



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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Blood Group Serology



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

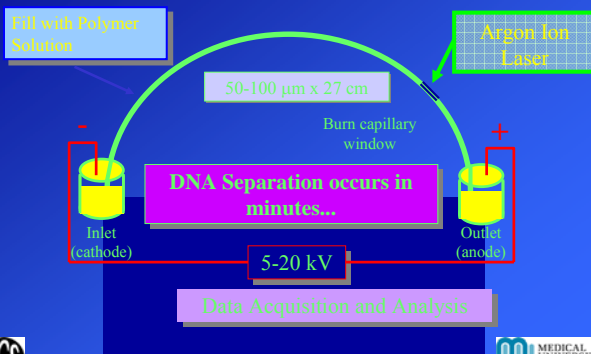


VNTR via PCR

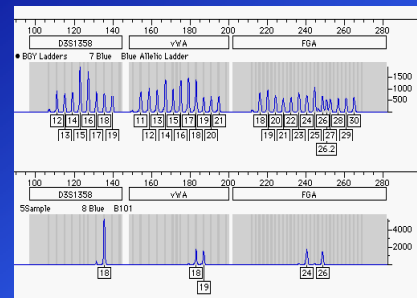
- Repetitive sequences with tandem-like organisation
- Microsatellites - STR: 2-7 bp repeats (e.g. AATG)
- Minisatellites: up to 100 repeats
- Length polymorphism: variable number of repeats
- Sequence polymorphism may exist in addition



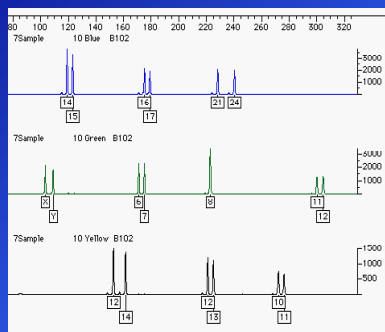
Capillary Electrophoresis (CE)



Genotyper 2.0 AmpFISTR



Display all Colors



Probability of Paternity Exclusion

Table 4-5 shows the Probability of Paternity Exclusion (P_e) values of the AmpFISTR Identifier kit STR loci individually and combined.

Table 4-5 Probability of paternity exclusion for the AmpFISTR Identifier kit STR loci

Locus	Caucasian
CSF1PO	0.406
D2S1338	0.725
D3S1358	0.630
D5S818	0.440
D7S820	0.582
D8S1179	0.680
D15S317	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 %



Blood Group Serology



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ARTIFICIAL

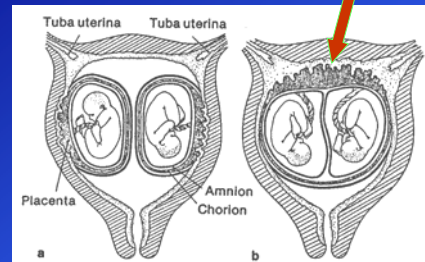
Transient

Permanent

- Blood transfusion
- Organ transplantation
- Bone marrow transplantation



Dizygotic twins



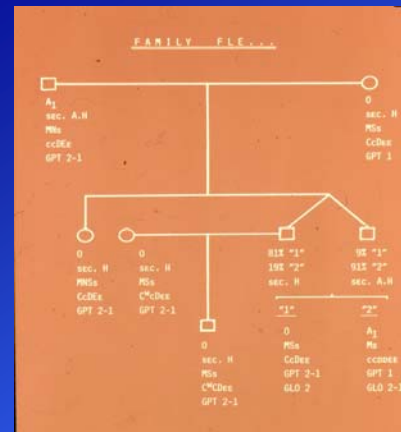
BLUTGRUPPENBESTIMMUNG F.F. (FAMILIE FLE...)

