INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Vzdělávání v oblasti forenzní genetiky
reg. č. CZ.1.07/2.3.00/09.0080

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.

HUMAN CHIMERISM

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CHIMERA

Organism with cells of 2 (or more) zygotes

SPONTANEOUS

Transient

- **Transplacental passage of blood cells**

  (mother ⟷ child)

Permanent

- **Blood (twin) chimerism**
- **Whole body (tetragametic or dispermic) chimerism**

ARTIFICIAL

Transient
⬇
Permanent

- **Blood transfusion**
- **Organ transplantation**
- **Bone marrow transplantation**

CHIMERISM ≠ MOSAICISM

.....both have more than one genetically distinct population of cells

but

CHIMERAS originate from more than one zygote

whereas

MOSAICS are formed of genetically different cells arising from a single zygote

ANALYSIS

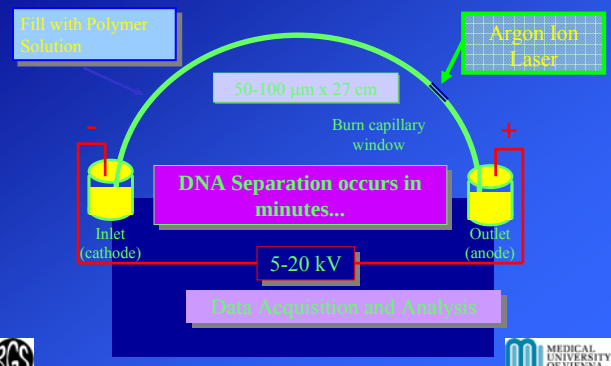
Detection of

- Blood groups
- HLA
- Platelet antigens
- DNA Polymorphisms

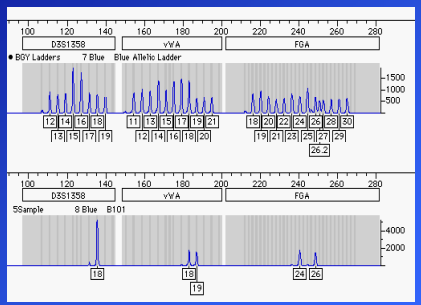
VNTR via PCR

- Repetitive sequences with tandem-like organisation
- Microsatellites - STR: 2-7 bp repeats (e.g. AATG)
- Minisatellites: up to 100 repeats
- Length polymorphism: variable number of repeats
- Sequence polymorphismus may exist in addition

Capillary Electrophoresis (CE)



Genotyper 2.0 AmpFISTR



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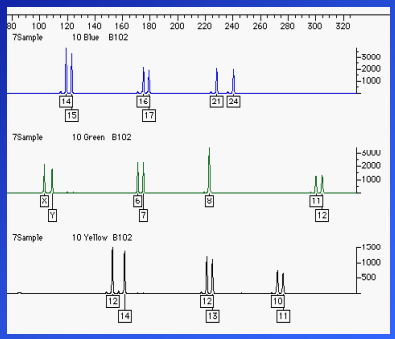

Probability of Paternity Exclusion

Table of Probability of Paternity Exclusion

Table 4-5 shows the Probability of Paternity Exclusion (P_E) values of the AmpFℓSTR Identifiler kit STR loci individually and combined.

Table 4-5 Probability of paternity exclusion for the AmpFℓSTR Identifiler kit STR loci

Locus	Caucasian
CSF1PO	0.496
D2S1338	0.725
D3S1358	0.630
D5S818	0.440
D7S820	0.582
D8S1179	0.680
D13S317	0.487
D16S539	0.566
D18S51	0.731
D19S433	0.531
D21S11	0.708
FGA	0.766
TH01	0.566
TPOX	0.329
vWA	0.625
SE33	0.861
D12S391	0.766

Cumulative CPE:

99.999997 %

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  (mother ⟷ child)

