Chimerism: An interesting topic and an interesting case!

Chimera: A Greek mythical animal with head of lion and goat and tail of serpent.

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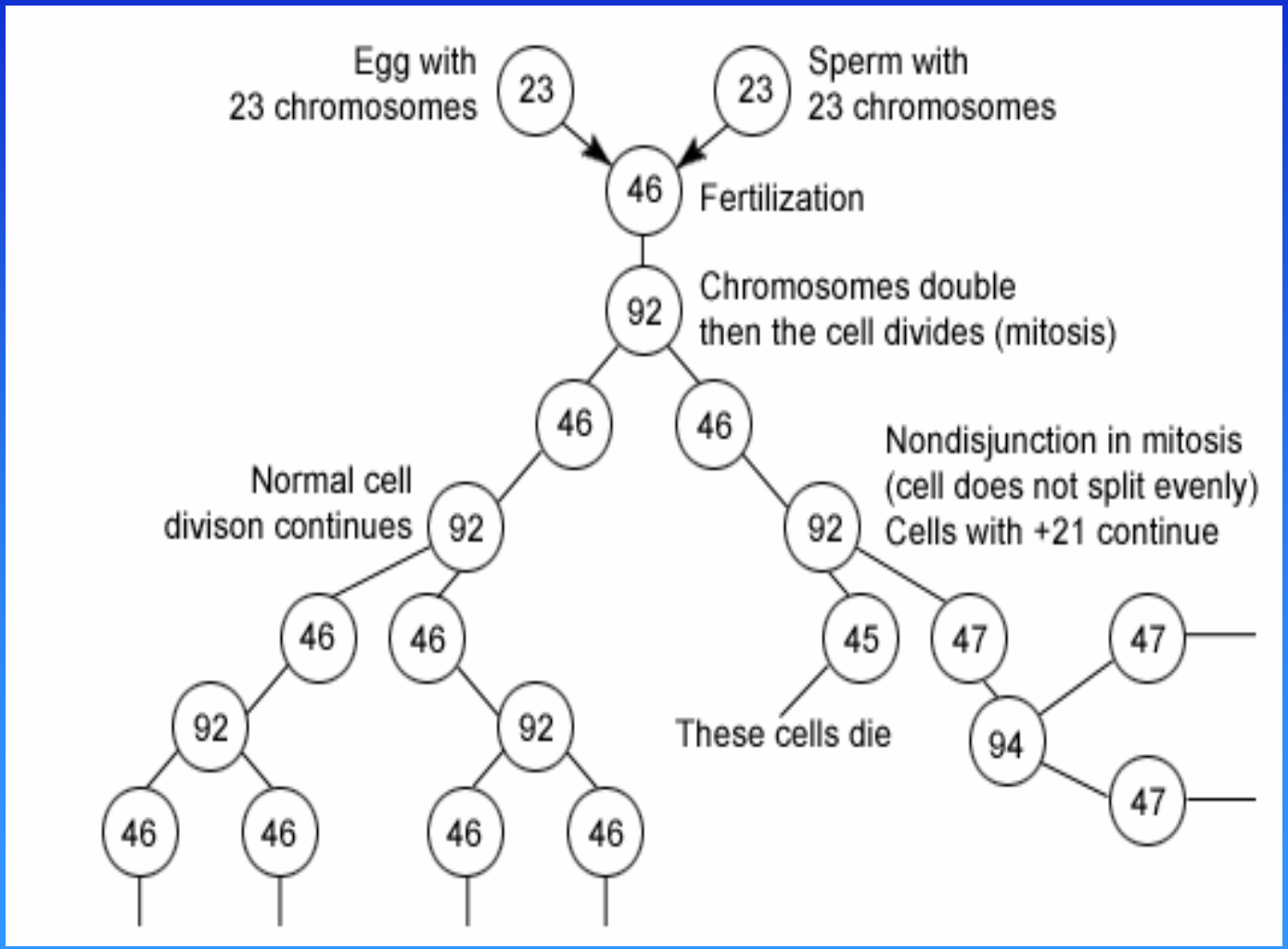
Introduction

Chimerism is the presence of two or more unrelated (genetically distinct) cell lines in an individual organism. The cell lines are derived from more than one zygote.

