

DOUBLE TROUBLE  
CHIMERISM IN TWIN A

# CASE STUDY

# TWIN A

1 DAY OLD NEWBORN TRANSPORTED TO CMH WITH  
IMPERFORATE ANUS AND CLEFT PALLET

Type and Screen received for surgery

History: 35 weeks gestation

C-Section due to hypertension in mother

Mother: O pos with negative ABSN

Twin B: History of O pos

No transfusions received

# SEROLOGY RESULTS

## Tube Method

### ▶ Specimen #1

- ▶ Anti-A: 0
- ▶ Anti-B: 0
- ▶ Anti-D: 4+
- ▶ A cells: Not performed
- ▶ B cells: Not performed
- ▶ Antibody Screen: Negative
- ▶ DAT: Negative

**Type: O pos**

### Specimen #2 and #3

- ▶ Anti-A 2+ Mixed Field
- ▶ Anti-B 0
- ▶ Anti-D 4+
- ▶ A cells: Not performed
- ▶ B cells: Not performed
- ▶ Antibody Screen: Negative
- ▶ DAT: Negative

**Type: A pos**

Confirmation ABO did not match initial type. Not enough specimen to repeat testing on sample #1.

**Sent to CBC Reference Lab**

# IRL Results

- ▶ Results: ABO Discrepancy
- ▶ Blood group: Indeterminate
- ▶ Repeat Blood type in 1 month for repeat typing or genotyping to determine blood type.
- ▶ Transfuse: O positive RBCs and AB plasma.
- ▶ Crossmatch: IS in tube

# Mixed Field: Double Trouble for the Blood Bank

- ▶ What is the patient's blood type?
- ▶ What is the origin of the mixed field results?



# What Causes *Mixed Field* Reactions?

# POSSIBILITIES:

- ▶ 1. Maternal-Fetal hemorrhage
- ▶ 2. Twin - Twin hemorrhage
- ▶ 3. Twin A is a Twin Chimera
- ▶ 4. Blood Transfusion
- ▶ 5. Subgroup of A
- ▶ 6. Mosaicism

Based on patient history #4 is excluded.

Other than the A subgroup and mosaicism, all possibilities involve a type of chimera.

What is a Chimera?

# Chimera

A person who has more than one distinct cell line that originates from two or more zygotes.<sup>1</sup>

Originally believed to be rare, twin chimeras have been detected in 8% of twins and 21% of triplets.<sup>2</sup>

# Mosaic vs. Chimera

- ▶ Both terms refer to one organism with two or more populations of cells.
- ▶ Both have more than one genetically distinct population of cells.
- ▶ A Mosaic forms from the same zygote and a chimera forms from two zygotes.
- ▶ Mosaics arise when a mutation occurs early in development and results in a mixture of cells, some with the mutation and some without.





Chimera D'Arezzo, c. 400 BCE. *Creative Commons*

# HISTORY OF CHIMERAS

- ▶ 1945: Owen discovers that RBCs of dizygotic twin cattle are a mixture of the two separate twins.<sup>3</sup>
- ▶ 1953: First Described in humans by Dunsford et al. Mrs. McK had 60% O cells and 40% A cells.<sup>4</sup>
- ▶ 1975: Race and Sanger divided Chimeras into either twin or dispermic.<sup>1</sup>

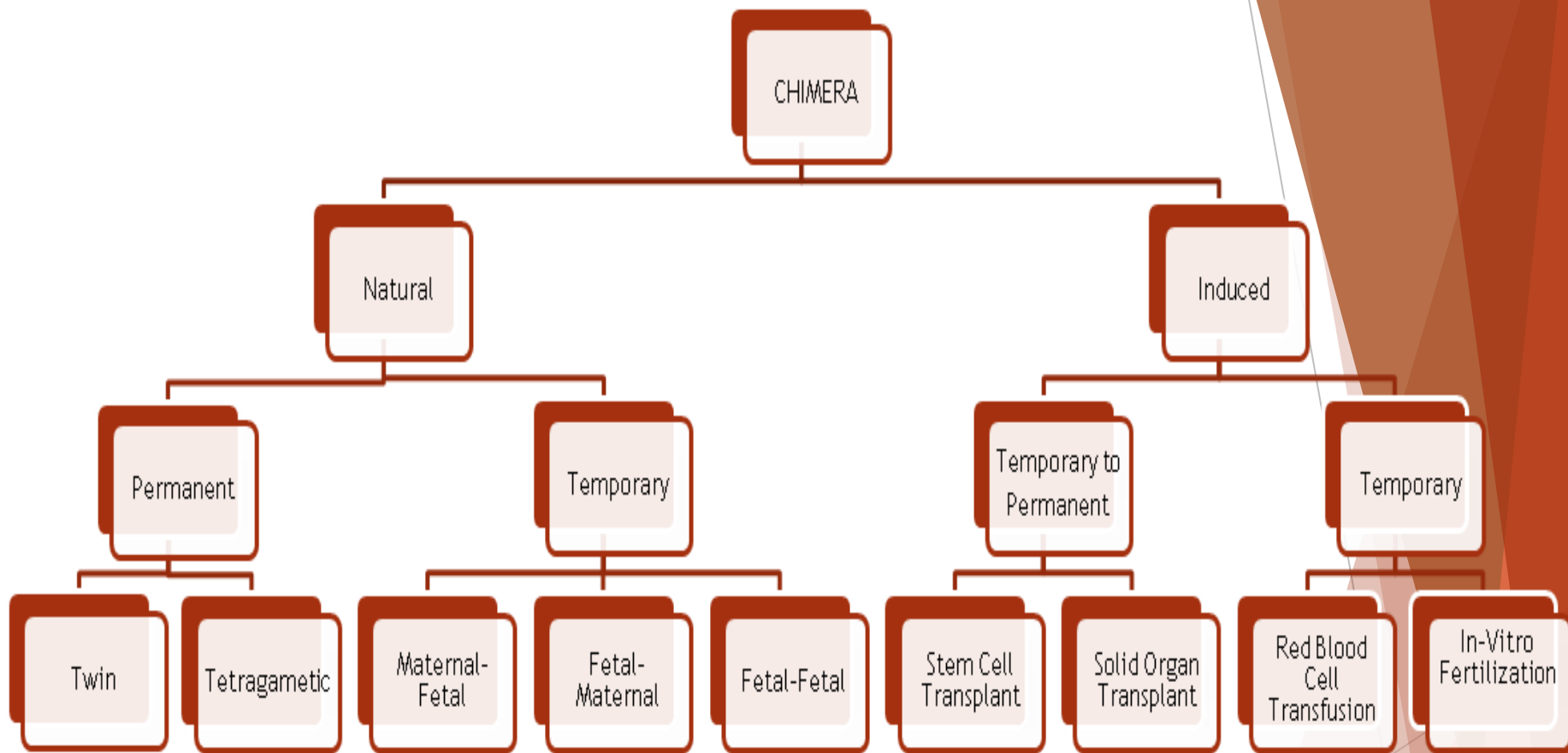
# Greek Mythology

## CHIMERA:

A mythological monster with the head of a lion, body of a goat, and the tail of a serpent. This symbol has been the symbol of an organism whose cells derive from two or more distinct zygotes.<sup>1</sup>

# CLASSIFICATION

The background features a series of overlapping, semi-transparent geometric shapes in various shades of orange and brown. These shapes are primarily located on the right side of the frame, creating a layered, abstract effect. The overall aesthetic is clean and modern, with a focus on geometric forms and a warm color palette.