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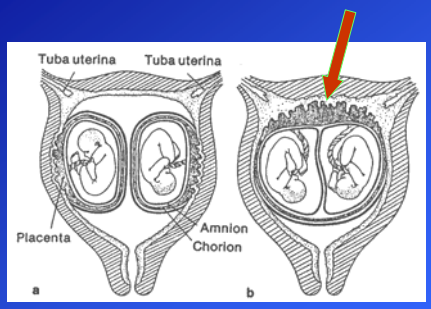
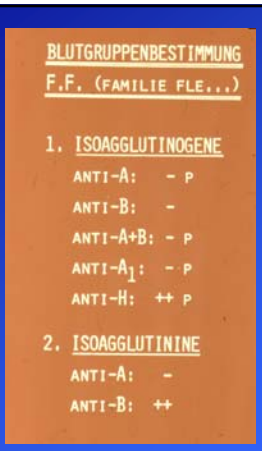
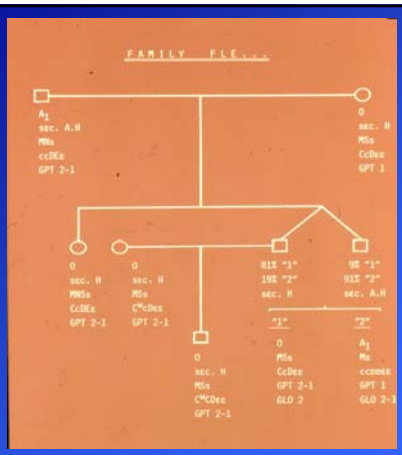
- Blood transfusion
- Organ transplantation
- Bone marrow transplantation

Dizygotic twins







percentage of different blood cell populations

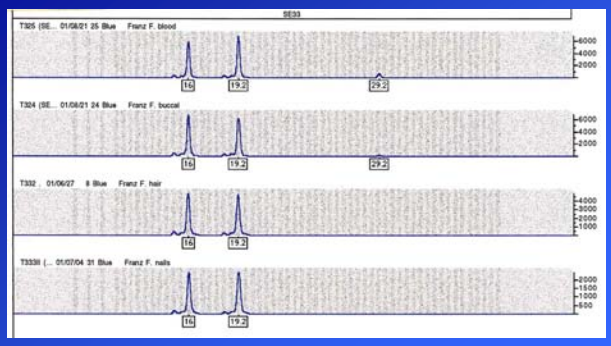
proband	genotype	erythrocyte population A_1	erythrocyte population 0	lymphocyte population I	lymphocyte population II
Franz F.	*00*	19 %	81 %	1 %	99 %
Johann F.	*$A_1$0*	91 %	9 %	42 %	58 %

(according to Pausch et al. 1979)

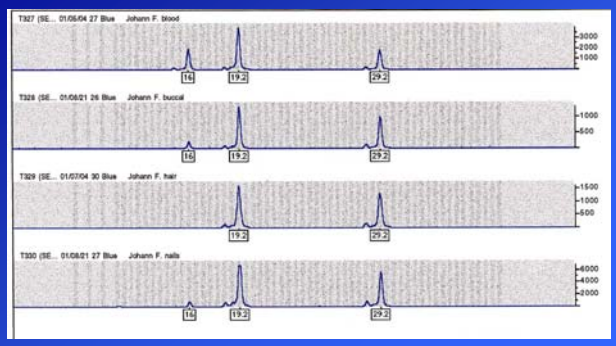
sample material

sample origin	DNA extraction
blood buccal swab	Chelex, Qiamp DNA Blood Mini Kit
eyebrow	Chelex
nail	Quiamp DNA Mini Kit Tissue Protocol

SE33 results Franz F.



