing
reaction is highly specific & under suitable conditions
occurs only between DNA or RNA strands which
have complementary or nearly complementary
base sequences. So if we wish to look for a
particular gene buried away in a large ~~amount~~
amount of DNA we might make a length of
DNA with a complementary sequence which will
anneal to the gene, but not to the rest of DNA.
This is the principle behind using gene probes
to screen for ~~DNA~~ genes we are interested in.
In 1970 an enzyme RNA-dependent DNA-poly-
merase or reverse transcriptase was isolated from
certain RNA tumor viruses. Reverse transcriptase
can be used to synthesize a DNA copy (complementary
DNA or cDNA) from any mRNA that can be
isolated from mammalian cells. If radioactive
bases are added to the reaction ~~[illegible]~~
~~[illegible]~~ the synthesized cDNA can be labelled
& hence used ~~[illegible]~~ as a hybridization probe to look
for complementary sequences in either genomic
DNA or in cellular RNA by DNA/DNA or DNA/RNA
hybridization. This can now be immobilized on
nitrocellulose.
Earlier cDNA probes were prepared from partially
purified RNA which might consist of several different
mRNA molecules. Now it is possible to clone
cDNA sequences in bacterial plasmids. This
is done by ~~[illegible]~~ synthesizing a second DNA
strand on a newly synthesized cDNA using a
bacterial DNA polymerase. It is now possible
to clone fragments of DNA into plasmids or
bacteriophage & to amplify individual genes
in E. coli making genomic probes. We have
three main sources of genomic probes: cDNA,
cloned genomic DNA, and DNA fragments pre-
pared from genomic DNA.

is copied into RNA. Chemically RNA is similar to DNA except for two differences; the sugar of DNA is deoxyribose while in RNA is ribose and instead of T thymine RN contains the closely related pyrimidine base Uracil. The synthesis of RNA on DNA template is very similar to DNA replication in that it involves the formation of complementary base pairs. As in the case of DNA duplicate G pairs with C but when mRNA is being made on a DNA template A pairs with U instead of T. Primary transcript is a large mRNA precursor which corresponds to the entire gene, including exons and introns. This molecule undergoes a series of processing steps before it is ready for delivery to the cell cytoplasm. The introns are cut out and exons spliced together. It is noteworthy that the 5' exon/intron junction always has the bases G & T whereas the 3' junction has A and G. Mesenger RNA does not contain intron sequences.

The splicing reaction mRNA undergoes further modifications while still in the nucleus. It is chemically modified at its 3' end by the addition of a so-called CAP structure, and at its 3' end by the attachment of a long string adenylic acid residues (poly A). The site of poly A addition is thought to be related to the highly conserved AATAAA sequence. It seems quite likely that during its time in the nucleus & for much of its time in the cytoplasm mRNA precursor and its processed product are associated with various protein molecules which may serve to stabilize & protect them from nucleus attack.

In Each human cell double stranded DNA, distributed among the 23 pairs of chromosome, consists of about $3-5.5 \times 10^9$ base pairs which corresponds to the total length of about two meters.

Two strands of DNA can be dissociated

It is now apparent that almost all mammalian genes have their coding regions interrupted by sequences of unknown functions called intervening sequences (IVS) or introns; at varying positions along their length. The number & ~~functions of these~~ size of these introns, which are generally considered longer than the coding sequences (exons) varies from gene to gene. At the 5' end of the gene there is a specific triplet (ATG) which determines the initiation of the protein synthesis on mRNA, and at the en end 3' end there is a termination triplet (TAA, TAG, TGA). Genes also have sequences of varying lengths at both ends which determine the structure of untranslated regions of mRNA. Most of these genes have blocks of non-translated regions of mRNA in their 5' flanking regions which are similar to those found in other species. The first of these which is about 20-30 base pairs upstream (to the left in a DNA strand reading left to right) from the RNA initiation or CAP site and which is now called ATA box, is a sequence which was originally found in the histone gene cluster of Drosophila; the second called CCAAT box is found about 70-90 base pairs upstream from the beginning of the gene - these regions and another which is well defined, & which is about 100 base pairs upstream from the beginning of the gene are involved in the control transcription of mRNA. They may be of particular importance in the ~~to~~ regulation of the level of transcription. In fact they can be called as signal boxes which determines the movement of traffic (polymerase) along the structural gene.

~~MRN~~ mRNA is synthesized on its DNA template in a 5'-3' direction by the action of the enzyme RNA polymerase II when the latter binds to both stranded DNA it causes a localized separation of the two DNA strands; but only one of it is

emulsion sensitive to the β-particles of ^{32}P adjacent to the nitrocellulose filter [illegible]. It is also possible to clone globin genes. This is done by fragments of DNA containing the genes are introduced into the DNA of certain bacteria by use of plasmids or bacteriophages. Replication of the bacteria in a medium for experiment generally large amounts of the introduced gene or genes.