# 2 TYPES OF CHIMERAS

- ▶ 1. NATURAL
- ▶ 2. INDUCED

# NATURAL CHIMERA

- ▶ 1. PERMANENT
- ▶ 2. TEMPORARY

# PERMANENT NATURAL

- ▶ 1. Twin Chimera
- ▶ 2. Tetragametic Chimera

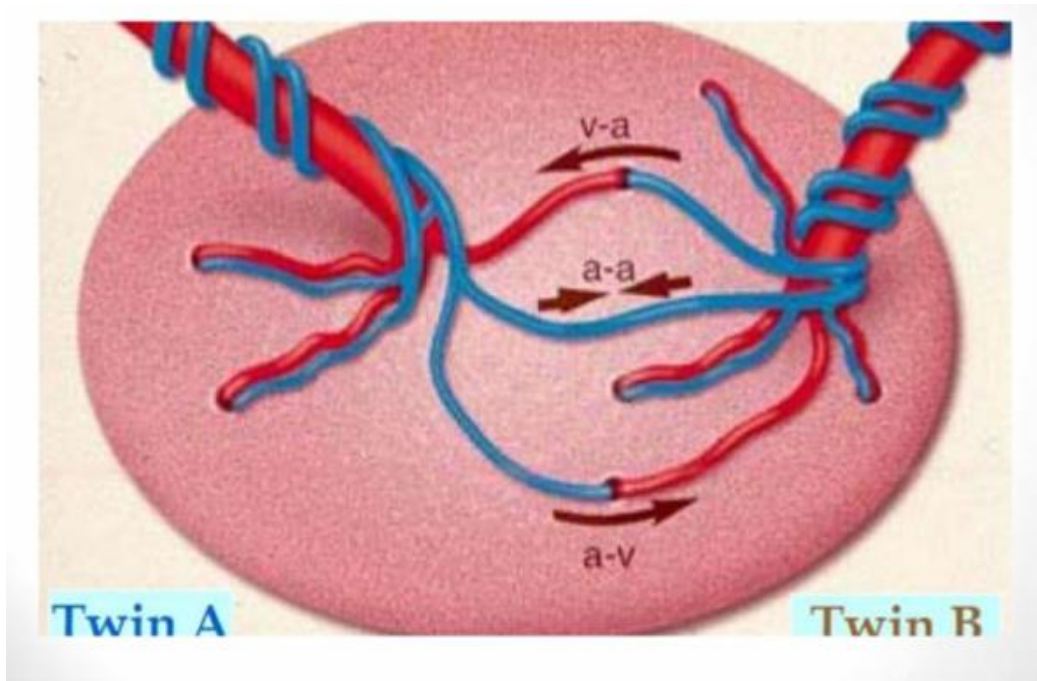


# TWIN CHIMERAS

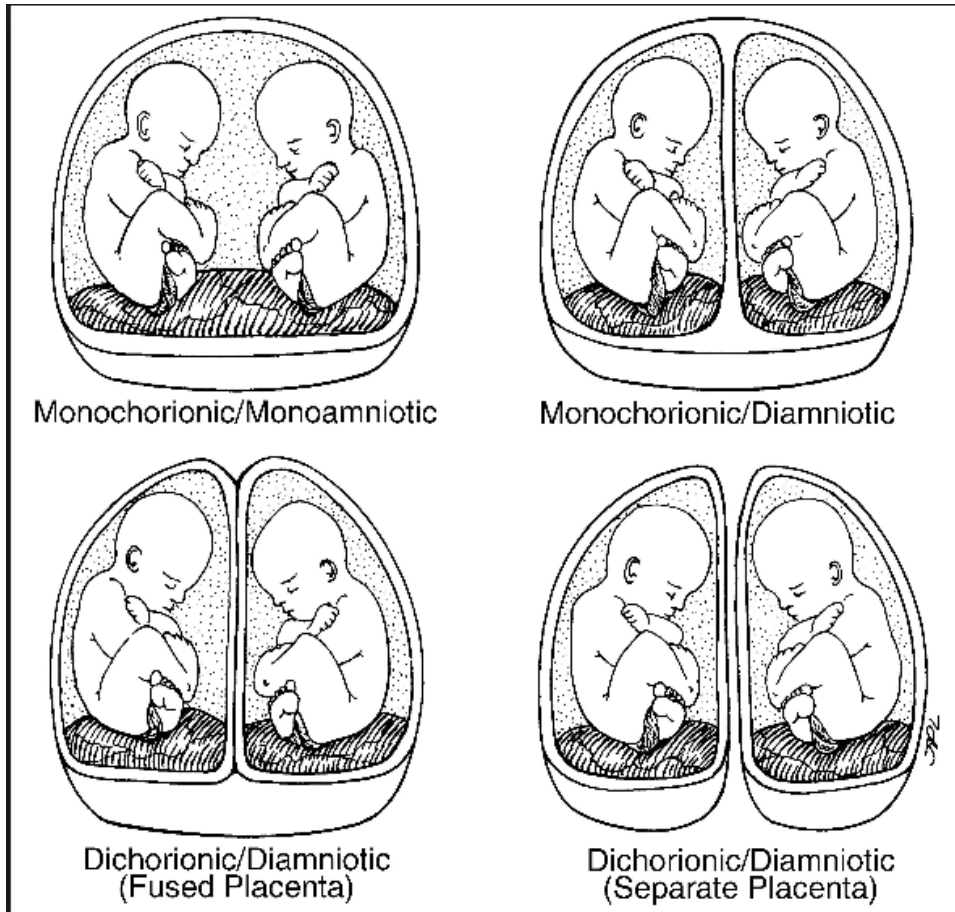
Caused by blood vessel anastomoses of nonidentical twins, resulting in exchange of blood cells, including hematopoietic stem cells. These blood cells travel to each others bone marrow. Due to the inability of the fetus to recognize foreign antigens, both cell lines are produced within each individual twin.

Isohemagglutinins may or may not be produced.<sup>5,6</sup>

# Blood Vessel Anastomosis



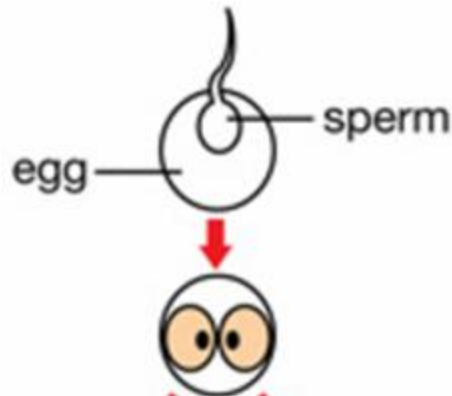
# Monochorionic vs. Dichorionic Placenta



<http://hdydi.com/category/multiple-types/fraternal>

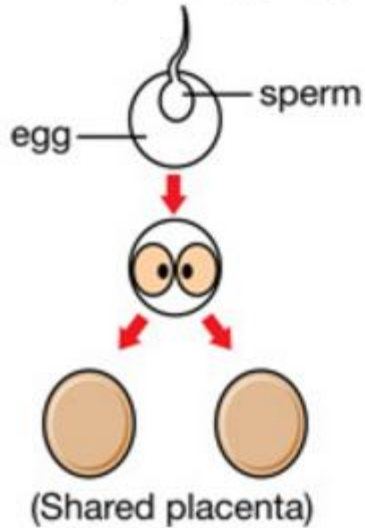
# Single Fetus

- ▶ **ZYGOTE:** The cell formed by the union of a male sperm and a female ovum.