NOT TO BE CONFUSED WITH MOSAICISM

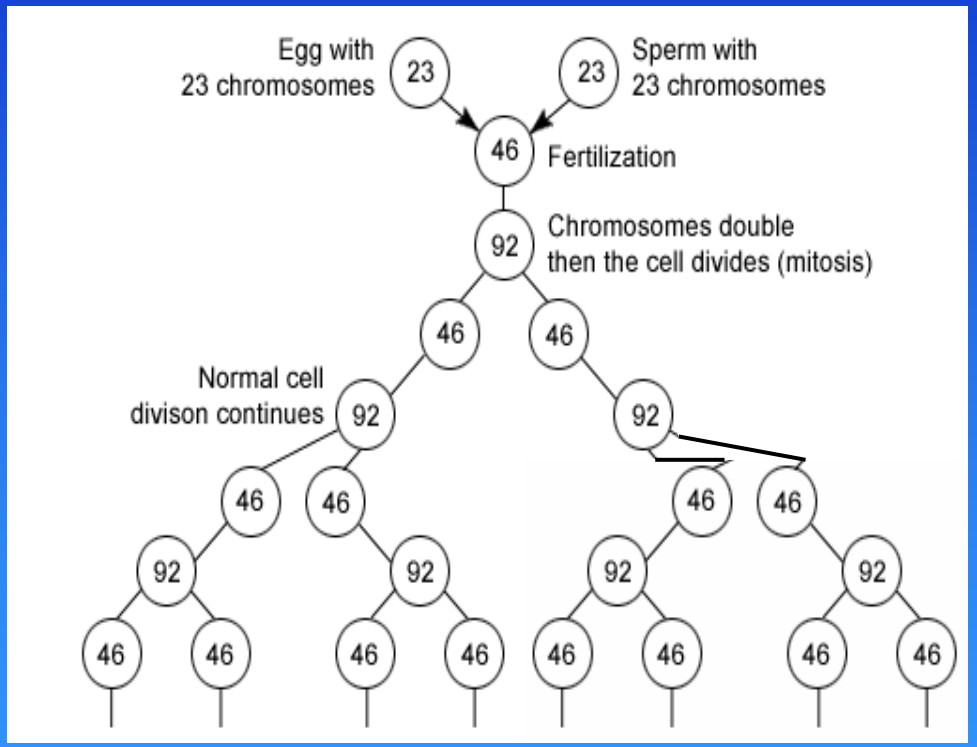
Mosaicism is the presence of two or more related cell lines in an individual organism. The cell lines are derived from a single zygote.

Mosaic Formation: All cell lines are derived from one zygote.



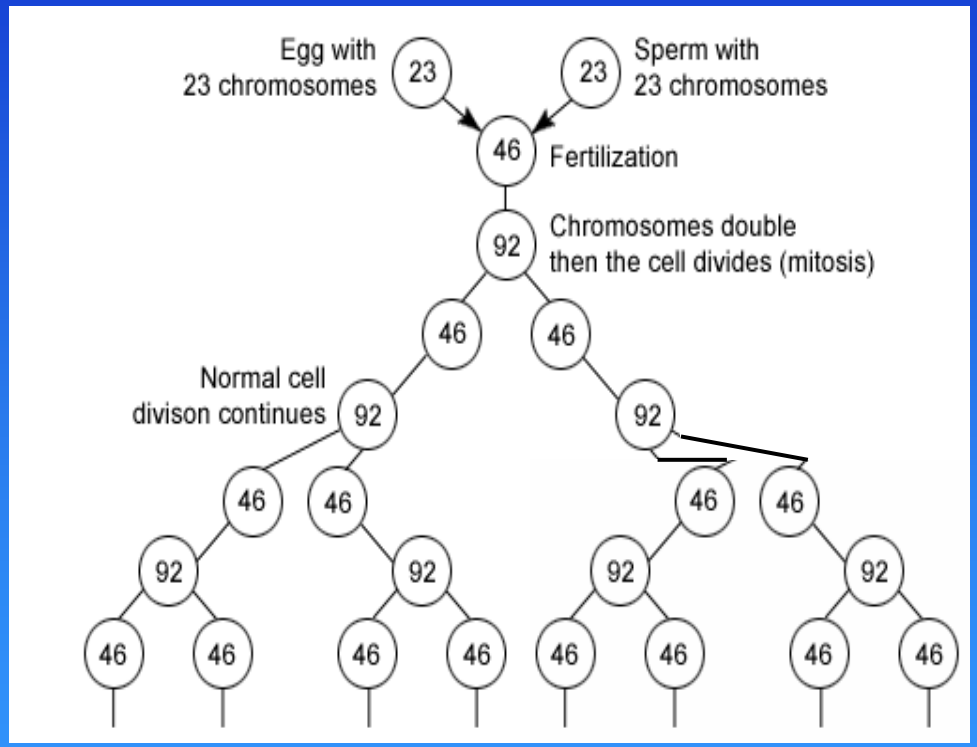
Egs. Mos 47,XX,+21/46,XX or mos 45,X/47,XXX/46,XX or mos 46,XY,del(18)(p10)/46,XY

Chimera Formation: Cells of more than one zygote mix within a single zygote.



Eg. Male [46,XY]

+



Eg. Female [46,XX]

NOTE: XX/XY chimerism is easiest form to detect cytogenetically but chimerism could be XX/XX or XY/XY.