In each Chromosome 16 (Cluster of two α genes + 2 ζ-like genes) two α genes loci are separated by approximately 3700 base pairs (3.7 kb) one is α1 and the other upstream or leftward (in the 5' direction) α2. Further upstream are two ψ (nonfunctional) α genes ψα1 and ψα2 and further upstream still are two ψ-like a ψ ζ-like (ψζ) and a ζ-like ζ gene. Downstream on the right ward (in a 3' direction) is the theta (θ) 1 gene. In each Chr 11 the δ-globin locus lies approximately 7 kb upstream or leftward (in the 5' direction) from the β-globin gene locus further upstream (approx 15 kb) is the Aβ-gene locus with a ψβ gene in between. Approx 5 kb upstream from Aγ gene is Gγ gene, the epsilon (ε) is further upstream still.

Analysis of globin gene p 507. Dacie
Haemolytic anemia Part 2. III Edn.
Obtain DNA from 10-20 ml of blood which
~~is enough to~~ will give enough DNA for analysis
~~The~~ Next extract DNA from Blood buffy coat
+ submit the extract to the action of restriction
endonuclease (bacterial origin) which will
cut up DNA into small fragments, different
enzymes cleaving DNA ~~into~~ nucleotide se-
quence at specific places known as restriction
sites. The product of this enzyme digestion
can be separated by electrophoresis in agarose
gel, the migration rates of the fragment being
inversely proportional to their molecular size.
Next, the fragments are transferred from the
agarose gel to a sheet of nitrocellulose by the
blotting technique originally described by Southern
(1975). Fragments are then submitted to possible
hybridization with 32 Platelet Complementary
DNA (cDNA) probes, prepared in the case of probes
for α- or β- ~~chain~~ globin genes from mRNA
obtained from Reticulocyte using enzyme
Reverse transcriptase that can synthesize
DNA on an RNA template (such enzymes
were first described by Baltimore 1970;
Temin & Mizutani, 1970) In this way it is
possible to locate the α- ~~and~~ or β globin genes
amongst the isolated + separated fragments
of DNA, the bound labelled cDNA being demons-
trated by autoradiography after placing an

Leave at R Temperature for some time (10 mts or so) First gelly like pple sea mix & the contents becomes cloudy. Centrifuge for 15 mts at 2500 rpm in cold.

Supernatant slightly coloured ~~but may be due to slight~~ ~~[illegible]~~ Precipitate at bottom proteins

Supernatant divided into 2 tubes, to each add double amount (9 ml) of dist ethanol, mix by inversion with a rubber stopper, DNA pple as a floating material seen, add the whole contents to the other tube with ~~[illegible]~~ ethanol, mix by inversion keep for ½ hr at ~~[illegible]~~ 4°C. Then, Centrifuge, add 70% ethanol, keep in refrigerator. Next day, Centrifuge, wash 2 washes with 70%.

Salting out method for the preparation of DNA (Miller)

5ml blood (EDTA) pH 7.5 Centrifuge ~~WBC (buffy coat)~~ remove plasma + Double Dist water 3 ml Centrifuge at 2500 rpm for 5 mts. Remove supernatant discard. To the (WBC, RBC stromaetc) add dist water (about 15 ml H_2O) mix. Centrifuge for 10 mts at 2500 rpm WBC pellet is broken by tapping To this added 6 ml of lysis buffer (Tris; HCl; Na EDTA pH 8.2 + SDS 0.2 ml of (20% SDS) + 0.5 ml Proteinase K (Proteinase K power - 25 mgm + 625 µl of SDS 20% + 50 µl of EDTA (S2) make vol to 12.5 ml with Double D. H_2O.

Mix Well. Keep at 37°C shaking water bath for overnight (or 48 hrs).

- as the above was 6 ml lysis buffer + 2 ml Satd. NaCl.

Mix well. You will find the content changing opaque

Case of 239-bp products of 30 cycles PCR amplification (Saiki as earlier 1988 Science). From experience, there can be a misincorporation when the smaller amplified fragment (eg 0.83 Kb) has been cloned and sequenced analysed (data not shown) In this paper, where as large as the 3.0-kb amplified fragment was cloned, complete sequencing of five independent clones (14,990 bp) derived from normal DNA revealed no error; thereby suggesting that the amplified products of relatively large size are less likely to be misincorporated during DNA synthesis with Taq DNA polymerase. Amplification of the 3.0-kb fragment facilitated screening by DNA blot-hybridization of all known mutations causing β-thal within only a single PCR reaction. Second, the amplified DNA can be sequenced directly to characterize an unknown mutation. Finally the DNA fragment can subsequently be cloned directly when cloned DNA is required. The approach described here is a rapid one for molecular characterization and molecular cloning for the β-globin gene cluster and other genes.

studies of the mutant allele. When a 110-bp segment of the β-globin gene was amplified using the Klenow fragment and directly cloned into M13 mp10 vector, only 0.9% of the clones corresponded to the direct fragment. To develop rapid entire β-globin gene cloning, we used Taq DNA polymerase and the primers G9 and G10 to amplify the 3.0-kb fragment (Fig. 1). This fragment contains the entire β-globin gene, from 823 bp 5' to the Cap site to 569 bp 3' to the poly(A) site of the β-globin gene. The primers G9 and G10 have within their sequence ~~artificial~~ restriction sites for HincII and PstI, respectively. Therefore after digestion with both ~~Hae~~ HincII and PstI, the 3.0 kb fragment can then be directly cloned into an appropriate vector (eg. pUC13 in this paper). Gel purification of the 3.0 kb fragment before making it more specific to the β-globin gene because amplification creates a trace of non-specific fragment especially with smaller ~~Ame~~ sizes (as in fig 2). In our work all 12 clones selected at random had β-globin gene inserts. Therefore, selection of the clone represents each allele is not difficult. Misincorporation due to the relative lack of fidelity of Taq DNA polymerase (Science 1988, 239, 487-91 Saiki RK et al) might be the only problem with this approach. The misincorporation rate/nucleotide/cycle for Taq DNA polymerase was estimated to be 2×10^{-4} in the