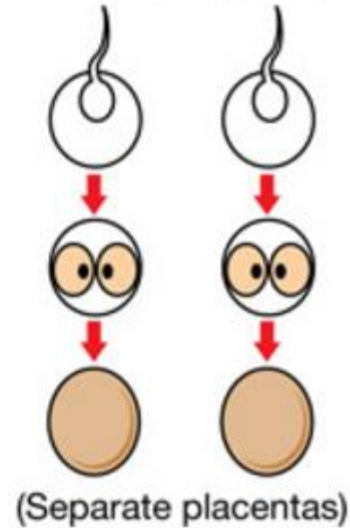


# Monozygotic vs. Dizygotic Pregnancy

a) Identical (Monozygotic) Twins



b) Fraternal (Dizygotic) Twins



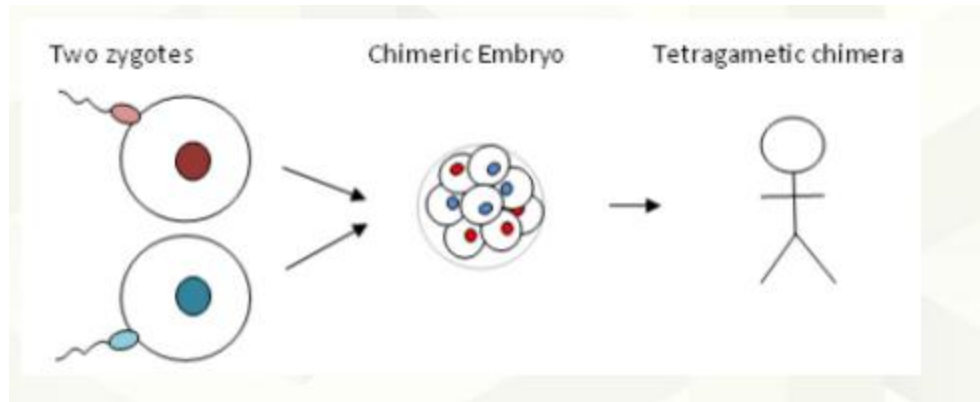
Identical

Fraternal

# TETRAGAMETIC CHIMERAS (Dispermic dizygotic)

- ▶ Fertilization of 2 maternal nuclei by 2 sperm.
- ▶ These 2 zygotes fuse into one person.
- ▶ If a male and female zygote are involved, the individual will have a combination of XX/XY chromosomes.
- ▶ This is a whole-body permanent chimera.
- ▶ May have patchy skin color, different colored eyes, and hermaphroditism.
- ▶ Which organs contain which DNA is random.
- ▶ Known as self-contained or vanishing twins.

# Tetragametic Chimera



# TEMPORARY NATURAL

- ▶ 1. MATERNAL-FETAL
- ▶ 2. FETAL-MATERNAL
- ▶ 3. FETAL-FETAL



# INDUCED CHIMERAS

- ▶ 1. Induced “Permanent”
- ▶ 2. Induced Temporary

## INDUCED PERMANENT CHIMERAS

- ▶ 1. ALLOGENEIC STEM CELL TRANSPLANT
- ▶ 2. SOLID ORGAN TRANSPLANT

# Allogenic Stem Cell Transplants

- ▶ 1. Standard myeloablative is transplantation where the donor's marrow is destroyed.
- ▶ 2. Nonmyeloablative transplantation allows both cell lines to coexist.

# Solid Organ Transplants

- ▶ Results in “Microchimerism”
- ▶ Amount of donor cells are too small to be seen in serologic testing.
- ▶ An interaction between donor and recipient leukocytes takes place.
- ▶ Dendritic donor cells have been found in the recipients lymph nodes, peripheral blood, skin, intestine and heart.<sup>7</sup>
- ▶ This balance can result in rejection, GVHD, or tolerance.

# INDUCED TEMPORARY CHIMERA

- ▶ 1. Blood Transfusion
- ▶ 2. In-Vitro Fertilization

# RBC Transfusions

Chronic transfusions and massive transfusions will create temporary chimeras and the donor cells will last for weeks.

# In-Vitro Fertilization

- ▶ IVF places more than one embryo into the mother.
- ▶ Results in a 33.9% increase in dizygotic twin deliveries when 2 embryos are implanted.<sup>8</sup>
- ▶ Causes an increase in multiple births with chimeras, twin chimeras, and tetragametic chimeras.

# Back to our Case Study

## Original Results:

- ▶ Anti-A 2+ Mixed Field
- ▶ Anti-B 0
- ▶ Anti-D 4+
- ▶ A cells: Not performed
- ▶ B cells: Not performed
- ▶ Antibody Screen: Negative
- ▶ DAT: Negative



## 3 Months Later:

- ▶ Patient returns for additional surgery.
- ▶ Type and screen is ordered.

# Results:

- ▶ Anti-A 3+
- ▶ Anti-B 0
- ▶ Anti-D 4+
- ▶ A cells: 0\*
- ▶ B cells: 0\*
- ▶ Antibody Screen: Not performed
- ▶ DAT: Not performed

\*Common reaction in children under 6 months

**No Mixed Field!**

**Blood Group A positive!**

Our patient does not appear to be a twin chimera or a subgroup of A.

What caused the mixed field  
in the initial sample?

## Most Probable:

- ▶ Maternal-Fetal hemorrhage
- ▶ Fetal-fetal hemorrhage

# Looking Back:





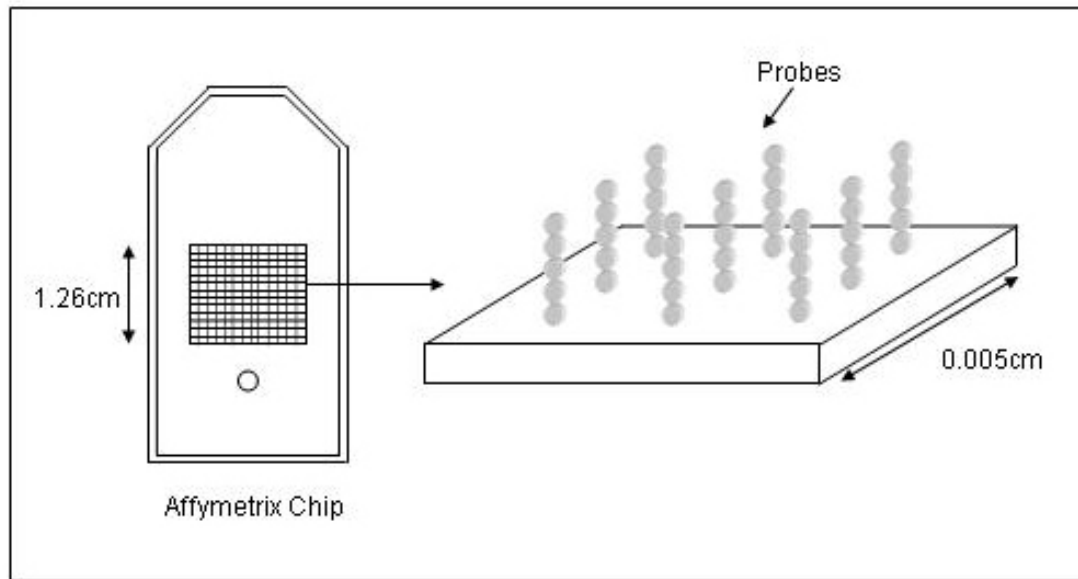
Original specimen sent to CMH Cytogenetics Lab for Microarray Analysis. These results support the conclusion that this patient is not a twin chimera.