1. ISOAGGLUTINOGENE

- ANTI-A: - P
- ANTI-B: -
- ANTI-A+B: - P
- ANTI-A₁: - P
- ANTI-H: ++ P

2. ISOAGGLUTININE

- ANTI-A: -
- ANTI-B: ++



percentage of different blood cell populations

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

(according to Pausch et al. 1979)

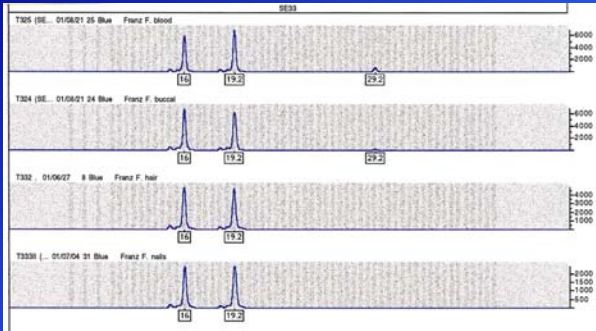


sample material

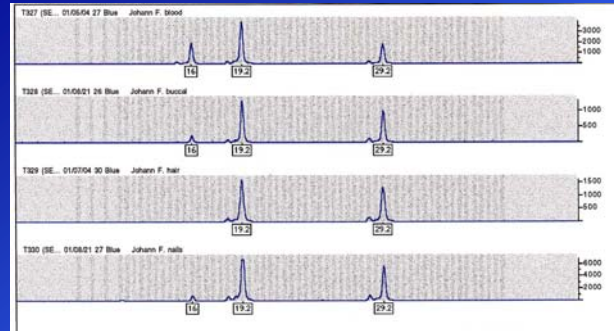
sample origin	DNA extraction
blood	Chelex, Qiamp DNA Blood Mini Kit
buccal swab	Chelex, Qiamp DNA Blood Mini Kit
eyebrow	Chelex
nail	Qiamp DNA Mini Kit Tissue Protocol



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
CSFIPO	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)	Pat. Fraternity (blood)
D8S1179	12,13	12,13	12,13	13,13	12,13	13,13
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	16,16	15,16
TC11	7,9	7,9	7,9	7,9	7,8	8,9
D13S317	11,12	11,12	11,12	11,12	8,12	8,12
VWA	14,17	14,17	14,17	13,17	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	16,16	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	XX



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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	O / 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	2,3,10,119
APO-B	29,47	311,396	29,396,478
D18S0	26M,39	18,31	18,26M,396
DNA STRs			
TC11	6,9,3	6,6	6,9,38
SE33	V20,V36	V15,V16	V15,V16,V36,V36M
FGA	23,26	23,23	23,23,306
FES	10,12	106,11	10,106,11,138

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

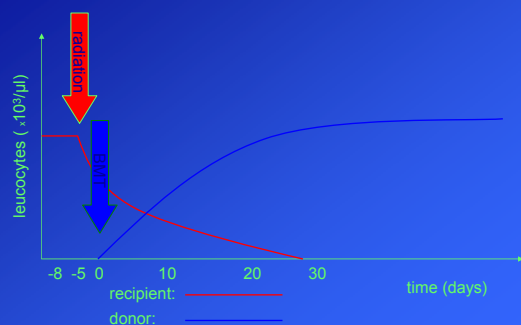


Aim of the study

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 lymphocytes (Chelex, Qiagen) or
 - DNA („salting out“ method from blood)

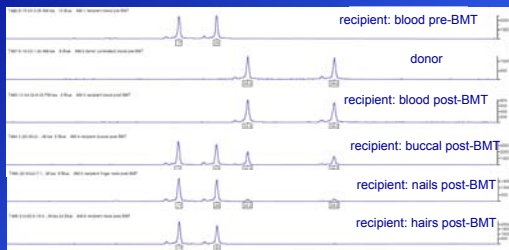


Materials and methods II

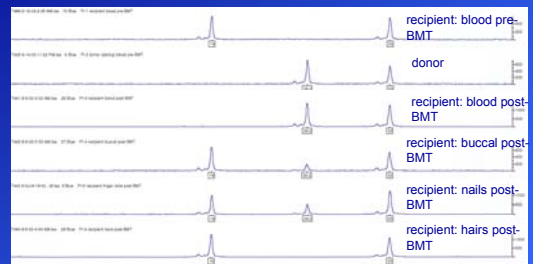
- DNA extraction and amplification
 - pre-BMT samples (donor + recipient) separately
 - post-BMT samples (recipient)
- Stratix PCR of highly polymorphic loci
 - SE33
 - D12S391
- Instruments (Applied Biosystems)
 - PCR Amplification System 9700
 - ABI Prism 310 Genetic Analyser