SE33 results Johann F.



summary

- a mixture of the genetic patterns of both twins was found in the blood samples
- a mixture of the genetic pattern of both twins in buccal swabs and nails is not to be expected for twin chimeras, but could be due to leucocyte contamination
- alleles of the true genetic line of each twin were identified in hair samples of both twins and in nails and the buccal swab of one of the twins.
- all alleles found in the blood and buccal swabs of both twins and in the nails of one of the twins derived from their own genetic line or from the other twin.

general aspects

! allelic patterns found in blood and buccal swabs can differ from the true genetic identity

! major component does not necessarily represent the true genetic line

! proportions of cell lines can change significantly during lifetime

Chimera Kärnten

- Genetic mother/child incompatibility
- Mother carries XY
- Blood of the mother showed DNA profile (and all blood markers) of her twin brother
- Analysis of the hair roots of the mother showed true DNA profile of the mother and a genetic mother/child compatibility

BLOOD CHIMERISM

System	Mother (blood)	Child (blood)	Put. Father (blood)
D8S1179	12,13	12,13	13,16
D21S11	***29,31.2***	***30,31***	31,31
D7S820	8,10	10,12	11,12
CSF1PO	9,12	12,12	12,12
D3S1358	***17,17***	***15,16***	15,16
TC11	7,9	7,8	8,9
D13S317	11,12	9,12	9,12
VWA	14,17	17,19	14,19
TPOX	8,11	8,8	8,8
D18S51	14,16	16,18	14,18
D5S818	12,12	12,13	11,13
FGA	21,22	21,22	20,22
SE33	***26.2,26.2***	***14,19***	19,19
AMEL	XY	XY	XY

BLOOD CHIMERISM

System	Twin Brother (of mother)	Mother (blood)	Mother (buccal swab)	Mother (hair)	Child (blood)	Put. Father (blood)
D8S1179	12,13	12,13	12,13	12,13	12,13	13,16
D21S11	29,31.2	29,31.2	29,30,31.2	30,30	30,31	31,31
D7S820	8,10	8,10	8,10,12	10,12	10,12	11,12
CSF1PO	9,12	9,12	9,12	12,12	12,12	12,12
D3S1358	17,17	17,17	16,17	16,17	15,16	15,16
TC11	7,9	7,9	7,9	7,9	7,8	8,9
D13S317	11,12	11,12	11,12	11,12	9,12	9,12
VWA	14,17	14,17	14,17	17,17	17,19	14,19
TPOX	8,11	8,11	8,11	8,8	8,8	8,8
D18S51	14,16	14,16	14,16	14,16	16,18	14,18
D5S818	12,12	12,12	12,13	12,13	12,13	11,13
FGA	21,22	21,22	21,22	21,21	21,22	20,22
SE33	26.2,26.2	26.2,26.2	14,26.2	14,26.2	14,19	19,19
AMEL	XY	XY	X(Y)	XX	XY	XY

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(mother ⟷ child)

Permanent

- Blood (twin) chimerism
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whole body chimerism: possible mechanisms

- Ovum nucleus and nucleus of second polar body — 2
- Two haploid daughters of the ovum nucleus — 2
- Early fusion of two embryos — 2
- Fusion of one daughter of zygote nucleus with nucleus of second polar body — 3 (2n/3n)
- Ovum nucleus and nucleus of first polar body — 3
- Suppression of second meiotic division — 4

Tetragametic chimerism

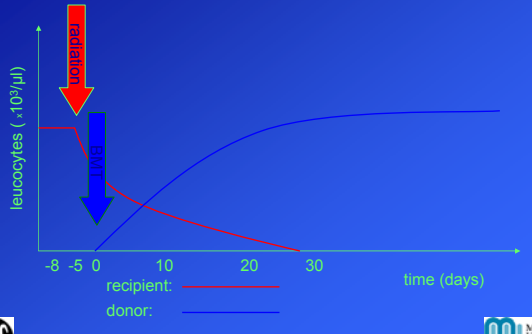
	Mother	Father	Proposita
RBC antigens			
AB0	A1	A1	0 / A1
MNS	MSs	MNs	Ms / MNSs
HLA			
Class I and class II	A32,B7,Cw7,DRB1*15/ A3,B15,Cw3,DRB1*13	A3,B51,Cw*15,DRB1*03/ A3,B8,Cw7,DRB1*16	A32,B7,Cw7,DRB1*15/ A3,B51,Cw*15,DRB1*03 A3,B15,Cw3,DRB1*13 A3,B8,Cw7,DRB1*16
DNA minisatellites			
YNZ22	3,10	2,11	2,3,10,11#
APO-B	29,47	31f,39s	29,39s,47#
D1S80	26M,39	18,31	18,26M,39#
DNA STRs			
TC11	6,9.3	6,6	6,9.3#
SE33	V20,V36	V15,V16	V15,V16,V20,V36#
FGA	22,26	25,25	22,25,26#
FES	10,12	10a,11	10,10a,11,12#

