IDENTIFICATION OF A PHENOTYPICALLY NORMAL TETRAGAMETIC CHIMERIC FERTILE WOMAN BY HLA AND STR TYPING

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Abstract

We studied a very unusual tetragametic phenotypically normal and fertile 46,XX Caucasian female, who was identified because her blood HLA typing showed what appeared at first to be maternal exclusion of two of her three children. The propositus, her mother, two brothers and three children were subsequently studied by advanced HLA molecular typing and DNA of 18 different STRs (Short Tandem Repeats). The propositus blood showed the presence of only two HLA haplotypes. Yet, other tissues included hair follicles, Bucal epithelium, skin fibroblasts, thyroid, and fibroblasts and epithelial cells from urinary bladder, showed evidence of four HLA haplotypes. Such chimerism was confirmed with a large number of autosomal STRs, while an extensive cytogenetics analysis showed a 46,XX chromosome constitution and lack of Y-specific DNA signal by amelogenin typing all indicating that the blood cells resulted from hematopoietic stem cells of the same genetic constitution. In some of the tissues, two haplotypes were found as preponderant, while the reverse was found in the other tissues. Furthermore, because two of the propositus's children received one HLA haplotype from the father and the second haplotype was not found in the blood of the propositus, this work suggests that it is possible to find cases that mimic parental exclusion unless one studies more than one tissue of the person under scrutiny. Our results are best explained by a chimerism in several tissues of the body, other than blood, which resulted from two maternal gametes, fertilized by two paternal spermatozoids forming a two cell zygote, with distinct genetic constitution, that differentially populated the body.

Introduction

Chimerism is the presence of two unrelated (genetically distinct) cell lines in an individual organism. Chimerism may be either acquired or congenital, and may involve either one or multiple organ systems. Thus, the infusion of hematopoietic cells during a hematopoietic or solid organ transplant may result in chimerism, and the transfusion of allogeneic blood may also be complicated by micro-chimerism, or by graft-versus-host disease. There are two known congenital causes of chimerism, one is twin-associated, in which hematopoietic cells are shared in uterus; and the other one is tri or tetragametic chimerism. Trigametic chimerism results from the fertilization of an ovum and polar body while tetragametic chimerism is the result of either fertilization and cleavage of oocytes from a binovular human ovarian follicle, or a fertilization of separate ova by different spermatozoids and the fusion of the two zygotes implanted very close one of the other. Tetraparental mice and rams have been used experimentally in which fusion of two fertilized eggs result in animals with cells that have a normal number of chromosomes, as well as chimerism. Individuals with spontaneous XX/XY chimeras have been found in humans that range from normal fertile males to males with sex abnormalities including hemaphroditism, and from fertile female hermaphrodites to phenotypically normal fertile females. Such type of chimerism was usually ascertained through cytogenetic and blood group analysis in previously reported cases, as well as with molecular HLA typing and RFLP analysis in more recently reported cases. In addition, there

is a report of one case of a 46XX/46XY hermaphrodite zygote conceived by in vitro fertilization, and ascertained by DNA polymorphisms. The spontaneous occurrence of a tetragametic individual with a 46,XX karyotype has not been previously described because they should generally be phenotypically normal. Here we report such a case in a phenotypically normal 46,XX female identified at first by HLA typing discrepancy during a kidney transplantation consideration who showed absence of a Y-specific DNA sequence in different tissues studied, has no blood chimerism.

HLA PCR-SSOP molecular typing

A 52 year old Caucasian woman, here called the propositus, was found by HLA typing to have an unusual chimerism in which her blood cells have two HLA haplotypes (A66, B41, DR4 and A2, B40, DR4 herewith designated as 1 and 3). Other tissues showed four haplotypes (1 and 3, plus A2, B8, DR8, and A11, B27, DR1 herewith designated 2 and 4). While the propositus showed haplotypes 1 and 3 in blood, and 1 and 3 and 2 and 4 in several other tissues, blood and hair follicles from her mother, two brothers and three sons showed a normal set of two haplotypes. Among the six tissues examined, that displayed the four haplotypes, hair and Bucal epithelium, as well as skin fibroblasts, all showed a predominant expression of the 1 and 3 haplotypes. On the other hand, thyroid, bladder epithelium and bladder fibroblasts showed a predominant expression of the 2 and 4 haplotypes.

Cytogenetics and molecular X and Y chromosome analysis.