# Microarray Analysis

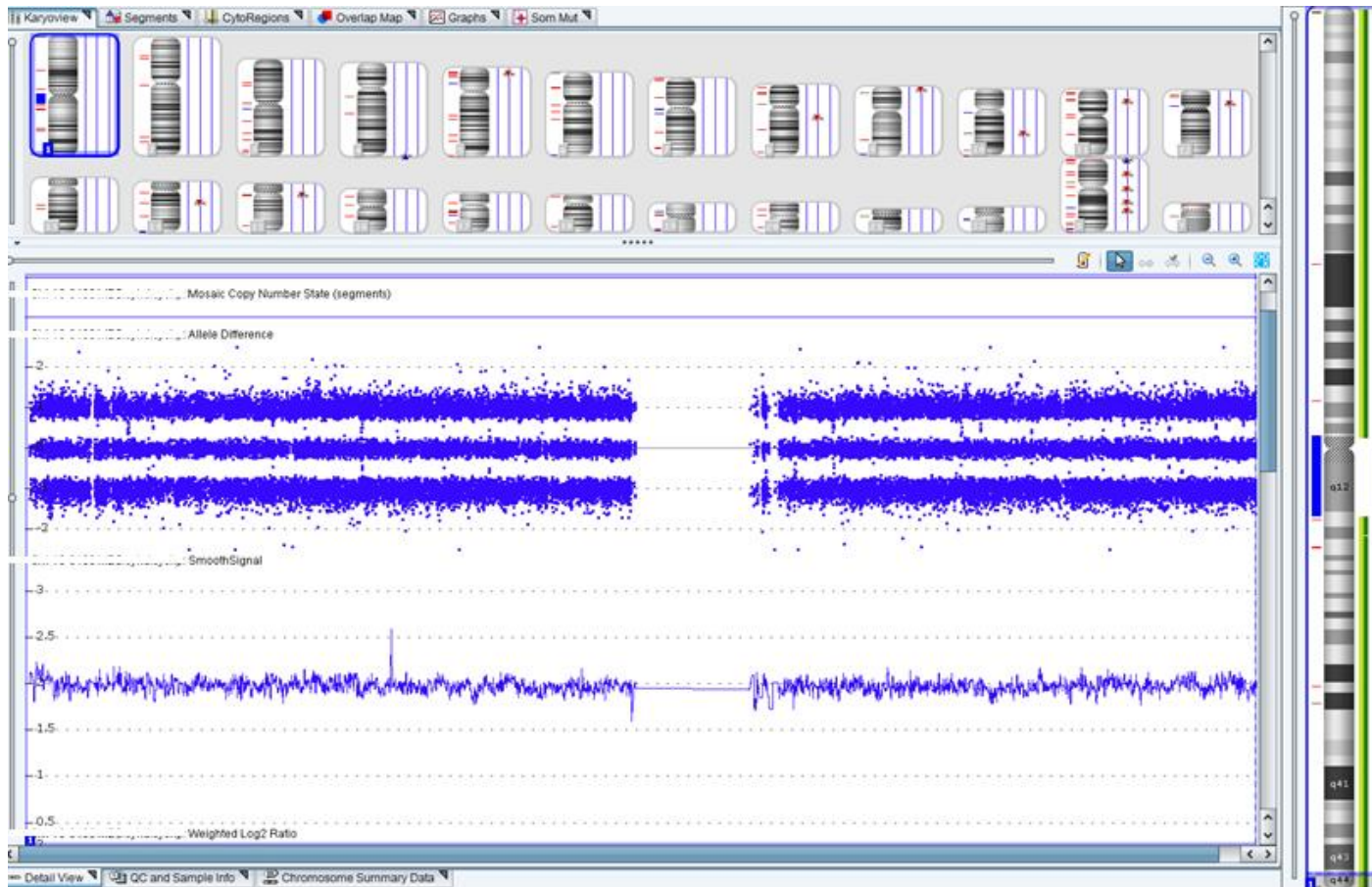
- ▶ Clones of interest (probes) are amplified by PCR and printed onto treated glass slides (chip) using a robotic printer. These gene chips contain thousands of known gene sequences.<sup>9</sup>
- ▶ DNA is extracted from the patient sample and amplified by PCR.
- ▶ The sample is hybridized to the microarray chip. Following washing, laser excitation is applied and the emitted light is measured in each color channel.
- ▶ Computer analysis is performed on fluorescence intensity and intensity ratios to each spot, which are then exported for statistical analysis to identify differentially expressed genes.
- ▶ The result is the ability to analyze the expression of many genes simultaneously in a single reaction.

# Illustration of a DNA GeneChip (Affymetrix)

Figure 1. Glass complementary DNA (cDNA) microarray produced by using high-speed precision robot. This type of DNA microarray can bear between 10,000 - 20,000 spots (genes) on an area of 3.6 cm<sup>2</sup>. Each spot represents the product of a specific gene and is generated by depositing a few nano liters of PCR product representing that specific gene usually at concentration of 100-500 µg/ml. The diameter of each spot is also typically 50-150 µm.



# Patient Microarray Results



## INTERPRETATION

Variant of Unknown Significance - Gain

arr 4q27(122,544,755-123,261,107)x3

Microarray analysis shows an ~716 kb gain within chromosome band 4q27 that contains ANXA5, TMEM155, PP12613, EXOSC9, CCNA2, BBS7, TRPC3 genes, and exons 1 through 70 of KIAA1109 gene. There are three copies of this region instead of two copies (normal) per diploid genome.

Duplication of the region as identified in this patient has not been previously reported in the literature.

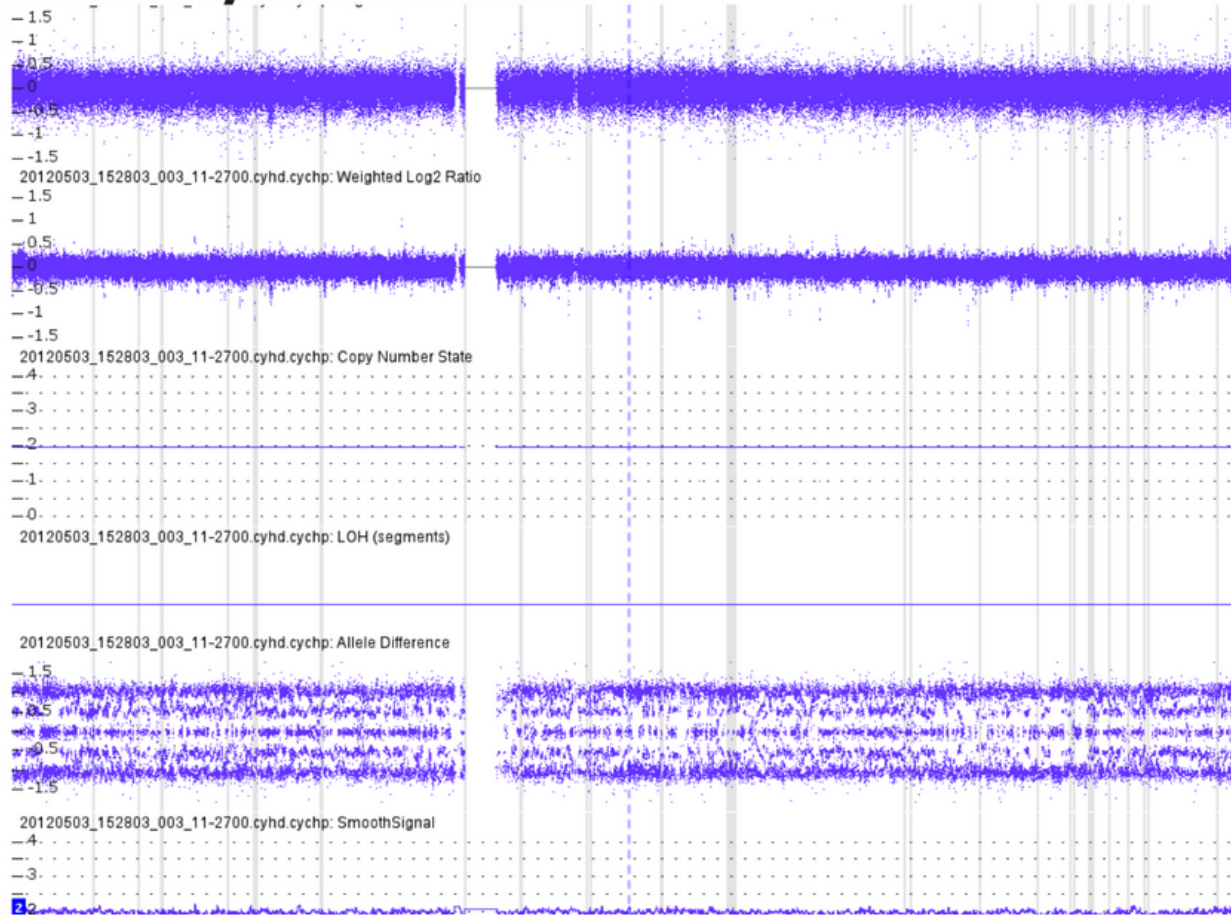
Of note, two of the genes within the duplicated region are known to be disease-associated. TRPC3 is considered a candidate gene for adult-onset cerebellar ataxia, and a heterozygous missense sequence variant was reported in an individual with this disease (1). However, the clinical significance of a duplication of TRPC3, as identified in this patient, is unknown. Homozygous or compound heterozygous mutations in the BBS7 gene are associated with Bardet-Biedl syndrome (OMIM: 615984). The clinical significance of a duplication of BBS7, as identified in this patient, is unknown.