identical results in blood, buccal swab, nails and hair roots

Aim of the study

Can donor cells also be found in tissues other than blood in bone marrow transplanted patients?

Bone marrow transplantation (BMT)



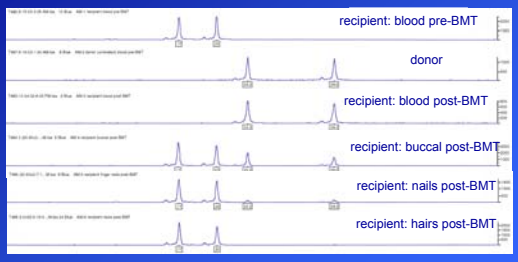
Materials and methods I

- 5 patients > 5 years after engraftment
  - blood (Chelex, Qiagen)
  - buccal swab (Chelex, Qiagen)
  - finger-nails (Qiagen)
  - eye brows (Chelex)
  - 2 out of 5 hair samples by alternative method
    Hellmann et al. 2001 Int J Legal Med (114):269-273
- 5 patients and donors prior to BMT
  - frozen lymphozytes (Chelex, Qiagen) or
  - DNA („salting out" method from blood)

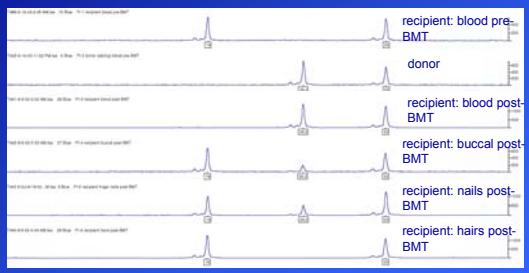
Materials and methods II

- DNA extraction and amplification
  - pre-BMT samples (donor + recipient) separately
  - post-BMT samples (recipient)
- Singleplex PCR of highly polymorphic loci
  - SE33
  - D12S391
- Instruments (Applied Biosystems)
  - PCR Amplification System 9700
  - ABI Prism 310 Genetic Analyser

SE33 results – unrelated donor



SE33 results-family donor (sibling)



CHIMERISM AFTER BONE MARROW TRANSPLANTATION

5 patients at least 5 years after successful BMT

Sample material	DNA
Blood	Donor
Buccal cells	Recipient + Donor
Finger nails	Recipient + Donor
Hairs	Recipient

Recipients >5 years after BMT (n=5)

Sample material	Genetic origin
blood	donor
buccal swab	32.1 % donor (16.6 - 76.3 %)
finger-nails	24.0 % donor (11.4 - 53.3 %)
hairs	recipient

Quantification based on peak areas according to:
Thiede et al. 1999: Bone Marrow Transplant (23):1055-1060

Summary

- Results of this study confirm the findings in the pair of chimeric twins
- Donor cells originating from hematopoietic tissue can migrate into other tissues
- Donor blood stem cells can transdifferentiate into cells other than blood in the recipient

Forensic aspects

- Mixed DNA profiles can originate from a single individual
- DNA profiles obtained from different tissues of the same individual can be discrepant
- DNA profiles obtained from samples taken at different times from the same individual can differ
- DNA profile obtained from hair samples exhibited the true genotype

Pre.../Pre...