C-A transversion at the Codon for amino acid 35. This mutation changes the normal tyrosine Codon TAC to TAA, an ochre termination Codon. This defect prevents β-globin Chain Synthesis by a premature termination and the β⁰-thal phenotype ensues. This mutation was confirmed by analysis of the β-globin gene, Cloned in a Conventional manner, dot blot hybridization and by direct sequencing of the amplified product. Since this mutation has hitherto not been identified in other ethnic groups, this may be the first report of mutation occurring in a Thai.

Conventional β-globin gene Cloning is time Consuming, labor intensive and a relatively large amount of genomic DNA is required. To avoid this problem, PCR have been used to generate a part of the β-globin gene fragment in vitro which can be directly sequenced or further Cloned into the M13 vector (Scharf S.J., Horn G.T. + Erlich HA - 1986 Science 233: 1076-78). At present, direct sequencing of the amplified DNA is the most rapid for Characterization of unknown mutation. However, a Cloned DNA fragment of the gene is favorable for analysis for the following reasons. Almost 200 bp from the sequencing primer can only be resolved by the direct sequencing. Direct sequencing analysis does not usually reveal an association of polymorphic sites with the specific mutation. DNA Cloning is required for the in vitro and in vivo expression

as codon 35. For further confirmation of the mutation, the amplified DNA of the patient and his parents were dot-blot-hybridized with specific oligonucleotide probes for either the allele of the β^{35} ochre mutation or the normal allele.

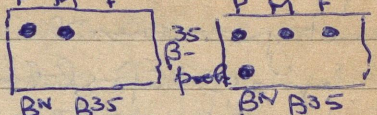


Fig 4

Dot blot-hybridization of amplified DNA of the patient (P), his mother (M) and his father (F) with specific oligonucleotide probes designed to detect β^{35} ochre mutation (See experimental procedure). β^N and β^{35} are the cloned cloned DNA used as controls for hybridization. The patient & his mother are heterozygotes for the mutation where as father does not carry this mutation

The result is, as expected, the patient and his mother are heterozygotes for this mutation whereas father who is heterozygote for β^E does not carry this ochre mutation (fig 4). This mutation was also confirmed by direct sequencing of the region in the vicinity of codon 35 of the 3.0-Kb amplified product of patient genomic DNA.

A molecular defect of this ~~form~~ β-thal was identified by determination of the nucleotide sequence of the clones of the PCR-amplified entire β-globin gene. The patient was a valid candidate for the investigation because extensive screening for known common mutations causing β-thal showed negative results. DNA sequence analysis of the β-thal gene revealed a

sequence of the corresponding region in Clone E is indicated by the open block.

The β-globin exhibits polymorphism limited almost exclusively to common sequence types and designated as β-globin gene frameworks. According to this definition, the Clone B and E sequences were framework 1 and framework 3 Asians, respectively. We analyzed the polymorphic restriction sites in the β-globin gene cluster (Hinc II site 5' to the ε-globin gene, the HindIII site in the $G\gamma$- and $A\gamma$-globin genes, the Hinc II site in the ψβ-globin gene, the Hinc II site 3' to the ψβ-globin gene, the Hgi AI, Ava II sites in the β-globin gene, and the Bam HI site 3' to the β-globin gene) to determine the haplotype of the chromosome bearing this ochre mutation. We found that the haplotype (+, −, −, −, −, −, −, +) was linked to this mutant allele.

Confirmation of the Ochre Mutation by Conventional DNA Cloning, Direct Sequencing and Dot-Blot Hybridization. The β-globin gene of the patient was also cloned conventionally using bacteriophage CHARON 28 as a vector. The 4.9-kb Bgl II fragment from positive recombinants was further subcloned at the Bam HI site of pUC13 before the sequencing. Sequence analysis of the β-globin gene cloned in a vector identified by the lack of an Ava II polymorphic site in the second intron revealed the same C→A transversion

β-globin alleles were selected by the presence of or absence of the AvaII polymorphic site in the second intron of the β-globin gene (Clones E and D). Sequence analysis of both Clones revealed that the HbE mutation (GAG → AAG at Codon 26) is located in Clone E but not in Clone D. Comparison of the nucleotide sequence of Clone D with that of the human β-globin sequence (Pontarotal 1983) revealed only one notable nucleotide substitution in Clone D, the C → A transversion within the codon (TAC) for the amino acid 35 (tyrosine) that produces a nonsense codon (TAA). This substitution

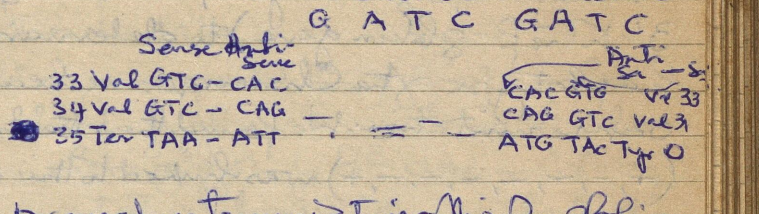


Fig 3. X

causes a premature termination of the β-globin chain synthesis and results in a β° - thalassemia phenotype.