The clinical significance of duplication of the remainder of the genes in this region is also unknown.

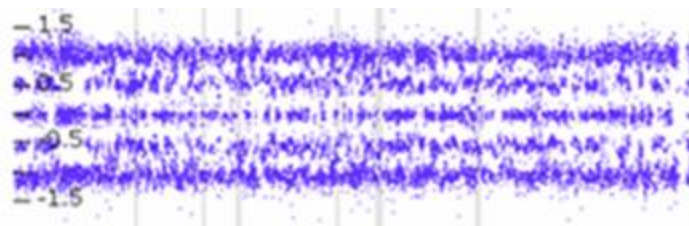
A single benign copy number gain has been reported for the duplicated region in the Database of Genomic Variants.

Parental testing may be useful to help determine the significance of this variant. Quantitative PCR analysis is available, as needed.

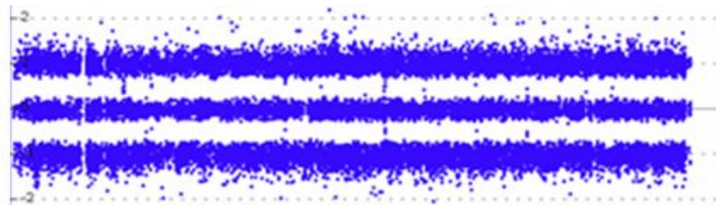
# Microarray of a Chimera



Chimera Microarray:



Patient Microarray:



What if the mixed field  
was still present at 3  
months?



Short Tandem Repeat Analysis  
would be performed.

# Short Tandem Repeats (STR)

- ▶ Short Tandem Repeats are unique to an individual
- ▶ Short, repetitive sequence elements 3-7 base pairs in length (1-4). These repeats are well distributed throughout the human genome.
- ▶ Think of it as a genetic fingerprint
- ▶ A person inherits one copy of an STR from each parent
- ▶ Homozygous: One peak at a given loci
- ▶ Heterozygous: Two peaks at a given loci
- ▶ Chimera: More than two peaks at a given loci
- ▶ Can be used to monitor BMT engraftment, twin zygosity, assessing maternal cell contamination in prenatal DNA specimens, and confirming the identity of histological specimens.<sup>10</sup>

# STR Process:

- ▶ Sample collection (EDTA blood or bone marrow)
- ▶ DNA extraction
- ▶ PCR amplification of DNA
- ▶ Color coded separation of fluorescein labeled STR alleles by capillary electrophoresis
- ▶ Data and profile interpretation using data collection software

# CMH STR Analysis

- ▶ Commercial kit: Promega Power Plex 16HS assay
- ▶ Co-amplification and three color detection of 16 loci, including
- ▶ Contains Amelogenin X/Y for gender determination
- ▶ Easily detects 1-5% residual cells