Spender	Empfänger vor KMT	Empfänger 2 1/2, 7 Mo nach KMT
CcDee	ccddee	CcDee
MNs	MNSs	MNs
Fy(a+b-)	Fy(a+b+)	Fy(a+b-)
Jk(a-b+)	Jk(a+b+)	Jk(a-b+)
Xg(a+)	Xg(a-)	Xg(a+)
EsD 1	2-1	1
Km(1+)	Km(1-)	Km(1+)
Gc 2-1S	2	2
Pi M1	M3	M3

Zim.../Zim...

Spender	Empfänger vor KMT	Empfänger 3, 4, 7 Mo nach KMT
O αβ	B	O α
Ms	MSs	Ms
Xg(a+)	Xg(a-)	Xg(a+)
SEP B	AB	B
PGM_1 3-1	1	3-1
EsD 2-1	1	2-1
Hp 2-1	2	2
Gc 1F-1S	1S	1S
Pi M3-M1	M1	M1

GvHD after LiverTX

- LiverTX early october 2004
- End november: deterioration of the clinical situation: diarrhoe, pancytopenia
- December: bone-marrow biopsy: no sign for GvHD
- Mid january 2005: chimerism in the blood of the patient (80% leucocytes of the donor)
- 2 days before exitus: 100% donor leucocytes
- retrospektive analysis of the bone-marrow biopsy: chimerism (15% donor lymphocytes)

GvHD after LiverTX

Material of 21 organs (autopsy):
1% - 62% (100%) leukocytes of the donor

Table 2 percentages of donor's cells in different post-mortem biopsies

sample origin	donor	sample origin	donor	sample origin	donor
prostata	3 %	brain	62 %	left lung	16 %
trachea	17 %	right kidney	7 %	liver	100 %
heart	3 %	left kidney	4 %	cardiac tissue	17 %
pelvic bone marrow	37 %	aorta	4 %	oesophagus	18 %
renal pelvis	13 %	right suprarenal gland	4 %	pancreas	24 %
colon	6 %	left suprarenal gland	7 %	stomach	10 %
vertebral bone marrow	29 %	right lung	22 %	thyroid gland	1 %

CHIMERISM ≠ MOSAICISM

.....both have more than one genetically distinct population of cells

but

CHIMERAS originate from more than one zygote

whereas

MOSAICS are formed of genetically different cells arising from a single zygote

Table 1. Characteristics of patients with spontaneous mixed RhD phenotype

Patient	Sex	Age, y*	Diagnosis	*RH* genotype†	Blood group phenotype‡ Rh D	C	E	c	e	Duffy Fy^a	Fy^b	D-positive RBCs, %	Number of D sites per D-positive RBC§	Follow-up, mo‖	Dynamics of D antigen positivity over follow-up interval
1	M	63	Idiopathic osteomyelofibrosis	*CcDdee*	±	±	−	+	+	+	+	56	11182	44	Complete loss
2	M	56	Rheumatoid arthritis	*CcDdee*	±	±	−	+	+	−	+	67	12073	52	Stable
3	M	60	Anal fistula	*CcDdee*	±	±	−	+	+	+	−	46	12578	40	Stable
4	F	86	Essential thrombocythemia	*CcDdee*	±	±	−	+	+	−	+	85	9838	5	Stable
5	F	33	Uterus myomatosus	*CcDdee*	±	±	−	+	+	+	±	81	11380	24	Stable
6	M	67	Acute myelogenous leukemia	*CcD^{Pet}/dee*¶	± w	±	−	+	+	−	+	56	4419	13	Progressive loss
7	F	43	Psychosis	*ccDdEe*	±	−	±	+	+	+	+	22	nd	65	Progressive loss
8	F	78	Colon carcinoma	*CcDdee*	±	±	−	+	+	−	+	nd	nd	33	Complete loss
9	F	86	Leg vein thrombosis	*CcDdee*	±	±	−	+	+	−	+	40	10950	12	Stable