X. DNA sequence of the β-thalassemia gene (Clone D) and β^E-globin gene (Clone E) obtained from direct cloning of amplified DNA. The ladder demonstrates the sequence of anti-sense strand in the region of Exon 2 which codes the sequence for codon 35. Triplet codons and their corresponding amino acids are also shown. The C → A terminal (G → T of anti-sense strand) within codon 35 in Clone D is denoted by a solid circle. The normal

Fig 2

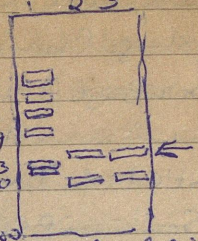


Fig. shows Agarose gel electrophoresis of amplified products. Three µl of amplified DNA was run on 0·7% agarose gel and visualized by ethedium bromide staining (0·5 µg/ml). Lane 1, is λ phage DNA digested with Hind III as size references. Lanes 2 and 3 represent PCR - amplified genomic DNA of the normal individual and the patient Compound heterozygote for β-thal and HbE. Arrow indicates the 3·0 Kb fragment containing the entire β-globin gene

The specific band is approximately 3·0 Kb was clearly seen with a trace of non-specific ~~band~~ fragment of a smaller size, a finding which may indicate a non-specific amplification of the non-β-globin gene region

Direct Cloning and DNA sequencing of the amplified DNA

The 3·0 Kb amplified fragments from both normal individual and the patient were excised from the gel after digestion with Hinc II and Pst I and further cloned into pUC 13 plasmid at an efficiency of about 10^6 transformants / µg of DNA (Maniatis). Clones containing the β-globin gene were easily selected by determining the presence of the 1·3 Kb Bam HI ~~ribosome~~ fragment spanning the Bam HI site in pUC 13 and the Bam HI site in exon 2 of the β-globin gene. The entire inserts of five clones of the normal β-globin gene selected at random were sequence analyzed. No nucleotide change was evident when the sequence was compared with the published sequence for the human β-globin gene except for known polymorphic changes (Lawn et al (Ponte et al JBC, 1983, 258 11594-11609). For the β-thal / HbE patient both

sequence as a 7.8 Kb HindIII fragment in a bacteriophage Charon 28 (Orkin et al 1981, JBC 256: 9782-84). Both β-thal + β^E globin genes from positive recombinants were subcloned as 4.9 Kb BglII fragment in pUC13 for DNA sequence analysis (Hatori 1986).

Southern Blot analysis for Genomic DNA - Restriction site polymorphisms within the β-gene cluster were detected by the Southern blotting procedure using appropriate probes (Fukumaki 1986, Blood, 67: 547-50).

Dot Blot hybridization with allele specific oligonucleotide probes. Two μl of Amplified DNA was dot blotted onto Nylon membrane, and membrane was prehybridized at 42°C for 1 hr in 3× SSPE (0.54 M NaCl, 30 mM NaH_2PO_4 (pH 7.4), 3 mM EDTA (pH 7.4)) 5× Denhardt's Soln, 0.5% SDS, and 1 mg/ml herring Sperm DNA. Each specific oligonucleotide probe, 5'-end labeled with [^{32}P] ATP, was taken added to the same buffer, and hybridization was performed at 42°C for 1 hr. The membrane was rinsed twice with 2 × SSPE, 0.1% SDS and washed once with the same Soln at 59°C for 10 min before exposure to x-ray film at -70°C for 2 hrs. The oligonucleotide probe specific for the mutant allele has the sequence 5' G A T G G T C T A A C C T T G G A C C 3' whereas the normal counterpart has the sequence 5' G T C C A A G G G T A G A C C A C C A 3'.

Results: The entire β-globin gene amplification by PCR demonstrated the electrophoretic pattern of the amplified products for the normal individual, +β-thal/β^E patient after 30 cycles of PCR using primer G9 and G10 (Fig 1)

50 mM KCl, 1.5 mM $MgCl_2$, and 0.01% gelatin. The mixture is incubated at 94°C for 7 min for strand separation before annealing for 2 min at room temperature. Ten units of Taq I DNA polymerase (Stratagene) were added & mixture incubated at 72°C for 7 min. The polymerase chain reaction was carried out on a DNA Thermal Cycler (Perkin-Elmer Cetus Inst). Each cycle of PCR consisted of incubation at 94°C for 1 min, 2 min incubation at 54°C and 7 min of incubation at 72°C. After 30 cycles of PCR, the amplified DNA was desalted and dNTPs eliminated using a Centricon 30 microconcentrator (Amicon Corp.). An aliquot of the amplified products was then double-digested with Hinc II and Pst I. The entire β-globin gene fragment approximately 3.0 Kb was purified from the gel before being ligated to pUC 13. Transformation was done under standard conditions using E. Coli JM83 as the host (See Maniatis 1982, Manual). Clone for β-globin gene easily screened by digestion of the plasmid DNA extracted from the white colony with BamH I which generates the fragment of 1.3 Kb. Nucleotide sequence of the cloned fragment was determined by the dideoxy chain termination method (Hattori & Sakaki, 1986, Anal. Biochem 152, 232-238). Direct sequencing of the amplified fragment was performed as described (Wong Kazazian & Skinner 1987, Nature 330, 384-386).