# STR

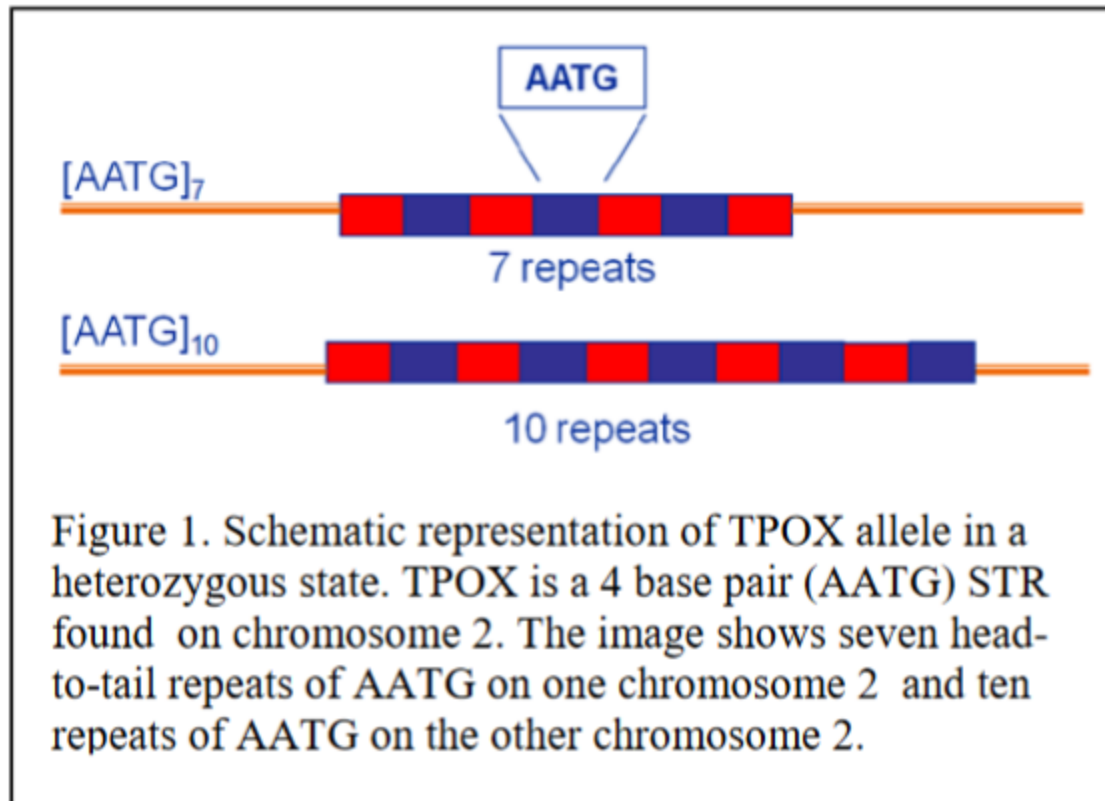
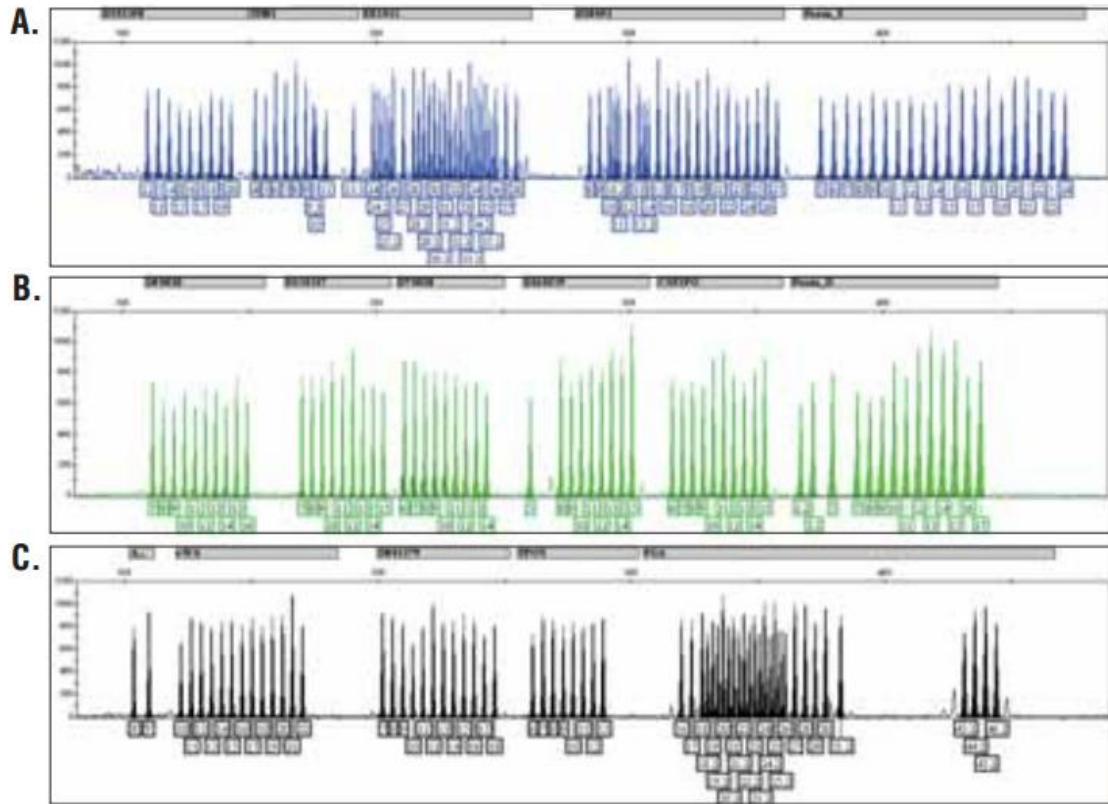
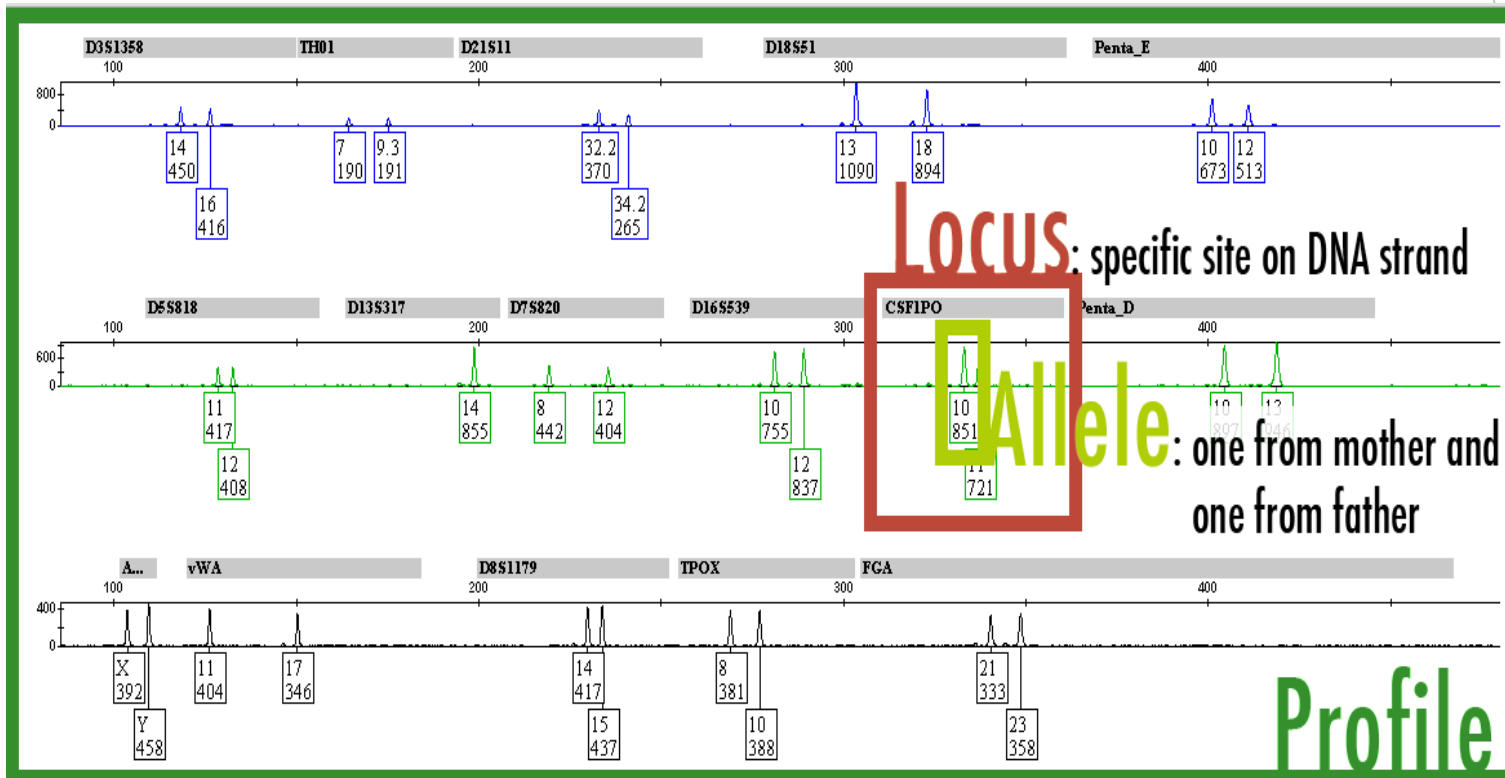


Figure 1. Schematic representation of TPOX allele in a heterozygous state. TPOX is a 4 base pair (AATG) STR found on chromosome 2. The image shows seven head-to-tail repeats of AATG on one chromosome 2 and ten repeats of AATG on the other chromosome 2.

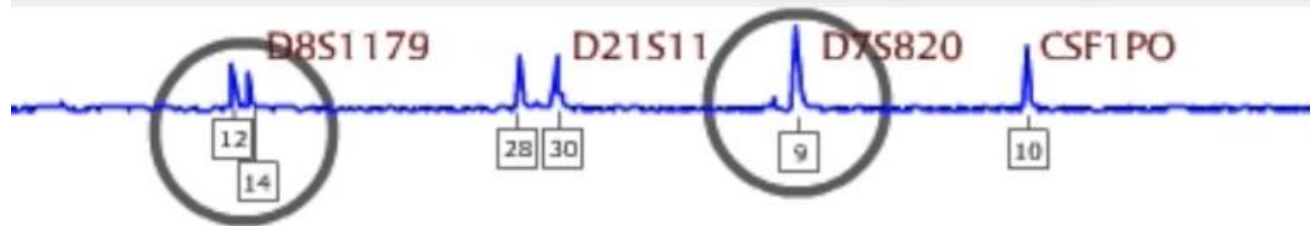
# STR Electropherogram Example:



# A Closer Look:



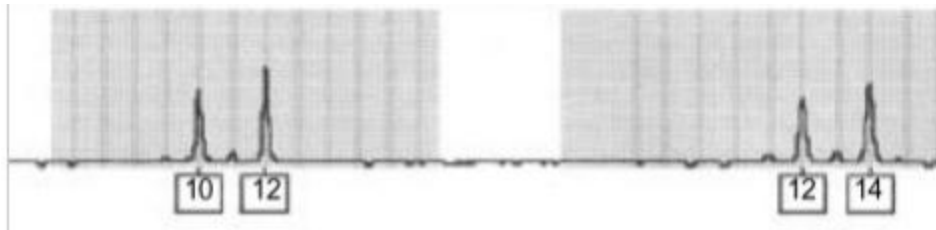
# Heterozygous vs. Homozygous



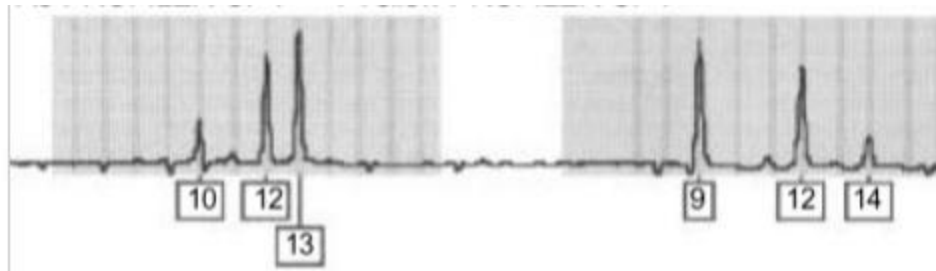


# Normal vs. Chimera

Normal



Chimera



# The Future of Chimeras

Due to the increase of artificial chimerism, via in-vitro fertilization, assisted fertility, hematopoietic stem cell and solid donor transplants, the occurrence of chimeras in transfusion services will continue to increase. These chimera patients provide a unique set of challenges for both the clinical blood bank and blood centers.