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

blood
buccal swab
finger-nails
hairs

Genetic origin

donor
32.1 % donor (16.6 - 76.3 %)
24.0 % donor (11.4 - 53.3 %)
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	2 1/2, 7 Mo nach KMT
CcD _{ee}	ccdde _e	CcD _{ee}
MNs	MNs	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
Pl M1	M3	M3



Zim.../Zim...

Spender	Empfänger	
	vor KMT	3, 4, 7 Mo nach KMT
O AB	B	O AB
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
Pl 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
prostate	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 ^a	Diagnosis	RH genotype ^b	Blood group phenotype					Number of D sites per D-positive RBCs ^c	Dynamics of D antigen positivity over follow-up interval			
					D	C	E	c	e					
1	M	63	Idiopathic colobocytelliosis	CcDde	±	±	-	-	+	+	58	11182	44	Complete loss
2	M	56	Rheumatoid arthritis	CcDde	±	±	-	-	+	+	67	12073	52	Stable
3	M	60	Anal fistula	CcDde	±	±	-	-	+	+	46	12578	40	Stable
4	F	88	Essential thrombocythemia	CcDde	±	±	-	-	+	+	85	9838	9	Stable
5	F	33	Uterus myomatous	CcDde	±	±	-	-	+	+	81	11380	24	Stable
6	M	67	Acute myelogenous leukemia	CcD ^{del} de	±	±	-	-	+	+	56	4419	13	Progressive loss
7	F	43	Psychosis	ccDDe	±	±	-	-	+	+	22	nd	65	Progressive loss
8	F	78	Colon carcinoma	CcDde	±	±	-	-	+	+	nd	nd	33	Complete loss
9	F	96	Leg vein thrombosis	CcDde	±	±	-	-	+	+	40	10950	12	Stable

RBC indicates red blood cell; ±, positive/negative mixed-field agglutination; -, negative; +, positive; ±, weak positive/negative mixed-field; and nd, not determined.
^aAge at initial recognition of mixed RHD status.
^bRH genotype and RHD zygosity as determined from blood DNA samples.
^cIn all patients, normal unreacted ABC, MNS, F, Lutheran, Kell, and Kidd blood group phenotypes were found.
 SD antigen details of the genotype-matched CcDde and CcD^{del}de (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^{del}de) displayed a partial D variant, D category IV type 4.

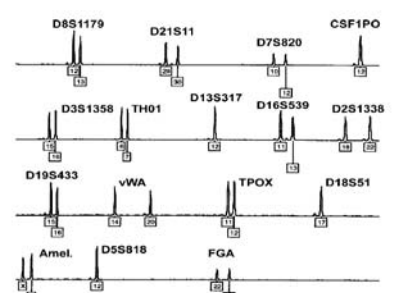


Figure 3. Exclusion of congenital or acquired chimerism by microsatellite marker analysis. Representative electropherogram showing the blood DNA profile of patient 1 after multiplex-PCR of 15 highly polymorphic autosomal short tandem repeat loci and amelogenin (Amel.). Numbers denote allelic designations 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 ^a	RHD/RHCE ^b specificities tested ^c	Number of BFU-E colonies				
			Tested	Excluded ^d	Interpretable	Positive for RHD/RHCE specificities ^e	
2	CcDde	C, c, D (Jaxon 7), e	30	0	30	13, 17, nd	0
3	CcDde	C, c, D (Jaxon 7), e	18	7	11	8, 3	nd, 0
6	CcD ^{del} de	C, c, D (Jaxon 5), e	40	4	36	29, 7	nd, 0
7	ccDDe	D (Jaxon 7), E, e	40	4	36	nd	15, 21

