

Full blood chimerism and de novo crossover at HLA in a phenotypically normal 46,XY woman

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Introduction

- Finding a 46,XY karyotype in a woman is rare and often linked to disorders of testicular development.

Objective

- We investigated a phenotypically normal, fertile woman with 46,XY restricted to peripheral blood and 46,XX in other tissues, demonstrating complete blood chimerism and a de novo HLA crossover, a combination not previously documented in a single individual.

Methods

- Cytogenetics: GTW-banding (≥ 20 metaphases; ISCN 2020); interphase FISH for X/Y.
- Molecular: PCR-STR (GlobalFiler) with capillary electrophoresis; High-resolution HLA typing by NGS (AlloSeq Tx17; 4-field phasing when available); HLA-antibody screen (Luminex).
- Comparators: Twin brother (blood, buccal); parents for HLA segregation.

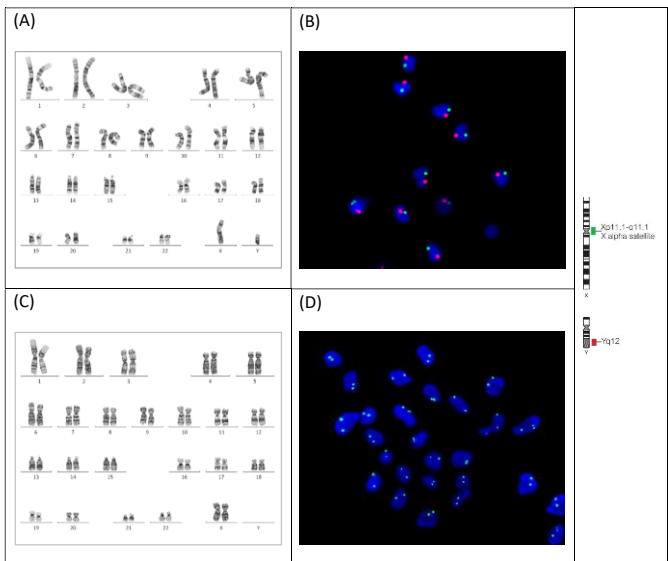


Figure 1. Cytogenetic study of the proband. The karyotype (A) and FISH (B) from proband's peripheral blood show XY chromosomes, while the karyotype (C) and FISH (D) from proband's skin culture show XX chromosome. FISH shows the centromere of chromosome X labeled in green fluorochrome and heterochromatic block of chromosome Y labeled in red fluorochrome.

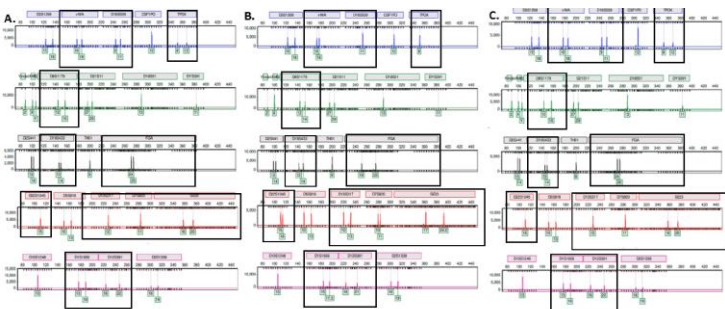


Figure 2. Electropherogram of STR run. The graphical representation of the DNA fragments generated by electrophoresis for the analysis of several STR (short tandem repeat) loci, which are specific regions of DNA characterized by short repeating sequences of nucleotides, reveals that the profile of the proband's peripheral blood (A) is identical to her twin brother (C), but different from the profile obtained from the proband's oral swab (B). Markers with divergence were highlighted.

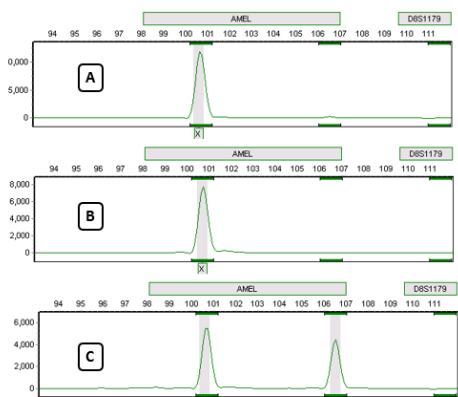


Figure 3. Electropherogram of Amelogenin locus. Graphical representation of the DNA fragments generated by electrophoresis for the Amelogenin gene locus, which is located on the X and Y chromosomes, shows a female (X-only) profile in proband's oral swab (A) and fibroblast culture samples (B); a male profile (XY) is observed in the proband's peripheral blood sample (C).