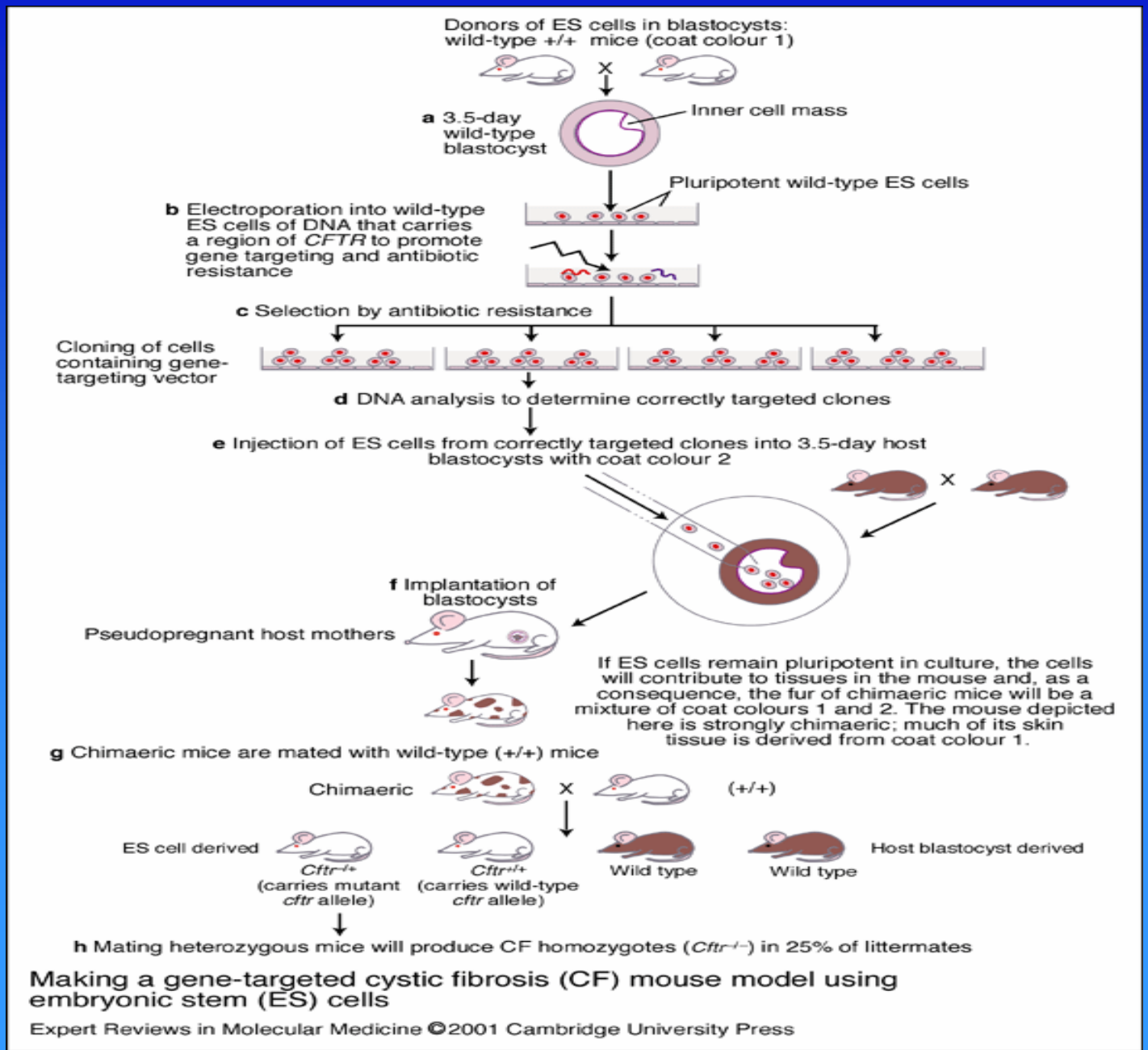
Chimeras may be:

1. Artificial:
   via transfusion/ transplantation of second cell line
   via experimental engineering in transgenic mice for example

2. Twin/Multiple Gestation:
   via transplacental passage of second cell line

3. Tetragametic:
   via fusion of two fertilized zygotes

Eg. of Chimerism via Transgene Engineering in Mouse. Detection of transgene is by coat color.



Making a gene-targeted cystic fibrosis (CF) mouse model using embryonic stem (ES) cells

This method is applied to the study of many human diseases to understand their processes: developmental biology and pathology.

Eg. CF, Parkinson disease

Facts about Naturally Occurring Chimerism

- Frequency: thought to be rare because it is generally recognized by sex chromosome discrepancy
  - Thus, at least 3 times more frequent than detected by XX/XY chimerism
  - Is likely to increase with increase in multiple births due to IVF

- Occurs in other species
  - Most common in cattle (results in free martins) but occurs in others
  - Frequency related to the biology of pregnancy; in cattle, anastomoses of early fetal circulation is common in multiple gestations

Misinterpretation of HLA typing in a woman with congenital chimerism due to postzygotic fusion of two embryos (NEJM 346:1545-1552,2002)