RBC indicates red blood cell; ±, positive/negative mixed-field agglutination; −, negative; +, positive; ± w, weak positive/negative mixed-field; and nd, not determined.
*Age at initial recognition of mixed RhD status.
†*RH* genotype and *RHD* zygosity as determined from blood DNA samples.
‡In all patients, normal unmixed ABO, MNS, P, Lutheran, Kell, and Kidd blood group phenotypes were found.
§D antigen densities of *RH* genotype–matched *CcDdee* and *CcD^{Pet}dee* (D category IV type 4) control samples were 10724 and 4327 D sites per red cell, respectively.
‖Time interval from initial serologic recognition of mixed RhD status to its latest determination; during this time, no patient received transfusions.
¶Patient 6 (*CcD^{Pet}dee*) displayed a partial D variant, D category IV type 4.

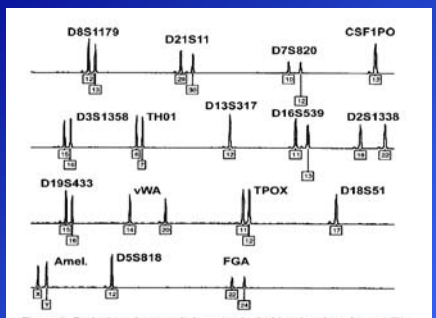


Figure 3. Exclusion of congenital or acquired chimerism by microsatellite marker analysis. Representative electropherogram showing the blood DNA profile of patient 1 after multiplex-PCR of 15 highly polymorphic autosomal short tandem repeat loci and amelogenin (Amel.). Numbers denote allelic designations of individual loci. No additional allelic peaks and only well-balanced heterozygous peaks are observed. Similar results were obtained with samples from the other 8 patients.

Table 2. Molecular genetic *RH* typing of single erythropoietic blast-forming units

Patient	*RH* genotype*	*RHD/RHCE* specificities tested†	Number of BFU-E colonies Tested	Excluded‡	Interpretable	Positive for *RHD/RHCE* specificities *CcDe*	*ce* only	*DEe*	*e* only
2	*CcDdee*	*C, c, D* (exon 7), *e*	30	0	30	13	17	nd	0
3	*CcDdee*	*C, c, D* (exon 7), *e*	18	7	11	8	3	nd	0
8	*CcD^{Pet}/dee*§	*C, c, D* (exon 5), *e*	40	4	36	29	7	nd	0
7	*ccDdEe*	*D* (exon 7), *E, e*	40	4	36	nd	nd	15	21

BFU-E indicates erythropoietic blast-forming unit; and nd, not determined.
*As determined from blood DNA samples.
†BFU-E samples of patients 2, 3, and 8 were genotyped by real-time PCR, whereas BFU-E samples of patient 7 were genotyped by PCR-SSP (as detailed in "Patients, materials, and methods").
‡Sample exclusion because of amplification failure of positive control.
§Partial D variant (D category IV type 4).

Table 3. Relative peak height ratios of chromosome 1 microsatellite markers in blood

Locus	Chromosome 1 position, Mb	Patient 1*	2*	3*	4†	5*	7† (initial)	7† (after 6 y)	8† (initial)	8† (after 2 y)	9*
	p telomeric end										
D1S468	3.57	0.19‡	0.66‡	1.10	0.37‡	ni	0.40‡	0.22‡	0.33‡	0.11‡	0.57‡
D1S607	14.90	ni	0.63‡	1.07	0.33‡	0.76‡	ni	ni	nd	nd	0.71‡
D1S2697	16.29	0.19‡	0.56‡	0.95	ni	0.69‡	0.34‡	0.23‡	ni	ni	0.63‡
D1S2644	18.90	ni	0.68‡	1.00	nd	ni	0.31‡	0.22‡	0.33‡	0.10‡	0.59‡
D1S199	19.83	0.41	0.44‡	ni	0.42‡	0.76‡	ni	ni	nd	nd	0.68‡
D1S2664	22.75	ni	0.71‡	0.24‡	0.24‡	0.74‡	ni	ni	ni	ni	ni
RHD	25.50	na	na	na	na	na	na	na	na	na	na
RHCE	25.59	na	na	na	na	na	na	na	na	na	na
D1S233	31.26	0.20‡	0.53‡	0.95	ni	0.69‡	0.44‡	0.28‡	0.34‡	0.17‡	ni
D1S2890	57.65	0.23‡	0.95	0.92	ni	ni	0.38‡	0.27‡	0.17‡	0.06‡	0.36‡
	Centromere										
D1S2635	157.44	0.94	0.99	1.16	0.94	0.65‡	0.70	0.87	0.99	0.97	1.02
D1S2836	244.94	0.92	1.01	ni	nd	0.85‡	0.61	0.69	0.60	0.56	1.00
	q telomeric end										