Conventional β-globin gene cloning and DNA sequencing

β-thal & βE globin genes were cloned with their flanking

Identification by Direct Cloning of entire β-globin gene using PCR. (1989) Fucharoen et al

Oligonucleotides were synthesized on an Applied Biosystems mode 380A DNA synthesizer & purified by high pressure liquid Chromatography using an AM-314 ODS YMC Packed Column (~~Yamamoto~~ Yamamoto Chemical Lab Co). Deoxyribonucleotides, dideoxyribonucleotides, [α-^{32}P] ATP and [γ-^{32}P] ATP were purchased from Amersham Corpn, T4 polynucleotide kinase, T4 DNA ligase, and restriction enzymes from Takara Shuzo Co, Taq DNA polymerase from Stratagene (Cetus).

Polymerase Chain Reaction, Direct Cloning of the entire β-globin gene, and Direct ~~[illegible]~~ ~~Sequencing~~ Sequencing — 1.0 μgm of genomic DNA is amplified in vitro by the polymerase Chain reaction using Taq DNA polymerase and primers G9 and G10 (G9 5'TCCCCAGTTAACCTCCT-ATT3' located at positions -823 to 804 from Cap site is Complementary to the antisense strand,) and primer ~~[illegible]~~ (G10, 5'AGACTAGCACTGCAG ATTCC3' is Complementary to the sense strand of the region from 550 to 569 bp 3' to the poly(A) site of the β globin gene. The size of the region to be amplified is 2998 bp. The restriction sites for Hinc II and Pst I are located within G9 and G10 respectively. One μgm of genomic DNA is mixed with 30 p mol of each primer, 200 μmol of dNTPs in a 100 μl reaction volume containing 10 mM Tris-HCl (pH 8.3), ~~50 mM~~

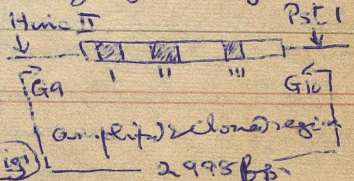


Fig 1

Weakly [illegible] will [illegible]

If we wish to look for a particular gene buried away in a large amount of DNA, we might make a length of DNA with a complementary sequence which will anneal to the gene, but not to the rest of the DNA. This is the principle behind using gene probes to screen for genes we are interested in. – Reverse transcriptase can be used to synthesize a DNA copy (complementary DNA) from any message RNA (mRNA) that can be isolated from mammalian cells. If radioactive bases are added to the reaction the synthesized cDNA can be labelled & hence used as hybridization probes to look for complementary sequences in either genomic DNA or in cellular RNA by DNA/DNA or DNA/RNA hybridization. When these techniques were first developed they were used to study hybridization in solution. Now it became possible to apply this method of fractionated DNA immobilized on nitrocellulose filters. It is possible to clone cDNA sequences in bacterial plasmids. This is done by synthesizing a second DNA strand on a newly synthesized cDNA using a bacterial DNA polymerase. In this way small cDNA duplexes are made which can be incorporated into plasmids and then grown in bacterial cells. It is now possible to clone fragments of genomic DNA into plasmids or bacteriophage and to amplify individual genes in E. coli making genomic probes. In order to label DNA radioactively to make hybridization probe a technique called nick translation is used. New approach has been developed to make short synthetic DNA fragments called oligonucleotides which construct specifically to detect single base changes in DNA

The DNA is allowed to dissolve for 2 l.w. at 37°C before quantitating. The DNA obtained is as good as phenol-chlor. extracted DNA. The OD 260/280 ratio were consistently 1.8–2.0 demonstrated good deproteinization.

A Simple Salting out procedure for extracting DNA from human nucleated cells
S.A. Miller, D.D. Dykes & H.F. Polesky
Nucleic Acids Res. (1988) 16 (3) 1216.
See also Dykes D. et al Electrophoresis (1986) 7: 278-282 for non-isotopic hybridization procedures rendering entire process of RFLP analysis free of toxic material
[Memorial Blood Centre of Minneapolis, 2304 Park Ave, South. Minneapolis. MN 55404. USA]

Buffy Coat of nucleated cells from EDTA blood suspended in Eppendorf centrifuge tube + 3 ml of nuclei lysis buffer (10 mM TRIS-HCl; 400 mM NaCl; 2 mM Na_2 EDTA, pH 8.2), Cell lysate digested overnight at 37°C with 0.2 ml of 10% SDS and 0.5 ml of Protease K Soln (1 mg Prot K in 1% SDS and 2 mM Na_2 EDTA). After digestion is complete, 1 ml of Saturated NaCl (approx. 6 M) added to each tube, Shake vigorously for 15 Seconds followed by Centrifugation at 2500 rpm for 15 min. The pptd Proteins pellet is left at the bottom of the tube & Supernatant containing the DNA was transferred to another 1.5 ml Eppendorf tube. Exactly 2 vol of Room temp Abs. ethanol is added & tube inverted several times until DNA is precipitated. The pptd DNA standard removed with wide mouth pipette & transferred to a 1.5 ml Tris-HCl - Na_2 EDTA (1.5 ml Tris HCl; 0.2 mM Na_2 EDTA pH 7.5)

3-4/8

Procedure of M.