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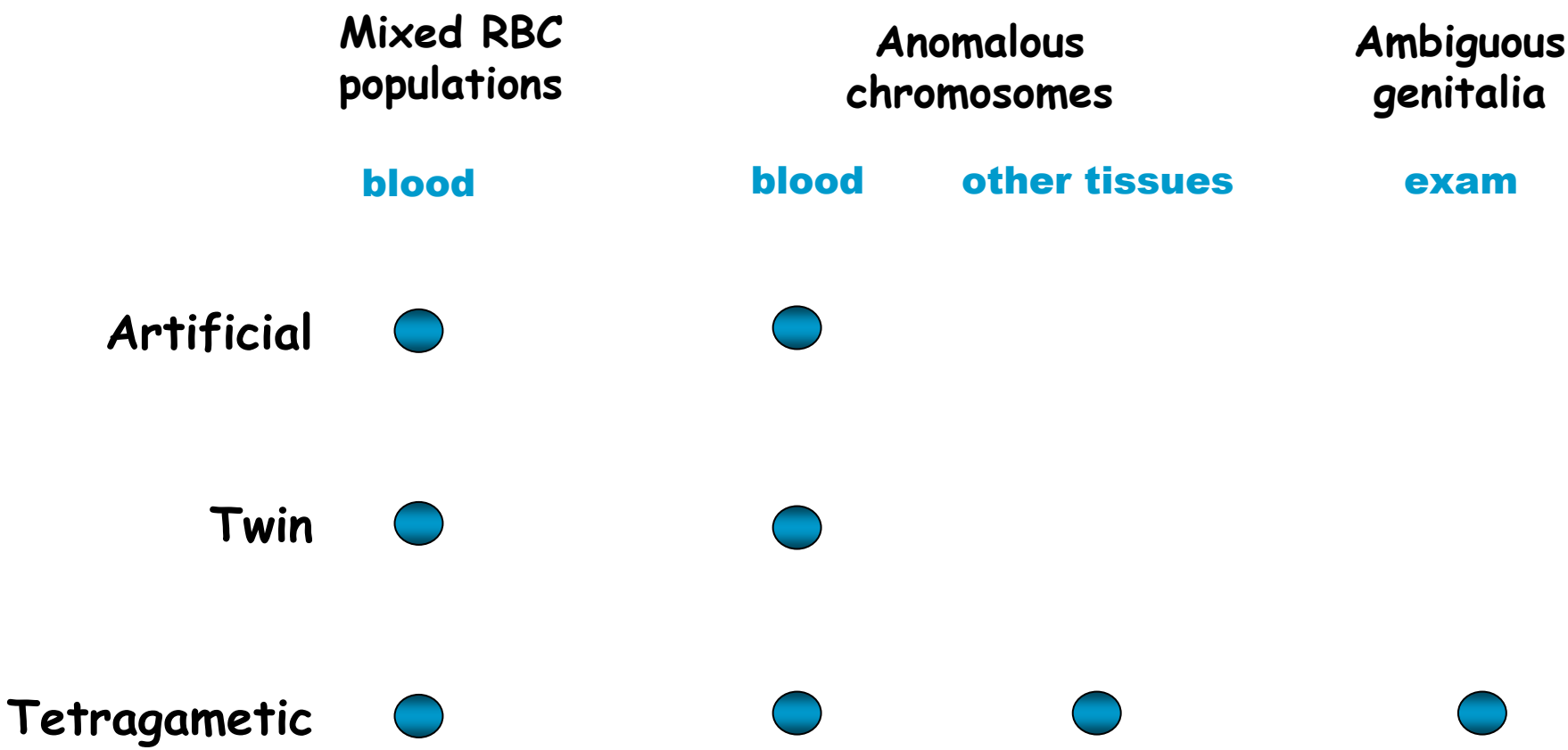
Detection of chimerism (in humans)



We report the identification of an unusual HLA typing dilemma that led to the diagnosis of tetragametic chimerism

CASE REPORT

K.K. presented to our institution at age 52, with renal failure secondary to focal sclerosing glomerulonephritis. She was considering a living related donor kidney transplant.

Pregnancy history: G3,P3

Normal physical exam

Blood type: A, Rh-positive

HLA studies of family members as possible donors were performed.

Family HLA typing (blood)

Name	Relationship	HLA haplotypes				
K.K	Proband	A	B			
P.K.	Husband			C	D	
M.K.	Son (age 29)			C		E
J.K.	Son (age 27)			C		E
B.K.	Son (age 19)	A			D	

A: A66, B41, DRB1*04, DRB4*01, DQB1*0301

B: A2, B60, Cw3, DRB1*04, DRB4*01, DQB1*0301

C: A3, B35, Bw6, Cw4, DRB1*0101, DQB1*0501

D: A24, B62, Bw6, Cw3, DRB1*04, DRB4*01, DQB1*0302

E: A25, B8, DRB1*08, DQB1*04

From these data, KK was told that she was "excluded" as the mother of two of her three children.

Extended family HLA typing (blood)

Name	Relationship	HLA haplotypes					
K.K	Proband	A		B			
P.K.	Husband			C		D	
M.K.	Son (age 29)			C			E
J.K.	Son (age 27)			C			E
B.K.	Son (age 19)	A				D	
E.H.A	Mother			B			F
D.H.A.	Brother	A		B			
T.A.	Brother			B			E
P.A.†	*Father*	*A†*					*E†*

† deceased; presumed type

F: A11, B27, Cw1, DRB1*0101, DQB1*0501

HLA haplotype of proband's tissues via PCR-SSOP

A haplotype probe: HLA-B41

E haplotype probe: HLA-B8

TISSUE \ HAPLOTYPE	A	B	E	F
Blood	*strong*	*strong*	NOT DETECTED	NOT DETECTED
Skin fibroblasts	*strong*	*strong*	weak	weak
Hair	*strong*	*strong*	weak	weak
Buccal mucosa	*strong*	*strong*	weak	weak
Thyroid	weak	weak	*strong*	*strong*
Bladder epithelium	weak	weak	*strong*	*strong*
Bladder fibroblasts	weak	weak	*strong*	*strong*

Extended family HLA typing

Name	Relationship	HLA haplotypes					
K.K.	Proband	A	B			E	F
P.K.	Husband			C	D		
M.K.	Son (age 29)			C		E	
J.K.	Son (age 27)			C		E	
B.K.	Son (age 19)	A			D		
E.H.A	Mother		B				F
D.H.A.	Brother	A	B				
T.A.	Brother		B			E	
P.A.†	*Father*	*A†*				*E†*	