Results and Discussion:

- Tissue specificity: Blood = 46,XY; other tissues = 46,XX $\rightarrow$ tissue-restricted discordance consistent with blood chimerism.
- STR: Proband's blood STR profile identical to twin brother, divergent from her other tissues (12 informative loci).
- HLA: Proband's blood shares brother's haplotype and harbors a maternal recombinant haplotype consistent with a de novo crossover within MHC (approx. between HLA-E/H and HLA-C/B, ~ 0.75 Mb).
- Immunology: Despite HLA mismatches between immune cells and tissues, no anti-HLA antibodies detected; no clinical signs of autoimmunity or GVHD.
- Fertility: Normal gonadal function and successful term pregnancy.
- This is, to our knowledge, the first report uniting complete blood chimerism with a documented de novo HLA crossover in the same individual.
- The coexistence of these phenomena likely reflects in-utero vascular anastomoses between dizygotic, sex-discordant twins, with recombination in the maternal MHC possibly contributing to lifelong immunotolerance.
- Crucially, human reproduction was not impaired, contrasting with freemartinism in other species.
- Multi-tissue testing is essential to avoid misinterpretation when blood karyotype suggests sex-chromosome discordance.

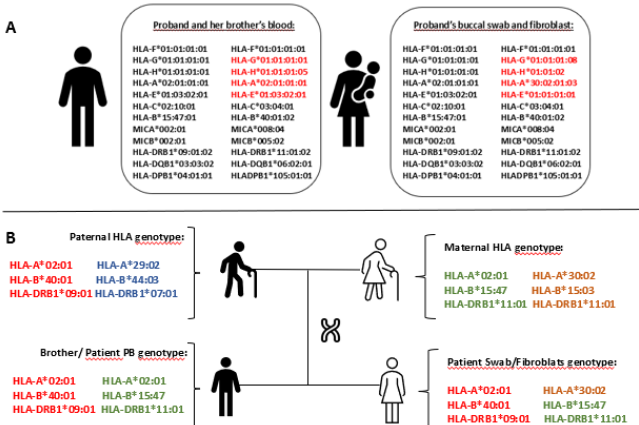
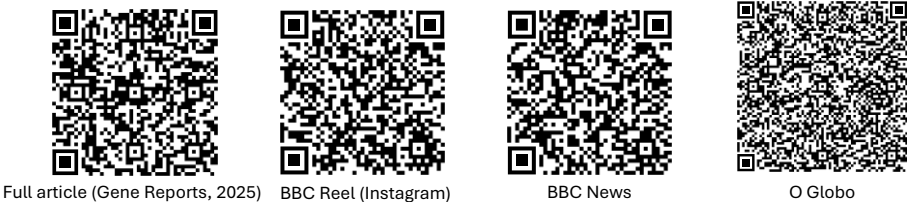


Figure 4. (A): Family HLA analysis of patient and her twin brother. The study of the human leukocyte antigen (HLA) system, whose genes are highly polymorphic, showed that the proband and her brother have HLA incompatibility in HLA-A locus and non-classical genes HLA-E, -G and -H. **(B): Segregation of parental haplotypes.** HLA typing of the patient's parents was performed. Familial segregation suggests that a genetic crossover occurred between the maternal haplotypes (each haplotype is color-coded); however, it is not possible to determine in which of the two offspring this event took place. Based on haplotype frequencies described in the Brazilian population, we infer that the crossover most likely occurred in the proband's haplotype.

Conclusion

- Complete blood chimerism with MHC recombination can be clinically silent and compatible with fertility.
- STR + high-resolution HLA + multi-tissue cytogenetics provided definitive origin tracing (blood derived from the male twin).
- These findings expand the understanding of tolerance mechanisms in natural human chimeras and inform genetic counseling in sex karyotype–phenotype discrepancies.

Global Impact & Media Coverage



References

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Ethics: Plataforma Brasil CAAE 63938922.0.0000.5474; Parecer 5.794.170.