WBC pellet from 10 ml of EDTA B'ood

+

6 ml lysis buffer (EDTA PH 8.2, 1M NaCl + 0.1M Tris)

+

0.2 ml (20%) SDS

+

0.5 ml Proteinase K (1 mg. + EDTA 8.2 SDS.)

WBC from 10 ml

+

9 ml lysis buffer

+

0.3 ml 20% SDS.

+

0.5 ml. proteinase K.

↓ incubated at shaking water bath 37°C overnight

lysate

+

2 ml 6M NaCl (supersat.) mix well

↓ ppt^n

centrifuged 15 min. 8000 rpm

↓

Supernatant + Double volume of Ethanol

70% ethanol wash

lysate

+

3 ml 6M NaCl

↓ ppt

Supernatant + Double volume of ethanol.

70% Ethanol

Bangalore Genei Private Ltd.
Flat No. 8, 50 Huligadri Seva
17th Cross, Malleswaram
Ph. 342091 Bangalore 560055

Ref: Current Science 1990. 59: (9)

III World Academy of Sciences (TWAS):
South-North Round Table on Haemoglobinopathies, Trieste, Italy, 6-8 Nov, 1986
Final Report Received from Dr. Galliero, Local Organizer, South-North Round table on Haemoglobinopathies, ICTP, P.O Box 586, Miramare, Strada Costiera 11, 34100 Trieste (Italy) I.J.H. (1986) IV: 384 p. 41-43.

Technical Report One tube Osmotic Fragility as an Useful Screening test for Thalassemia Carriers - A field experience. Chitra Mahadik, Chhaya Kapadia; Harsha Yagnik, P.K. Sukumar & S.M. Merchant. Ind. Jl. Haemat (1986) 4: 62-64.

Haemoglobins D, E and Hereditary Persistence of Foetal Haemoglobin in a Maratha family - Haemoglobin DE disease in two members. Desai S.S.; Master, Homai; Chavan D.S.; and Sukumaran P.K. Ind. Jl. Haemat (1986) 4: 71-74

Haemoglobin SD Disease - A Case report Chitra Mahadik; J.Y. Barve, R. Chittal

Normal Values
Serum Iron. 60-160 µg/100 ml
TIBC — 250-400 µgm/100 ml
Transferrin Saturat — >15%

and P.K. Sukumaran. Ind. Jl. Haemat (1986) 4: 75-76.

X. Bam HI; Bgl III; EcoRI; Hind III; Sal I; Sau 3A I; Taq I; DNA polymerase I (Klenow); Lambda DNA; Lambda DNA digest (EcoRI; Hind III; EcoRI/Hind III)
Various Plasmid Vector
VRC Complex (RNase Inhibitor)
Nuclease free BSA
Immochemicals
(a TDICI Venture Company)
Society for Study of Human Biology
"Blood Groups and Smallpox"
J.P. Garlick (London).
Recent publications have suggested that individuals of blood groups A and AB are especially likely to experience severe reactions to smallpox infections. No such effect was found in a series of 158 smallpox cases tested during an epidemic in South-West Nigeria.

X. Abstracts of Communications

Scientific & special General Meeting, Univ. College, December 1st, 1962

Letter 07 5.4.90.

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Human ~~AR~~ APRT gene
2.2 Kb. Bam HI
90% of Human APRT gene
PUCHU AP/B

PLASMID

includes most exons I and
extends into 3' untranslated
region

3rd Nov 1986 - 3-14 Nov.
Health Monitoring of Human Papula
exposed to Mutagens & Carcinogens.
W.H.O. Sponsored Course

Nucleic Acid Research
Vol 16 No 3 1988: 1215
A simple salting out procedure
for extraction of DNA from
nucleated cells.
S.A. Miller, DD-Dykes & H.F.
Polesky TIFR-4952311

S.A. Miller, Memorial
Blood Centre of Minneapolis,
2304, Park Avenue South,
Minneapolis MN-5504
USA

(1) Thal alleles in a dot blot format by hybridization with allele-specific radio labelled oligonucleotide probes
(2) (or) by direct sequence analysis of the amplification product. studied in Spanish β thal carriers in Spanish pop (Goosens et al, Creteil France)
Nonsense Codon 39 was predominant (64%) whereas the IVSI position 110 mutation, most common mutation on the east part of Mediterranean is underrepresented in these samples (8.5%)
Genomic DNA (1 μgm) was amplified by PCR using E coli DNA polymerase (Klenow Reagent) & PCR Primer