† deceased; presumed type

F: A11, B27, Cw1, DRB1*0101, DQB1*0501

HLA haplotypes: blood versus hair
K family

Source \ Sample	Blood	Hair
KK (proband)	A, B	A, B, *E, F*
MK (son)	C, E	C, E
JK (son)	C, E	C, E
BK (son)	A, D	A, D
DHA (brother)	A, B	A, B
TA (brother)	A, F	A, F

Additional laboratory studies

Red cell phenotype studies: A, Rh-positive; no mixed field agglutination (ABO, Rh, Kell, Duffy, Kid, Lewis, P, MNSs)

Peripheral blood: 46 XX karyotype

Skin fibroblasts: 46 XX. Interphase FISH for chromosome 6 centromeres: two per cell with no evidence of aneuploidy

DNA VNTR (microsatellite) analysis

LOCUS	KK (proband) Blood	KK (proband) Skin	MK (son) Blood	DA (brother) Blood	TA (brother) Blood
D2S2216	211/211	215/221	215/221	211/221	211/221
D2S160	217/219	213/217	213/215	217/219	217/219
D20S195	139/149	139/147	139/147	139/149	139/149
DXS1073	311/313	313/313	311	313	313

DNA VNTR (microsatellite) analysis

LOCUS	KK (proband)				
	Blood	Skin	Hair	Thyroid	Buccal mucosa
D16S539	13/9	13/9	13/11/9	13/11/9	13/9
D4 (FGA)	22/20	23/22/20	23/22/20	23/22/20	22/20
D9S304	12/4	12/4	12/9/4	12/4	12/9/4

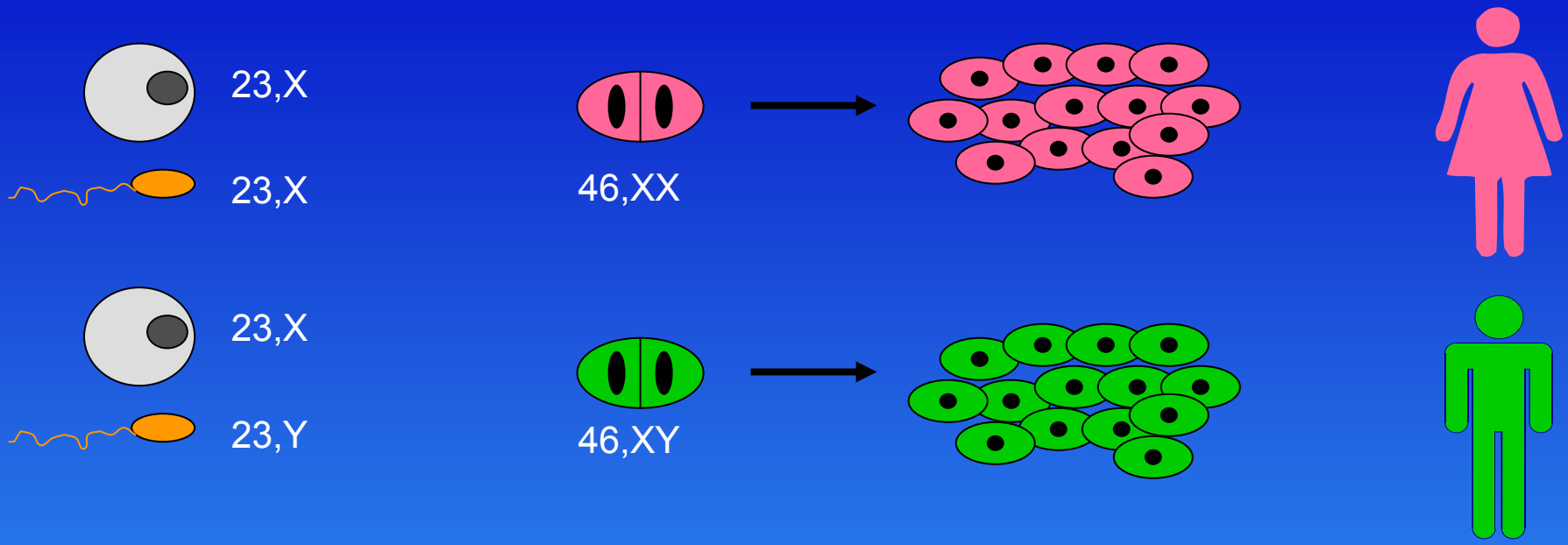
Table 1. Microsatellite analysis for 17 chromosomes (NEJM 346:1545-1552,2002)