One hundred G-banded pro-metaphases from blood and 100 pro-metaphases from skin fibroblasts showed that the propositus had a normal 46 XX chromosome complement (data not shown). No evidence of triploid or tetraploid mitoses, or of chromosome spreads with extra chromosomes, was found. The fibroblasts tested originated from the same material that showed approximately 80% signal for haplotypes 1 and 3 and 20% signal for haplotypes 2 and 4. Molecular detection of Y specific DNA sequences was also performed on blood, skin fibroblasts, thyroid and bladder tissue from the propositus. All the PCR reactions obtained from the different samples for AMEL Y/AMEL X, which simultaneously amplify X and Y specific fragments of different sizes failed to yield a Y specific DNA product.

Analysis of STRs

In addition to HLA, which has been mapped to the short arm of chromosome 6, to determine the presence of chimerism in the propositus, we analyzed 18 STRs from 16 different autosomes. The results of the analysis on the propositus and various family members are summarized in Table 1, It included DNA from blood, hair follicle, thyroid, Bucal mucosa, and skin fibroblasts from the propositus and blood from the propositus's mother, the two brothers and three sons. Thirteen STRs demonstrated the presence of chimerism in different tissues of the propositus (D16S539, D7S820, D13S317, FGA, D1S533, D9S304, Penta E, D18S51, D21S11, D3S1358, D8S1179, D12S1090, and D3S1744). Lane trace analysis for D16S539 shows alleles 13 and 9 in the propositus blood and absence of allele 11, while hair follicles, in addition to alleles 13 and 9, a minor component of allele 11 was detected. In the thyroid tissue the predominant alleles for D16S539 were 11 and 9 with a minor component for allele 13. In the case of STR D7S820, the thyroid tissue showed alleles 10 and 8, while in blood, only allele 10 was present. Hair follicles sowed allele 10 and a minor component for allele 8. For D13S317, alleles 11 and 8 were present in the blood sample. In addition to these two alleles, a minor component for allele 13 was found in hair follicles. On the contrary, in thyroid tissue alleles 13 and 11 were the predominant alleles with a minor component for allele 8. (Table 1).

A segregation analysis based on the results obtained from the propositus's mother, her two brothers and the different tissues typed allowed us to reconstruct the genetic make up of the propositus's father in order to derive the genetic composition of the two zygotes that gave origin to this chimerism. Six of the STRs typed were fully informative (D7S820, D9S304, Penta E, D18S51, D12S1090 and D3S1744, Table 2). For example, for D9S304, the propositus's mother is a 4/4 homozygous. Her two brothers were heterozygotes 9 and 4, being allele 4 and obligated maternal allele. The propositus blood showed alleles 12 and 4, therefore the propositus's father was 12 and 9. Thus, the two zygotes were 12/4 and 9/4. The fusion of these two zygotes explains the chimerism found in different tissues such as hair and Bucal swab (12 / 9 / 4) for that locus.

It is to note that, if only STRs of blood cells from the propositus had been used, maternal exclusion for two of her three sons would have been demonstrated. These results corroborated the maternal exclusions found by HLA typing (see shadowed cells in Table 1).

Discussion

The case reported here represents the first report of body chimerism in a phenotypically normal and fertile XX female, resulting from a fusion of two fertilized eggs. It resulted in what at first appeared to be a maternal exclusion of two of her three children.

Several mechanisms have been postulated to give rise to genetic chimerism. Among them are: 1) Fertilization of a secondary oocyte and first polar body. 2) Fertilization of an ovum and a first polar body. 3) Fertilization of the ovum and second polar body. 4) Fusion of a normal zygote and a parthenogenic cell. 5) Fusion of two zygotes. 6) Double fertilization of a binovular nucleus.

During the first meiotic division a secondary oocyte and a first polar body are normally formed, each containing a bivalent chromosome complement in which each chromosome has double chromatids. Fertilization by a sperm of either one, or both, of these two theoretical possibilities, would generate a triploid cell in each case, and would be either lethal or result in phenotypic abnormalities not found in the present study, therefore, that possibility was ruled out (mechanism #1). The fertilization of and ovum and any of the polar bodies are in the present case ruled out by the presence of 4 HLA haplotypes and by the segregation analysis carried out for several STRs since only a trigametic individual would have been originated if any of those fertilization mechanisms had taken place (mechanisms 2 and 3). In addition, in the case of a second meiotic division, it is theoretically possible that instead of the formation of an ovum and a second polar body, there would be an immediate cleavage, and the first meiotic division leading to the formation of two cells with haploid products. This possibility was excluded in our case once again because the two maternal pronuclei would contain a similar genetic material and their fertilization would give rise to a unimaternal contribution (trigametic chimerism), instead of the bimaternal HLA and STR alleles contribution found, as mentioned before.