BFU-E indicates erythropoietic blast-forming unit; and nd, not determined.
^aAs determined from blood DNA samples.
^bBFU-E samples of patients 2, 3, and 6 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").
^cSample exclusion because of amplification failure of positive control.
^dPartial 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 ^a	2 ^a	3 ^a	4 ^a	5 ^a	7 ^b (initial)	7 ^b (after 6 y)	8 ^b (initial)	8 ^b (after 2 y)	9 ^a
p telomeric end											
D15A08	3.57	0.191	0.568	1.10	0.371	ni	0.404	0.228	0.331	0.111	0.571
D15S07	14.90	ni	0.038	1.07	0.338	0.701	ni	ni	ni	ni	0.713
D15S097	16.29	0.191	0.568	0.96	ni	0.694	0.344	0.351	ni	ni	0.633
D15D44	18.90	ni	0.092	1.00	ni	0.312	0.221	0.302	0.102	0.592	
D15I99	19.83	0.41	0.442	ni	0.612	0.761	ni	ni	ni	ni	0.688
D15D84	22.75	ni	0.711	0.242	0.242	0.742	ni	ni	ni	ni	
RHD	25.50	ni	ni	ni	ni	ni	ni	ni	ni	ni	
RHCE	25.59	ni	ni	ni	ni	ni	ni	ni	ni	ni	
D15D33	31.26	0.208	0.534	0.96	ni	0.691	0.442	0.281	0.242	0.172	ni
D15D90	37.65	0.231	0.96	0.82	ni	ni	0.382	0.272	0.172	0.062	0.302
Centromere											
D15D26	107.24	0.94	0.99	1.16	0.94	0.651	0.79	0.87	0.99	0.87	1.00
D15D56	244.94	0.92	1.01	ni	ni	0.862	0.61	0.69	0.60	0.56	1.00

ni indicates not informative (homozygous); nd, not determined; and na, not applicable.
^aPeak height ratios of blood samples by peak height ratios of hair samples.
^bPeak height ratios of blood samples for other tissues tested; unusual values were determined by comparison with normal controls.
^cValues indicating unusual peak imbalance.



Division of Blood Group Serology

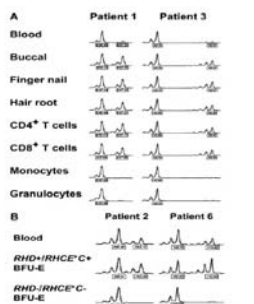


Figure 4. Loss of heterozygosity (LOH) on chromosome 1 in patients with spontaneous RH phenotype. **A**, Patient 1 (CcDde) and Patient 3 (CcDde) with LOH on chromosome 1 in Buccal, Finger nail, Hair root, CD4⁺ T cells, CD8⁺ T cells, Monocytes, and Granulocytes. **B**, Patient 2 (CcDde) and Patient 6 (ccDDe) with LOH on chromosome 1 in Blood. **RHD-IRHCE⁺ BFU-E** and **RHD-IRHCE⁻ BFU-E** indicate the presence of RHD and RHCE⁺ and RHCE⁻ alleles, respectively. **ni** indicates not informative. **ni** indicates not informative (homozygous); **nd**, not determined; and **na**, not applicable. **†**Peak height ratios of blood samples by peak height ratios of hair samples. **††**Peak height ratios of blood samples for other tissues tested; unusual values were determined by comparison with normal controls. **†††**Values indicating unusual peak imbalance.