Chromosome No. and Locus	Variable	Type and Source of Sample											
		Blood, patient	Hair, patient	Thyroid, patient	Buccal mucosa, patient	Skin, patient	Blood, son 1	Blood, son 2	Blood, son 3	Blood, father†	Blood, mother	Blood, brother 1	Blood, brother 2
1, D1S533	No. of repeats	15/11	*15/11/(8)*	*15/11/(8)*	15/11	15/11	15/9	**9/8**	ND	8/11	15/14	14/8	15/11
2, TPOX	No. of repeats	11/8	11/8	11/8	11/8	11/8	11/11	12/11	ND	8/11	11/11	11/8	11/11
2, D2S160	Size of allele (bp)	217/219	ND	ND	ND	*213/217*	**213/215**	ND	ND	ND	ND	217/219	217/219
2, D2S2216	Size of allele (bp)	211/211	ND	ND	ND	*215/221*	**215/221**	ND	ND	ND	ND	211/221	211/221
3, D3S1358	No. of repeats	17/14	17/14	*17/(15)/14*	17/14	17/14	**15/15**	17/15	ND	14/18	17/15	17/15	18/15
3, D3S1744	No. of repeats	20/17	*20/(19)/17*	*20/19/(17)*	*20/(19)/17*	*20/(19)/17*	**19/18**	20/18	18/17	17/19	20/20	20/17	20/17
4, FGA	No. of repeats	22/20	*(23)/22/20*	*23/22/(20)*	*22/20*	*(23)/22/20*	22/20	22/20	ND	ND	23/20	23/22	23/22
5, D5S818	No. of repeats	12/11	12/11	12/11	12/11	12/11	12/11	11/11	11/11	ND	11/11	12/11	12/11
7, D7S820	No. of repeats	10/10	*10/(8)*	*10/(8)*	10/10	*10/(8)*	**11/8**	**11/8**	11/10	10/12	10/8	10/10	12/8
8, D8S1179	No. of repeats	16/13	*16/13/(11)*	*16/11/(13)*	16/13	16/13	16/13	16/11	ND	13/16	13/11	16/11	16/11
9, D9S304	No. of repeats	12/4	*12/(9)/4*	*9/4*	*12/(9)/4*	12/4	12/4	12/9	ND	9/12	4/4	9/4	9/4
11, THO1	No. of repeats	9.3/9	9.3/9	9.3/(9)	9.3/9	9.3/9	9.3/9.3	9.3/9.3	ND	10/13	9.3/9	10/9.3	9.3/9.3
12, vWA	No. of repeats	17/14	17/14	17/14	17/14	17/14	17/17	19/17	ND	ND	17/14	17/14	18/14
12, D12S1090	No. of repeats	23/20	*23/(22)/20*	*22/22*	*23/(22)/20*	*23/(22)/20*	**24/22**	**24/22**	24/23	22/23	22/20	22/22	22/22
13, D13S317	No. of repeats	11/8	*(13)/11/8*	*13/11/(8)*	11/8	11/8	13/11	11/11	11/11	8/11	13/11	11/11	11/11
15, Penta E	No. of repeats	15/14	*15/14/(13)*	*14/13*	15/14	15/14	17/14	**13/5**	ND	13/14	15/14	14/13	14/14
16, D16S539	No. of repeats	13/9	*13/(11)/9*	*(13)/11/9*	13/9	13/9	**12/11**	11/9	13/11	9/11	13/11	11/9	13/11
18, D18S51	No. of repeats	14/11	14/11	11/11	(14)/(11)	14/11	13/11	19/11	ND	11/14	14/11	14/14	11/11
18, D18S849	No. of repeats	18/16	18/16	*18/17*	18/16	18/16	18/16	**17/17**	18/17	ND	18/17	17/16	17/16
20, D20S195	Size of allele (bp)	139/149	ND	ND	ND	*139/147*	139/147	ND	ND	ND	ND	139/149	139/149
21, D21S11	No. of repeats	31/28	31/28	*31/30.2*	31/28	31/28	31/29	34.2/31	ND	ND	31/30.2	31/30.2	31/28
X, DXS1073	Size of allele (bp)	311/313	ND	ND	ND	313/313	311	ND	ND	ND	ND	313	313

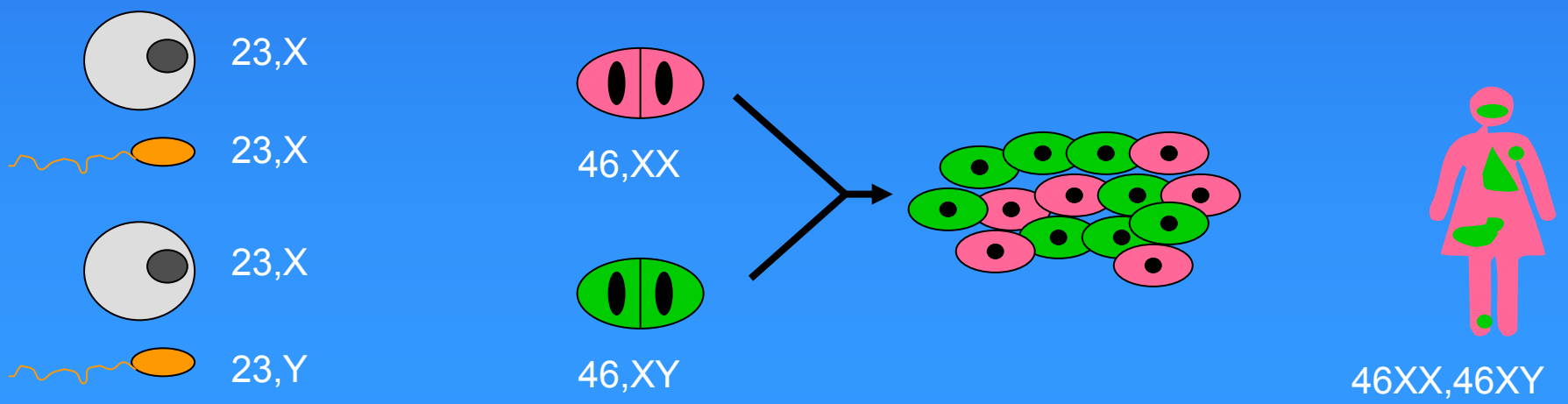
*Values in parentheses refer to minor-intensity alleles (for which the height of the curve was less than 50 percent of the height of the curves for other alleles at the same locus). Discrepancies in results between blood samples and tissue samples from the patient are italicized. Results in two of the patient's sons that appeared to rule her out as their mother are shown in boldface type. ND denotes not done.