The mechanisms of fusion of a zygote and a parthenogenic cell (#4) has been invoked in a 1 year old XX/XY patient with facial asymmetry and sex reversal. Such chimeric mechanism is ruled out in our case because neither of the two types of cells found in the propositus showed a maternal uniparental disomy, which would be an obligatory outcome in parthenogenic cells. In the propositus, the two maternal HLA haplotypes segregated into the two types of cells found, each in combination with a paternally derived haplotype, the same mechanism was found with some informative STRs as described before.

In the present case, only the fusion of two zygotes or the double fertilization of a binovular ovum are possible. However, our results do not provide evidence to indicate which of those two mechanisms were responsible for the chimerism detected. In favor of the first mechanism are the reports of humans with spontaneous XX/XY chimeras, and at least one case of an XY/XX true hermaphrodite that resulted from such a mechanism after in vitro fertilization. In addition, tetraparental mice and rams have been produced experimentally by fusing two fertilized zygotes. On the other hand, the second mechanisms remains as a possibility sin approximately 85% of a large number of serially sectioned human ovaries have shown the presence of binovular follicles. Due to the finding of human binovular cells of which one of the pronuclei was fertilized, it is theoretically possible to result from a double sperm fertilization of a binovular egg. If such a chimerism could prove to be viable in humans, it may prove indistinguishable from one produced by two fertilized zygotes that subsequently fused into one embryo.

The existence of four different HLA haplotypes, two from her father and two from her mother as well as two different STR sets of allele constitution, which were found in different proportions in different tissues of the propositus, strongly suggest a bi-parental (double fertilization) tetragametic origin of the chimera. The tetragametic chimera resulted from the fertilization of two maternal pronuclei by two sperms, whose descendant cells differentially populated the body. In the case of the blood, however, only one type of Stem Cell populated the blood cells in this adult chimeric individual. On the other hand several tissues of

the propositus, are populated in different proportions, with two different HLA cell types. Such a differential body population of two different types of cells, has been previously demonstrated in cases of cell mosaicism and chimerism. The HLA chimerism found in several tissues was confirmed with the use of 18 STRs from 16 different autosomes, confirming that there are two different kinds of dizygotic cells, in the propositus tissues, such as hair follicles, thyroid, skin fibroblasts and Bucal swabs.

Materials and Methods

The patient was 52 years old at the time of presentation to our institution. She had chronic renal failure due to focal-sclerosing-glomerulonephritis, and was studied to receive a related renal transplant. She was otherwise in good health. She had three pregnancies and three children. Her physical exam was unremarkable. Her blood group was A, Rh-positive. Samples of blood from the patient and her immediate family were collected for HLA typing. We also collected fresh and stored tissue samples from the patient, with her informed consent. Samples included hair; Bucal mucosa; skin biopsies, from which fibroblasts were cultured; archived thyroid tissue from a benign nodule removed twenty years earlier; and bladder tissue, from which epithelial cells and fibroblasts were separated and cultured.

HLA Typing

DNA was extracted by QIAAMP Blood kit (QIAGEN). HLA typing was performed by sequenced-specific primer amplification (24) and sequence-specific oligonucleotide probe hybridization (Lifecodes Corporation, Stanford, CT). For blood and tissue samples, typing was done using 30 PCR cycle. In order to increase the possibility to detect two additional haplotypes in the blood of the propositus we used sequential PCR amplification. The initial PCR was done using 30 PCR cycles and 10 ul amplicon were then used as template for another 30 cycles PCR amplification. DNA extracted from 1 ul whole blood was used as control for sequential PCR amplification.

Molecular X and Y chromosome analyses.

The Amelogenin sex identification marker was amplified by PCR following manufacturer recommendations (GenePrint® STR systems, Promega Corporation, Madison, Wis). The amplification products were resolved in 4% acrylamide-Bis-Acrylamide denaturing gels and stained with silver nitrate. A normal XX chormosme constitution will show a single 212 bp fragment while a XY constitution will show a 212 and 218 bp fragments.