ni indicates not informative (homozygous); nd, not determined; and na, not applicable.
*Peak height ratios of blood divided by peak height ratios of hair samples.
†Peak height ratios of blood samples (no other tissues tested; unusual values were determined by comparison with normal controls).
‡Values indicating unusual peak imbalance.

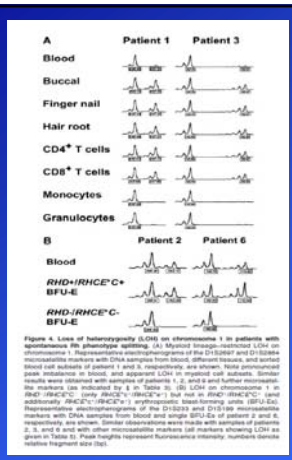


Figure 4. Loss of heterozygosity (LOH) on chromosome 1 in patients with spontaneous Rh phenotype splitting. (*A*) Myeloid lineage–restricted LOH on chromosome 1. Representative electropherograms of the D1S2697 and D1S2864 microsatellite markers with DNA samples from blood, different tissues, and sorted blood cell subsets of patient 1 and 3, respectively, are shown. Note pronounced peak imbalance in blood, and apparent LOH in myeloid cell subsets. Similar results were obtained with samples of patients 1, 2, and 8 and further microsatellite markers (as indicated by ‡ in Table 3). (*B*) LOH on chromosome 1 in *RHD*−/*RHCE*$^{+}$C^{-} (only *RHCE*$^{+}$c^{-}/*RHCE*$^{+}$e^{+}) but not in *RHD*$^{+}$/*RHCE*$^{+}$C^{+} (and additionally *RHCE*$^{+}$c^{-}/*RHCE*$^{+}$e^{+}) erythropoietic blast-forming units (BFU-Es). Representative electropherograms of the D1S233 and D1S199 microsatellite markers with DNA samples from blood and single BFU-Es of patient 2 and 6, respectively, are shown. Similar observations were made with samples of patients 2, 3, and 8 and with other microsatellite markers (all markers showing LOH as given in Table 3). Peak heights represent fluorescence intensity; numbers denote relative fragment size (bp).

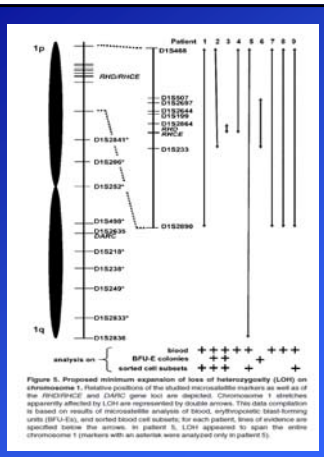


Figure 5. Proposed minimum expansion of loss of heterozygosity (LOH) on chromosome 1. Relative positions of the studied microsatellite markers as well as of the *RHD/RHCE* and *DARC* gene loci are depicted. Chromosome 1 stretches apparently affected by LOH are represented by double arrows. This data compilation is based on results of microsatellite analysis of blood, erythropoietic blast-forming units (BFU-Es), and sorted blood cell subsets; for each patient, lines of evidence are specified below the arrows. In patient 5, LOH appeared to span the entire chromosome 1 (markers with an asterisk were analyzed only in patient 5).