Southern Blotting 32P labelling and autoradiography account for the about 60% of the total material cost which is approximately £250.00 a family (Old at 1986). But Taq polymerase (US 0.79 unit & oligonucleotides (Commercially available (US 15.0) probes & 260 unit amount to total £10.00 per sample
Amplipriners (oligonucleotides) were synthesized on "BIOSEARCH CYCLONE DNA) Synthesizer
Taq DNA polymerase from Stratagene
Oligonucleotide Synthesis — Applied Bio Systems Model 38A — DNA Synthesizer
Polymerase C Reaction — Perkin Elmer Cetus Inst

VIL
11-50

Chitra M.
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Hl
600N WOLFE STREET,
Baltimore. Maryland
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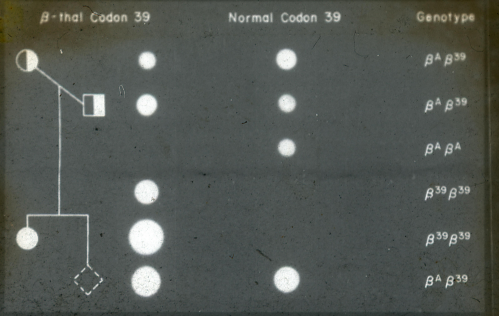
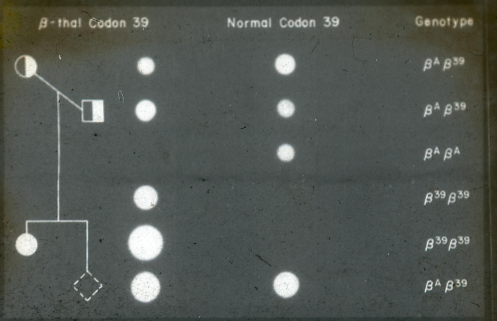
Waghmare
Indian Federation
against Copyright
Thef
INFACT
Block D; 5th Floor
Shivasagar Estate
Annie Besant Rd
Worli-400018
Tel 4929822/
4929096

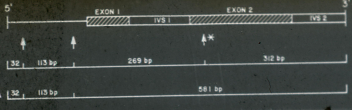
Bittles AS, ARR Devi,
N.A. Rao (1988)
Consanguinity trivial &
Secondary sex ratio
in the population of
Karnataka (S. India)
Annals of Human Biology
In press

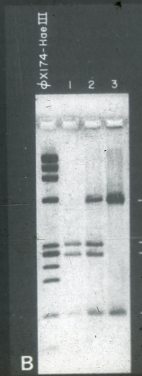


Dot blot showing the use of ASO probes on amplified β-globin DNA for prenatal diagnosis of β-thalassemia. Both parents in the pedigree at the left carry the nonsense codon 39 mutation as demonstrated by hybridization of their amplified DNAs to the mutant ASO. Control samples, essential for monitoring the specificity of the wash, are in the third and fourth dots from the top and represent amplified DNA from individuals homozygous for normal β-globin alleles and homozygous for the nonsense codon 39 β-thalassemia allele, respectively. Amplified DNA from this couple's affected child hybridizes to mutant ASO only and from the fetus to both the mutant and normal ASOs, which demonstrates that the fetus is a carrier of β-thalassemia.



Dot blot showing the use of ASO probes on amplified β-globin DNA for prenatal diagnosis of β-thalassemia. Both parents in the pedigree at the left carry the nonsense codon 39 mutation as demonstrated by hybridization of their amplified DNAs to the mutant ASO. Control samples, essential for monitoring the specificity of the wash, are in the third and fourth dots from the top and represent amplified DNA from individuals homozygous for normal β-globin alleles and homozygous for the nonsense codon 39 β-thalassemia allele, respectively. Amplified DNA from this couple's affected child hybridizes to mutant ASO only and from the fetus to both the mutant and normal ASOs, which demonstrates that the fetus is a carrier of β-thalassemia.





(A) *Mae*I map of a β-globin region 726-bp amplified DNA product that contains the sequence altered by the codon 39 β-thalassemia mutation. The 5' and 3' end points of amplified product are determined by the two primers used in the amplication reaction. Arrows represent *Mae*I sites, and the arrow with an asterisk represents the *Mae*I site created by the codon 39 mutation.[14] (B) Distinguishing codon 39 β-thalassemia allele by EtBr staining after *Mae*I digestion of amplified DNA. Ethidium bromide stain of *Mae*I-digested, amplified DNA product from (*a*) an individual homozygous for the nonsense codon 39 β-thalassemia allele, (*b*) an individual with β-thalassemia in whom one allele is of the nonsense codon 39 type, and (*c*) an individual with normal β-globin alleles. Staining is carried out after electrophoresis in a 3% Nu-Sieve agarose, 1% agarose minigel.

ease and speed with which a diagnosis can be made in the fetus.

single nucleotide changes in genomic DNA have led to the reality of prenatal diagnosis by direct mutation detection

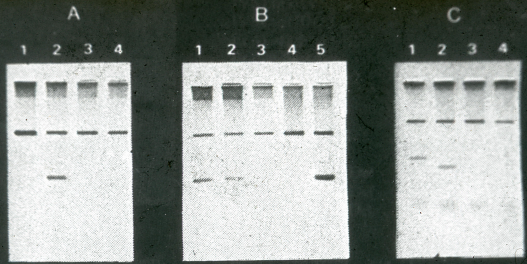


Fig 1—ARMS analysis of Indian mutations.