Communication with the physician for accurate patient history is extremely important when investigating mixed field reactions.

# The future of Genetics: Next Generation Sequencing

High-throughput sequencing technologies that screen large amounts of genetic material at lower cost than traditional sequencing technologies, such as automated Sanger sequencing.<sup>12</sup>

# Three Types of NGS

	Availability	Cost	Advantages	Disadvantages
<b>Targeted gene panels</b>	Commercially available	~\$5000	-Highest resolution of NGS approaches -Contains a number of well-characterized ASD susceptibility genes (syndromic and non-syndromic)	-Unable to detect variants in genes not included in the panel
<b>Whole exome sequencing</b>	Available only in research settings	~\$1000	-Estimated to detect the majority of disease-covering variants	-Unable to detect variants in non-coding regions
<b>Whole genome sequencing</b>	Available only in research settings	~\$4000-\$5000	-Greatest coverage of the genome (both coding and non-coding regions)	-Lowest resolution of NGS techniques -Higher cost than whole exome sequencing



Genetic testing is the key to identifying Chimeras!



# Interesting Chimera Examples

The background of the slide features an abstract design of overlapping, semi-transparent geometric shapes in various shades of orange and brown. These shapes are primarily located on the right side of the slide, creating a layered, modern aesthetic. The text is centered on the left side of the slide.

# Case Study #1:

- ▶ 6 yr. old female having surgery
- ▶ Pre-operative Type and Screen shows Mixed Field
- ▶ Anti-A: 4+
- ▶ Anti-B: Mixed Field
- ▶ A cells: 0
- ▶ B cells:0
- ▶ Phenotype reveals mixed field reactions with Anti-E

# Patient History

- ▶ Patient is a dizygotic triplet (one girl and two boys) born at 32 weeks after IVF.
- ▶ Three quadricellular embryos were implanted.



# Results of genetic testing:

- ▶ Both males blood type: A
- ▶ The propositus female blood type: AB
- ▶ All three children revealed dual population of A and AB RBCs with the same proportion (90% A and 10% AB).
- ▶ The female patient was shown to have more male DNA than female DNA(90% vs. 10%)! She was normal and showed no signs of hermaphroditism.
- ▶ Three zygotes were implanted. One survived (female), one split to form identical twins (males), and one did not survive.

Female is a triplet chimera  
following In-Vitro fertilization.<sup>13</sup>

# Case Study #2

- ▶ 70-year old woman admitted to the hospital for surgery
- ▶ Presurgical Type and Antibody Screen ordered.
- ▶ Anti-A: 2+ Mixed Field
- ▶ Anti-B: 0
- ▶ A cells: 0
- ▶ B cells: 3+
- ▶ RhD: 1+ Mixed Field
- ▶ RhE: 1+ Mixed Field

# Patient History

- ▶ No history of transfusion or transplantation
- ▶ 7 previous pregnancies

Patient was interviewed for  
more information and...

She had a twin brother  
that died in infancy!

# Results of Genetic Testing:

- ▶ Genotyping was performed and the ABO type was an A2/O chimera.
- ▶ STR analysis was performed and more than two alleles were present in 6 of 21 loci, confirming chimerism.
- ▶ Genomic DNA analysis for the presence of SRY (Sex determining Region Y) was performed and found positive for male DNA.

Patient is a Twin Chimera.<sup>14</sup>



# Case Study #3

- ▶ 39-year old normal Korean male
- ▶ Demonstrated mixed field hemagglutination on B cell typing at the time of blood donation
- ▶ Genotyping was performed to identify the ABO subtype B3. This subtype is relatively common in Korea

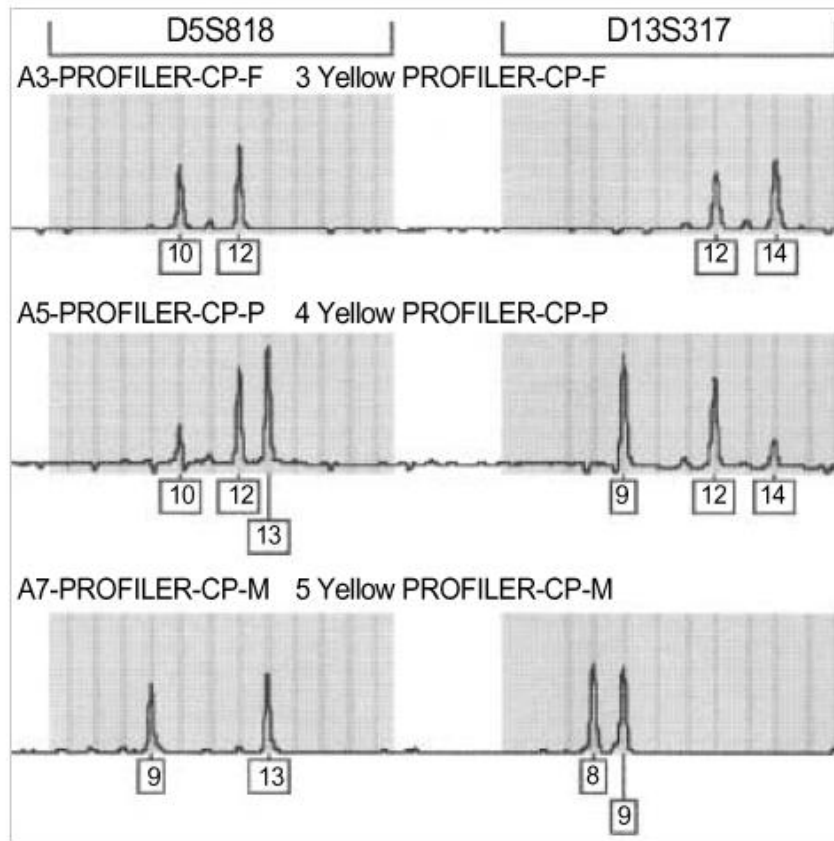
# Patient History

- ▶ Morphological normal male and father of one child
- ▶ No history of transfusion or transplants
- ▶ No history of a twin

# Results of Genetic Testing:

- ▶ Direct sequencing of exons 6 and 7 of the ABO gene showed two distinct RBC populations, group B and group O and not the B3 subgroup
- ▶ STR analysis demonstrated more than two alleles in 4 of 9 loci
- ▶ STR analysis of the parents and propositus demonstrated a pattern consistent with a double paternal DNA contribution
- ▶ His karyotype revealed a mosaic pattern: 32/50 metaphases were 46XY and 18/50 metaphases demonstrated 47XYY

# STR Electropherogram Results



Father

Propositus

Mother

These results indicate the propositus is most likely a dispermic chimera resulting from the division of one ovum and its fertilization by two spermatozoa.<sup>15</sup>

I LEAVE YOU WITH....