Mother–child exclusion due to paternal uniparental disomy 6

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Table 1 Mother–child exclusions in the first investigation

System	HLA	SE33 (ACTBP2)
Mother	A2,29; B56,57; Cw1,w6; DRB1*01,*07; DQB1*03,*05	*17,*28.2
Child	A3; B13; Cw6; DRB1*07; DQB1*02	*14

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26 out of 42 chromosome 6 loci: mother/child exclusion

Child: „homozygous“ for all chromosome 6 loci tested

Table 2 Chromosome 6 typing in mother, child, and father (from 6p25 to 6q27)

Locus	Mother	Child	Father
D6S1574	*156, 158*	*168*	160,168
F13A	**4,*6*	**7*	**7*
D6S309	311, 321	321	321,323
D6S470	*124*	*132*	132,134
D6S289	*163, 167*	*171*	171,175
D6S422	302, 318	302	302,310
D6S276	211, 223	223	223
HLA-A	*A2, 29*	*A3*	A1,A3
D6S2960 (C3_4_13)	*286, 298*	*302*	302,310
D6S2906 (C3_3_6)	**9, *12*	**15*	**13,*15*
D6S2939 (C2_4_4)	**10, *18*	**10*	**9,*10*
HLA-C	Cw1, w6	Cw6	Cw6, Cw7
HLA-B	*B56,57*	*B13*	B8,B13
D6S2931 (C1_4_4)	**8, *9*	**8*	**8,*10*
HLA-DR	B1*01, *07	B1*07	B1*07,*15
HLA-DQ	*B1*03, *05*	*B1*02*	B1*02,*06
D6S1610	*205, 207*	*201*	201,205
D6S1549	191, 199	199	191,199
D6S282	*119, 123*	*121*	121,127
D6S1650	*118, 120*	*122*	116,122
D6S432	*279*	*285*	273,285
D6S272	*185, 193*	*187*	183,187
D6S1573	*286, 290*	*296*	286,296
D6S257	*171, 177*	*161*	161,173
D6S460	*286*	*294*	278,294
D6S1609	*83, 99*	*97*	97
SE33	**17, *28.2*	**14*	**14,*28.2*
D6S462	112	112	112,114
D6S300	*199*	*197*	197,209
D6S1671	*268, 284*	*267*	267,276
D6S434	206, 210	210	210,218
D6S1698	*174, 182*	*172*	172,180
D6S287	*124*	*122*	122,126
D6S262	*172, 180*	*182*	176,182
D6S292	158, 162	158	158,166
D6S308	342	342	342
D6S441	*171, 183*	*177*	177,181
D6S1581	263, 271	271	271,273
D6S264	115	115	113,115
D6S1697	252, 254	252	252,254
D6S446	216, 222	222	222,226
D6S281	*140*	*142*	142

Table 3 Demonstrated heterozygosity of the child in chromosomes 1–5, 7–22, and X

Chromosome	Locus	Genotype/phenotype
1	D1S80	*22F, *24
	RH	CcDee
2	D2S1338	*17, *18
	TPOX	*8, *11
3	D3S1358	*16, *17
4	FGA	*21, *24
	MNS	MNs
5	D5S407	97, 99
	D5S644	92, 98
	D5S406	170, 182
7	D7S820	*10, *12
8	D8S1179	*11, *14
9	D9S1810	206, 208
	D9S1818	200, 206
10	D10S1649	136, 140
	D10S1655	252, 260
11	TH01	*9, *9.3
12	D12S391	*21, *22,
	VWA	*19, *20
13	D13S1322	96, 98
	D13S1243	253, 257
14	D14S990	141, 149
15	Penta E	*7, *12
16	D16S539	*11, *12
17	D17S30 (YNZ22)	*4, *8
18	D18S51	*15, *16
19	D19S433	*12, *13
20	D20S902	309, 313
	D20S906	96, 102
21	D21S11	*28, *33.2
	Penta D	*10, *13
22	D22S1170	203, 212
X	DXS1223	154, 158

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