Gel A shows analysis of an unknown DNA sample with the primer for the IVSI nt5 mutation (lane 1), IVSI nt1 (2) Fr 41–42 (3), and Fr 8–9 (4). Gel B shows a prenantal diagnosis for the IVSI nt5 mutation: lanes 1 and 2 = maternal and paternal DNA, respectively, with IVSI nt5 mutant primer; lane 3 = normal DNA with IVSI nt5 normal primer; lane 4 = chorionic villus DNA with IVSI nt5 normal primer; lane 5 = chorionic villus DNA with the IVSI nt5 mutant primer. Gel C shows chorionic villus DNA with IVSI nt5 mutant primer (lane 1) and Fr 8–9 mutant primer (2), and normal DNA with IVSI nt5 mutant primer (3) and Fr 8–9 mutant primer (4). The upper bands in each gel are the 861 bp control bands.

Ethnic distribution of β-thalassemia mutations from the Indian subcontinent

Mutation	Ethnic Group						Total
	Sindhi	Punjab Sikh	Gujarati	Indian Muslim	Pakistani Muslim	Others	(%)
IVS1n5	1	5	13	4	10	6	39(35.4)
619 bp deletion	7	4	1	5	6	2	25(22.7)
Fr c8/9	2	3	2	4	5	2	18(16.4)
Fr c41/42	3	5	1	0	0	1	10(9.0)
IVS1n1	3	1	1	0	1	0	6(5.4)
Fr c16	0	0	1	0	4	0	5(4.5)
Nons c15	0	0	2	1	0	0	3(2.7)
Cap site (+1)	0	2	0	0	0	0	2(1.8)
-88	0	1	0	0	0	0	1(0.9)
25 bp deletion	0	0	0	0	1	0	1(0.9)
Total	16	21	21	14	27	11	110

THALASSEMIA VARIANTS ※

BETA GENE CLUSTER	
β°	THALASSEMIA
β^{+}	THALASSEMIA
$\delta\beta$ -	THALASSEMIA
$\delta\beta$-β-	THALASSEMIA
$\delta\beta$-HPFH	THALASSEMIA
HPFH-β-	THALASSEMIA
γ -	THALASSEMIA
HbS - β°	THALASSEMIA
HbS - β^{+}	THALASSEMIA
HbD - β	THALASSEMIA
HbQ - β	THALASSEMIA
HbJ - β	THALASSEMIA
HbE - β	THALASSEMIA

ALPHA GENE CLUSTER
Hb - H DISEASE
Hb - BART'S
Hb - S - H DISEASE
HbS - α THALASSEMIA

※ Seen in Bombay

HAEMOGLOBINOPATHIES IN SOUTH EAST ASIA.

Country	Pop. Millions	Birth rate	Annual births x 10³	% for pop. het for				Homozygous births per thousand		Annual births of homozygotes with					Homozygote annual bi	
				S	Th	E	Total	Total	Total patn.	SS	STh	Tm	E/Th	EE	Total	Tot Pat
South Asia																
Bangladesh	92.6	47	4,353	-	1-3	1-7	< 5	.625	.41	-	-	462	1,306	952	2,720	1,
Bhutan	1.4	42.7	58	-	-	4	4	.4	-	-	-	-	-	23	23	
INDIA	712.0	33.3	23,698	0-40	1-17	0-30	3.9	.92	.92	14,810	+	7,040	+	+	21,850	21,
Maldives	.16	44.5	7.3	-	3	-	3	.23	.25	-	-	2	-	-	2	
Nepal	15.0	43.7	656	-	1	3	4	.4	.2	-	-	42	94	149	262	
Pakistan	87.1	48.2	4,200	+	2-65	+	6	.9	.9	+	+	7,182	378	+	7,560	7,
Sri Lanka	15.2	28	425	-	2-3	1-10	3.5	.31	.25	-	-	44	64	22	130	

HAEMOGLOBINOPATHIES IN SOUTH EAST ASIA.

Country	Pop. Millions	Birth rate	Annual births x 10^3	% of pop. het for				Homozygous births per thousand		Annual births of homozygotes with					Homozygote annual births	
				S	Th	E	Total	Total	Total path.	SS	STh	Tm	E/Th	EE	Total	Total path.
South Asia																
Bangladesh	92.6	47	4,353	-	1-3	1-7	5	.025	.41	-	-	462	1,306	952	2,720	1,768
Bhutan	1.4	42.7	58	-	-	4	4	.4	-	-	-	-	-	23	23	
INDIA	712.0	33.3	23,698	0-40	1-17	0-30	3.9	.92	.92	14,810	+	7,040	+	+	21,850	21,850
Maldives	.16	44.5	7.3	-	3	-	3	.23	.25	-	-	2	-	-	2	2
Nepal	15.0	43.7	656	-	1	3	4	.4	.2	-	-	42	94	149	262	136
Pakistan	87.1	48.2	4,200	+	2-69	+	6	.9*	.9	+	+	7,192	378	+	7,560	7,560
Sri Lanka	15.2	28	425	-	2-3	1-10	3.5	.31	.25	-	-	44	64	22	130	108