STR studies

All STR analyses were carried out using commercially available kits used routinely in parentage testing and forensic analysis. The TPOX, D3S1358, FGA, D8S1179, THO1, vWA, Penta E, D18S51 and D21S11 were simultaneously amplified (Powerplex® 2.1GenePrint® STR systems, Promega Corp, Madison, Wisconsin), electropheresed in a 5% Long-Ranger, 1X TBE buffer and analyzed in a Hitachi FMBIO II Fluorescent scanner as recommended by the manufacturer. The D16S539, D7S820, D13S317 and D5S818 (GammaStar, GenePrint® STR systems, Promega Corp, Madison, Wisconsin) were simultaneosly amplified and resolved in 4% acrylamide-Bis-Acrylamide denaturing gels and analyzed in a Hitachi FMBIO™ II Fluorescent scanner as recommended by the manufacturer. The FGA, D7S820, D1S533, and D9S304 (Multiplex II) and D12S1090, D3S1744, and D18S849 (Multiplex I) (Lifecodes Corporation, Sanford, CT) were amplified and resolved in 4% Acrylamide-Bis-Acrylamide denaturing gels, and stained by silver nitrate following manufacturer recommendations. Allele designations were made according the recommendations of the DNA Commission of the ISFG with the aid of the allele ladders provided by the manufacturer.

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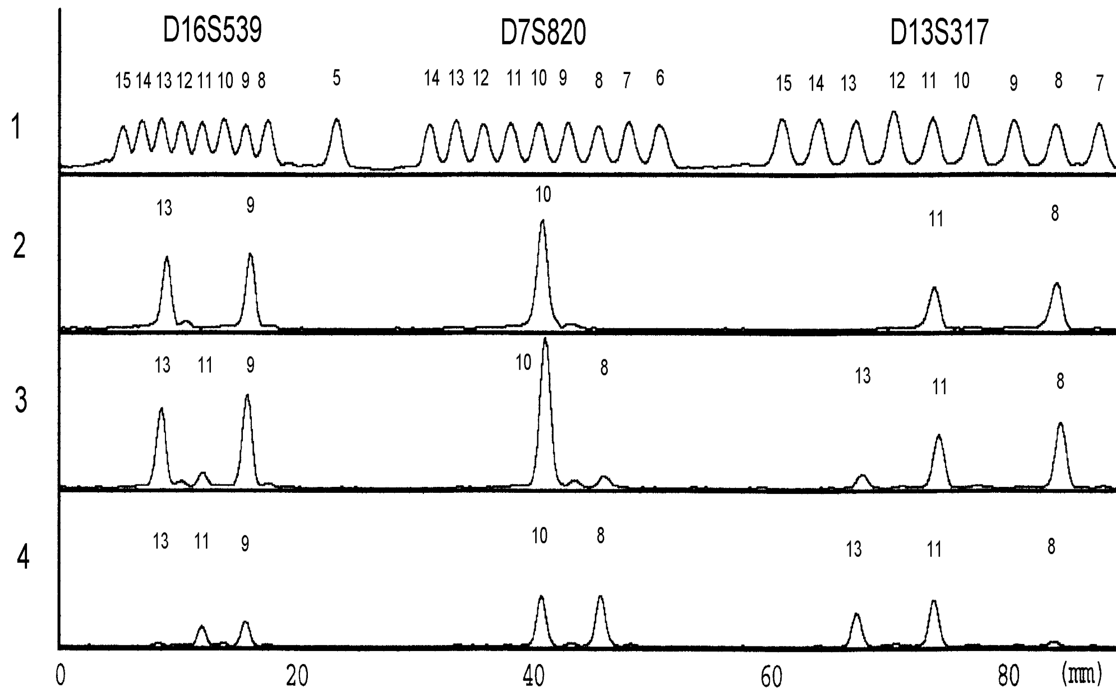

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recommendations of the International Society for Forensic Genetics (ISFG) and by the ladder provided by the manufacturer (Promega Corporation, Madison, WI).

Table 1. Eighteen STRs from 16 different autosomes detecting chimerism in tissue, as well as maternal exclusion in two of the three sons. The most informative STRs for the detection of the propositus tetragametic chimerism are indicated in bold letters on the left. The STRs vWA, TPOX, THO1 and D5S8189 were not informative. Minor component alleles detected by STR typing are shown in brackets. Notice that STRs D16S539, D7S820, D3S1358, D12S1090 and D3S1744 would have excluded maternity for Son #1 and STRs D7S820, D1S533, Penta E, D12S1090 and D18S849 would have excluded maternity for Son #2 (Shown shadowed), if only blood was considered.

Table 2. Segregation analysis of 6 STRs demonstrating the tetragametic origin of chimerism in the propositous. The genotype for each loci for the propositus´s father was deduced from the information obtained from the propositus´s brothers and mother.