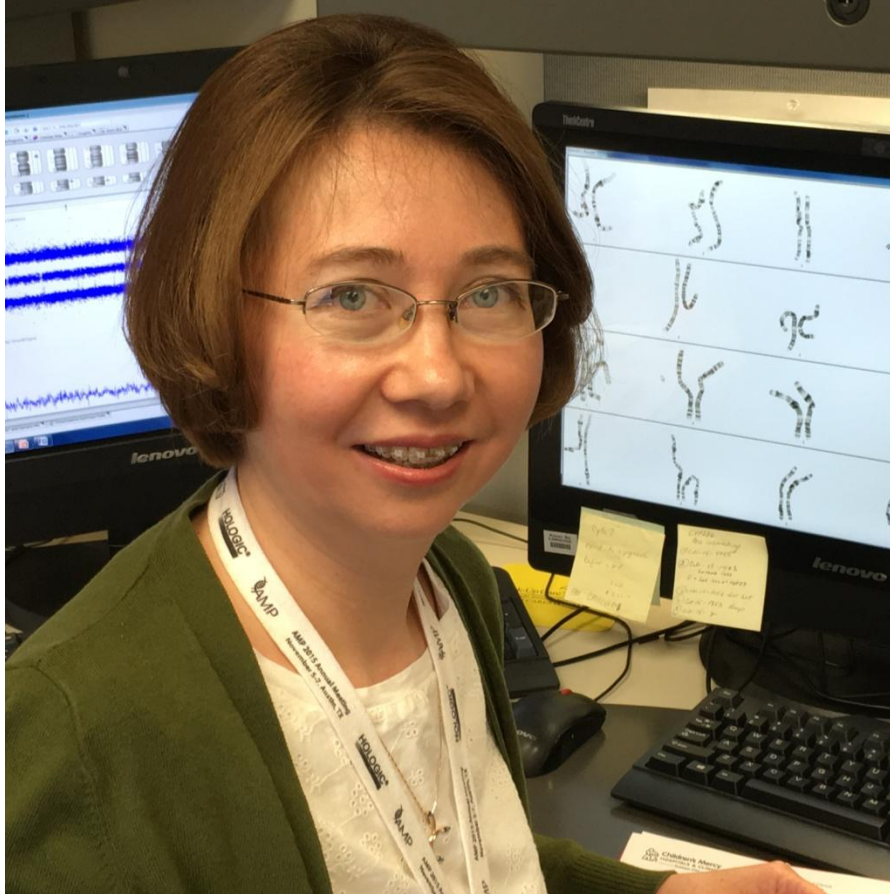
- ▶ There may be a link with microchimerism and some autoimmune diseases. (Body sees the foreign DNA and attacks itself).<sup>18</sup>
- ▶ Women with sons have been found to contain some Y chromosomes in peripheral blood mononuclear cells for decades.<sup>19</sup>
- ▶ Stem cells passing from fetus to mother have been shown to have a healing affect. For example, fetal stem cells have been found in a pregnant mother's diseased heart.<sup>20</sup>
- ▶ Researchers studied the brains of 59 women ages 32 to 101 and found male DNA in 63% of the women's brains.<sup>21</sup>

## DEFINITIONS:

- ▶ ZYGOTE: The cell formed by the union of a male sperm and a female ovum.
- ▶ MONOZYGOTE: Developed from a single fertilized ovum (identical twins)
- ▶ DIZYGOTE: Developed from two fertilized ovum (fraternal twins)
- ▶ TETRAGAMETIC CHIMERA: Two fertilized eggs fuse together
- ▶ DISPERMIC CHIMERA: One ovum splits and fertilized by two sperm
- ▶ early in development and one baby forms.
- ▶ MOSAIC: Two or more populations of cells with different genotypes in one individual who has developed from a single (zygote).
- ▶ ANASTAMOSIS: Interconnecting blood vessels
- ▶ Hermaphrodite: Containing both male and female DNA
- ▶ Microchimerism: The presence of a small number of cells that originate from another individual (zygote)



# A Special Thanks to Dr. Elena Repnikova!



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## References:

- ▶ 1. Race R.R., and Sanger R.: Blood groups in man. Oxford (England): Blackwell Scientific Publications, 1975.
- ▶ 2. Van Dijk B.A., Boomsma D.I., and De Man A.J.M.: Blood group chimerism in human multiple births is not rare. *Am J Med Genet* 1996; 61: pp. 264-268
- ▶ 3. Owen RD. 1945. Immunogenetic consequences of vascular anastomoses between bovine twins. *Science* 102: 400- 407. Palmer JF, ed. 1835.
- ▶ 4. Dunsford I., Bowley C.C., Hutchinson A.M., et al: A human blood-group chimera. *Br Med J* 1953; 2: pp.80-81.
- ▶ 5. Bluth M. Reid M., Manny N. Chimerism in the Immunohematology Laboratory in the Molecular Biology Era: *Transfusion Medicine Reviews*, 2007-04-01, Volume 21, pp. 134-46.
- ▶ 6. Tipett P.: Blood group chimeras, A review. *Vox Sang* 1983; 44: pp.333-359.
- ▶ Cross Ref (<http://dx.doi.org/10.1111/j.1423-0410.1983tb06357.x>)
- ▶ 7. Starzl TE, Demetris AJ, Murase N, et al. Chimerism after organ transplantation. *Current opinion in nephrology and hypertension*. 1997;6(3):292-298.
- ▶ 8. Centers for Disease Control and Prevention, American Society for Reproductive Medicine, 2009 *Assisted Reproductive Technology Success Rates: National Summary and Fertility Clinic Reports*. Atlanta: U.S. Department of Health and Human Services; 2011. <[http://www.cdc.gov/art/ART2009/PDF/ART\\_2009\\_Full.pdf](http://www.cdc.gov/art/ART2009/PDF/ART_2009_Full.pdf)>
- ▶ 9. <http://grf.lshtm.ac.uk/microarrayoverview.htm>
- ▶ 10. Butler J.: Short tandem repeat typing technologies used in human identity testing. *BioTechniques*; 43: Sii-Sv.
- ▶ 11. [http://www.childrensmercy.org/library/uploadedFiles/childrensmercyorg/Clinics\\_and\\_Services/Clinics\\_and\\_Departments/Pathology\\_and\\_Laboratory\\_Medicine/News0314-Dr%20Selvarangan-final\(1\).pdf?n=8917](http://www.childrensmercy.org/library/uploadedFiles/childrensmercyorg/Clinics_and_Services/Clinics_and_Departments/Pathology_and_Laboratory_Medicine/News0314-Dr%20Selvarangan-final(1).pdf?n=8917)
- ▶ 12. 15. [http://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina\\_sequencing\\_introduction.pdf](http://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf)
- ▶ 13. Kuhl-Burmeister R., Simeoni E., Weber-Matthiesen K., et al: Equal distribution of congenital blood cell chimerism in dizygotic triplets after in-vitro fertilization. *Hum Reprod* 2000; 15 pp. 1200-1204
- ▶ 14. Sharpe C., Lane, D., Cote J., et al: Mixed field reactions in ABO and Rh typing chimerism likely resulting from twin haematopoiesis. *Blood Transfus* 2014; 12: pp. 608-610.

## References:

- ▶ 15. Duck C., Sang K., Yazer, M., et al: A dispermic chimera with mixed field blood group B and Mosaic 46, XY/47, XYY Karyotype: J Korean Med Sci 2007; 22: 553-6
- ▶ 16. Lo Y. M.D., Corbetta N., Chamberlain P.F., et al: Presence of fetal DNA in maternal plasma and serum. Lancet 1997; 350:pp. 485-487 Cross ref ([http://dx.doi.org/10.1016/S0140-6736\(97\)02174-0](http://dx.doi.org/10.1016/S0140-6736(97)02174-0))
- ▶ 17. <http://genetics.thetech.org/ask/ask208>
- ▶ 18. Adams K. M., and Nelson J.L.: Microchimerism: An investigative frontier in autoimmunity and transplantation. JAMA 2004; 291: pp. 1127-1131
- ▶ 19. Evans P.C., Lambert N., Maloney S., et al: Long-term fetal microchimerism in peripheral blood mononuclear cell subsets in healthy women and women with scleroderma. Blood 1999; 93:pp. 2033-2037.
- ▶ 20. Rina J. Kara et al. "Fetal Cells Traffic to Injured Maternal Myocardium and Undergo Cardiac Differentiation." *Circulation Research*, published online November 14, 2011. DOI: [10.1161/](https://doi.org/10.1161/)
- ▶ 21. <http://www.independent.co.uk/news/science/male-dna-found-in-womens-brains-8181068.html>
- ▶ 22. Bianchi D.W., Zickwolf G.K., Weil G.J., et al: Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. Proc Natl Acad Sci USA 1996;93 pp. 705-708

Questions?