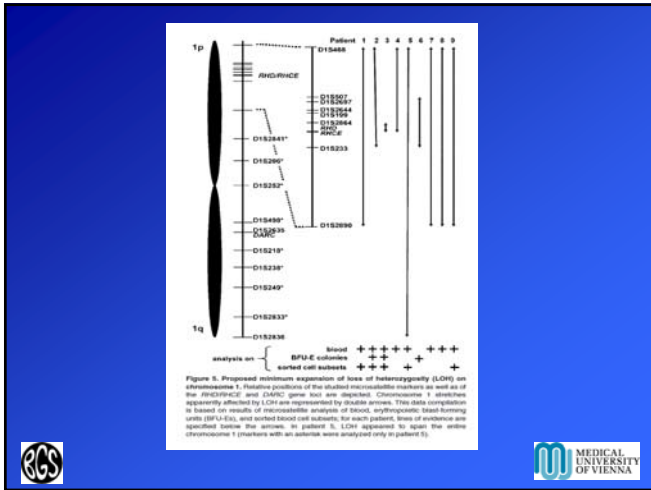


Figure 5. Proposed minimum expansion of size of heterozygosity (LOH) on chromosome 6. Relative positions of the studied microsatellite markers are used as the *RFLP/ACE* and *CAFC* gene loci are depicted. Chromosome 1 distalities are equally affected by LOH are represented by double arrows. This table compilation is based on results of microsatellite analysis of blood, arthrocytic blast-forming units (BFU-E), and nested blood cell subsets for each patient. Sites of analyses are specified below the arrows. In patient 5, LOH appeared in sperm cells (the entire chromosome 6) (patients with an asterisk were analyzed only in patient 5).

Mother-child exclusion due to paternal uniparental disomy 6

R. Wegener, V. Weirich, E. M. Dauber, W. R. Mayr
Int J Legal Med 2006; 120: 282-285

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

Mother-child exclusion due to paternal uniparental disomy 6

R. Wegener, V. Weirich, E. M. Dauber, W. R. Mayr
Int J Legal Med 2006; 120: 282-285

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 p25 to fq27)

Locus	Mother	Child	Father
DMS1774	158, 158	168	160, 168
PIA	*4,*6	*7	*7
DMS309	311, 321	321	321, 323
DMS470	124	132	132, 134
DMS209	163, 167	171	171, 175
DMS422	302, 318	302	302, 310
DMS276	211, 223	223	223
HLA-A	42, 29	43	A1, A3
DMS2960 (C3_A_33)	268, 298	302	302, 310
DMS2960 (C3_A_30)	*8, *12	*12	*12, *15
DMS2939 (C2_A_4)	*10, *18	*10	*9, *10
HLA-C	Cw1, w6	Cw6	Cw6, Cw7
HLA-B	056, 57	013	00, 013
DMS2931 (C1_A_4)	*8, *9	*8	*8, *10
HLA-DQB	01*01, *03	01*07	01*07, *15
HLA-DQ	01*03, *05	01*02	01*02, *06
DMS1610	205, 207	207	201, 205
DMS1549	191, 199	199	191, 199
DMS282	116, 123	121	121, 127
DMS1850	118, 120	122	116, 122
DMS412	278	283	273, 283
DMS272	183, 183	187	183, 187
DMS1713	206, 206	206	206, 206
DMS257	171, 177	167	161, 173
DMS460	286	294	278, 294
DMS1689	83, 99	87	87
SE33	*17, *28.2	*14	*14, *28.2
DMS462	112	112	112, 114
DMS308	196	197	197, 209
DMS1877	268, 264	267	267, 276
DMS434	206, 210	210	210, 218
DMS1698	174, 182	172	172, 180
DMS287	124	122	122, 126
DMS262	172, 180	182	176, 182
DMS292	158, 162	158	158, 166
DMS308	342	342	342
DMS441	171, 183	177	177, 181
DMS1581	263, 271	271	271, 273
DMS264	115	115	113, 115
DMS1697	252, 254	252	252, 254
DMS446	216, 222	222	222, 226
DMS297	140	142	142

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

Chromosome	Locus	Genotype/phenotype
1	D1S80	*23f, *24
	RH	CdDe
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	D7S829	*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
	D13S1245	253, 257
14	D14S990	141, 149
15	Penta E	*7, *12
16	D16S539	*11, *12
17	D17S10 (YN222)	*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

Mother-child exclusion due to paternal uniparental disomy 6

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