†Because samples were not available from the patient's father, the results were deduced from studies of other family members.

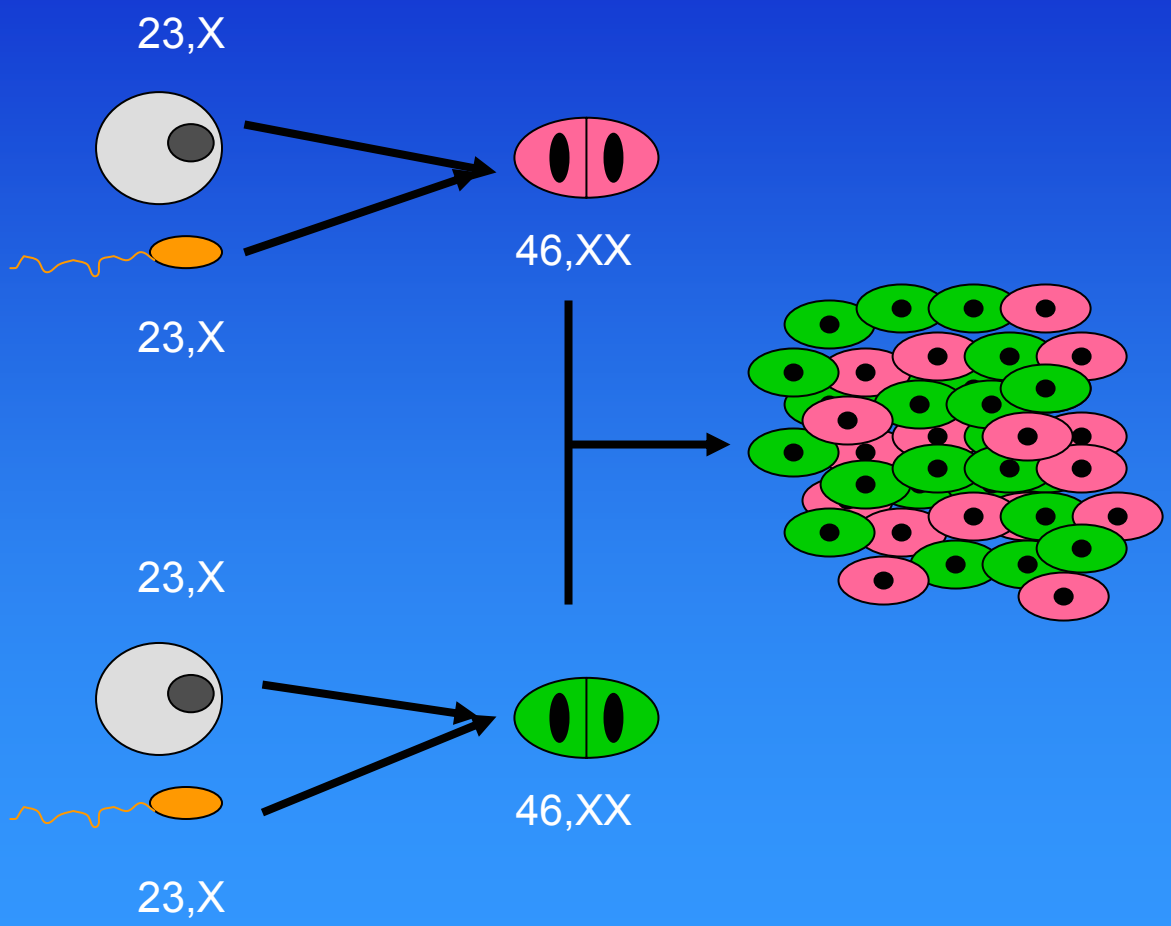
Normal fertilization and development

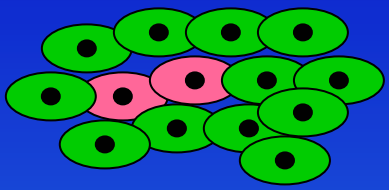


Tetragametic chimerism

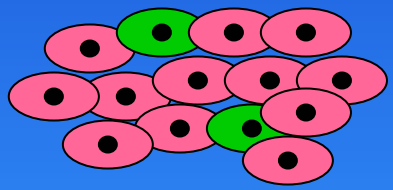


Tetragametic chimerism – this case

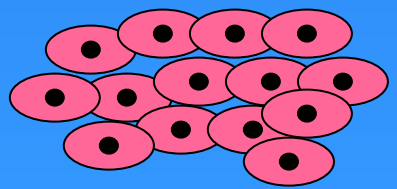




Bladder epithelium
Bladder fibroblasts
Thyroid



Buccal mucosa
Hair
Skin fibroplasts



Blood

TETRAGAMETIC ("DISPERMIC") CHIMERISM

Diagnosis established by:

- Four HLA haplotypes in tissues
- Segregation of haplotype pairs via PCR
- DNA microsatellite analysis
- FISH and cytogenetics

Diagnosis difficult to make due to:

- Presence of only one cell line in blood
- XX/XX fusion

CONCLUSIONS

1. This patient is a tetragametic chimera, from the fusion of two 46 XX zygotes early in development.

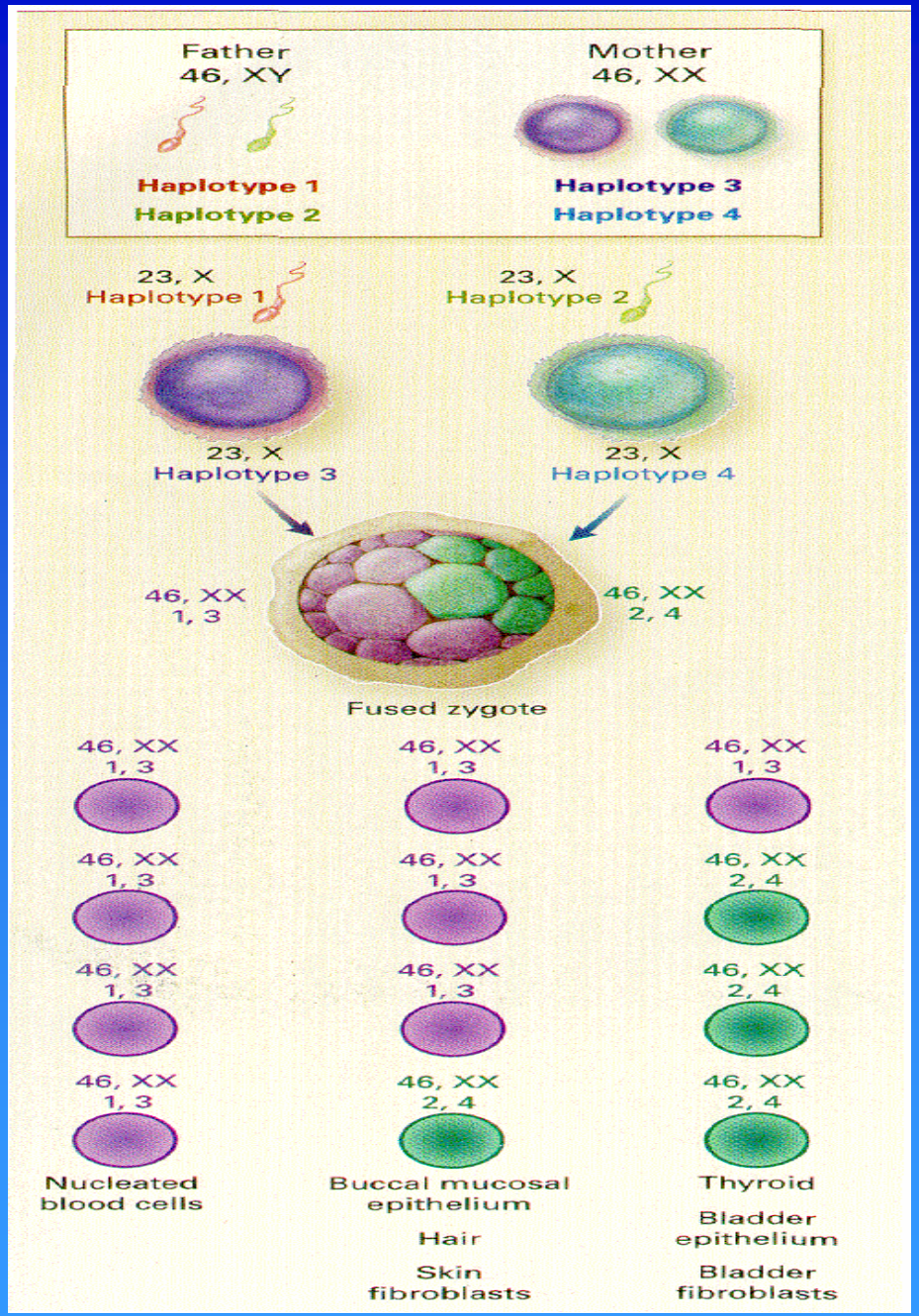
2. Tetragametic chimerism, while rare, may be underdiagnosed, expecially with same-sex zygote fusions.

3. The absence of a second cell line in blood contributed to the diagnostic dilemma. This finding may represent extreme population skewing, or the disappearance of one line during development.

4. The patient exhibits tolerance to all four HLA haplotypes, and her options for transplantation are widened by her genetic make-up.

Implications for Disease

- Mosaicism and chimerism outcomes could overlap
  - requires DNA analysis generally to detect in other tissues/cells unless cell lines are chromosomally distinct (male-female, distinguishing heteromorphisms)
- No way to predict recurrence risk (rare but probably higher than population risk if mutation related)
- Greater tolerance for organ/tissue transplantation
- Cause dilemmas in identity testing
  - Possible false negative results if wrong tissue selected
  - Could appear as apparent mixed DNA sample
  - Identify DNA source as coming from 2 individuals instead of 1
- Cause dilemma in gender assignment (ie. hermaphrodite)



(NEJM 346:1545-1552,2002)

Cell-mediated lysis using KK's lymphocytes