Cytogenetic and Genome Research

Novel Insights from Clinical Practice

Cytogenet Genome Res 2022;162:378–385
DOI: 10.1159/000528737

Received: November 22, 2022
Accepted: December 15, 2022
Published online: December 19, 2022

Intersexual Twins due to Tetragametic Chimerism

Rainer Wimmer[a] Uta Neumann[b] André Weber[a] Melanie Isau[a]
Heike Renner-Luetzkendorf[c] Petra Zschieschang[d] Oliver Wachter[e]
Kaimo Hirv[e] Markus Stumm[a]

[a]Medicover Genetics GmbH, Berlin, Germany; [b]Klinik für Kinderendokrinologie und -diabetologie, Charité-Universitätsmedizin Berlin, Berlin, Germany; [c]Gynäkologie und Geburtshilfe, Brustzentrum/Perinatalzentrum, Helios Klinikum Berlin-Buch GmbH, Berlin, Germany; [d]Medicover Humangenetik – Berlin-Lichtenberg, Berlin, Germany; [e]Zentrum für Humangenetik und Laboratoriumsdiagnostik, Martinsried, Germany

Established Facts

- Dizygous twins may show confined blood chimerism due to bidirectional exchange of hematopoietic stem cells through placental anastomoses.
- Heterogoneic division after triploid conception, parthenogenesis of the ovum, fertilization of the second polar body or embryo amalgamation are theoretical mechanisms for XX/XY chimeras.

Novel Insights

- Chimerism is a possible explanation for sexual ambiguity or discrepancies in sexual differentiation of monochorionic twins.
- Embryo amalgamation or fertilization of polar body 2 can explain the tetragametic pattern found in these twins.

Keywords
Intersexuality · DSD · Monochorionic twins · Monozygous twins · Tetragametic chimerism

Abstract
Disorders of or differences in sexual development (DSD) are defined by congenital conditions in which development of chromosomal, gonadal, or anatomic sex are atypical. Here, we report on monochorionic diamniotic twins delivered by caesarean section in the 36th week of pregnancy. Monochorionic twins are usually monozygous and thus should have the same sexual differentiation. In this case, one twin had female external genitalia, while the other showed ambiguous genitalia. At first, a diagnosis of mixed gonadal dysgenesis was proposed because of the obvious sexual discrepancy between the supposedly monozygous twins. Cytogenetic analyses were performed to assure the sex chromosome status for both children. Male and female cells were found subsequently in both children. While hematopoietic chime-

Karger@karger.com
www.karger.com/cgr

Correspondence to:
Rainer Wimmer, rainer.wimmer@medicover.de

rism of monochorionic dizygous twins as a result of twin-to-twin blood transfusion is a rare but already well-documented phenomenon, to our knowledge this is the first case description of tetragametic chimerism that led to intersexuality.

Introduction

Human individuals with chimeric constitutions are rarely detected. Some chimeras are found in the hematopoietic system during blood typing or parental analysis and can often be traced back to a dizygous twin and bidirectional exchange of hematopoietic stem cells through placental anastomoses. When the respective cell lines differ in the gonosomal composition (XX and XY) and are not confined to the hematopoietic system, their distribution in the body can cause certain health issues. Especially in the gonads the chimeric distribution may result in reproductive problems, cause gonadoblastomas or dysgerminomas, or lead to differences of sexual development or intersexuality [Madan, 2020; Tate et al., 2022].

Case Report

A 34-year-old woman pregnant with naturally conceived gemini was referred to the hospital at the gestational age of 31+3 weeks to schedule caesarean section when the ultrasound results were found ambiguous concerning the external genitalia of one child. A monochorionic diamniotic placenta was documented and confirmed by macroscopic evaluation after birth. Although the parents knew about the possible variance of sexual development in one child, they expected the birth of two girls.

One of the newborns showed ambiguous genitalia. On its right side, there was a palpable testicle within a scrotum/labial fold and, on the left, a labium without any palpable gonad. A phallus/clitoromegaly was measured with a length of 2 cm. The vagina and urethra shared a single opening in the urogenital sinus. Further laboratory investigations during minipuberty within the first week of life revealed an elevated testosterone level (8.16 nmol/L) as seen in boys [Johannsen et al., 2018], while estradiol (8 ng/L), FSH (0.5 U/L), and LH (<0.3 U/L) were low, indicating the existence of testicular tissue with hormone producing function. Ultrasound exams of the internal genitalia revealed an inconspicuous looking testicle and epididymis on the right side and on the left a small uterus (1.1 mL) with no accompanying gonad. Perineally a vagina was detectable with an atresia of the distal part. Normal results were revealed for the heart, kidneys, urinary system, and upper abdominal organs.

The other newborn had female external genitalia. Further investigations with ultrasound revealed a normal uterus in midline (3.4 mL), but no detectable ovaries. Blood sampling at the age of 6 months showed no testosterone. Oestradiol was detectable (27.6 pmol/L), as well as an elevated FSH (19.7 U/L) and a low LH/FSH quotient, hinting toward the existence of hormone producing ovaries.

Heparinized blood samples from both children were analyzed to clarify their sex chromosome constitution. Fluorescence in situ hybridization (FISH) with sex chromosome centromere-specific probes on native blood interphase nuclei, as well as chromosomal analysis was performed on lymphocyte cultures. Surprisingly, FISH analysis showed a very low percentage of blood cells with an XY constitution on a largely XX background in both twins (online suppl. Fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000528737). In the twin with ambiguous genitalia, 0.46% of the blood cells were found with XY and 0.48% in the twin with female appearance (Table 1). The presence of male cells in both twins' hematopoietic systems was confirmed by chromosomal analysis of stimulated lymphocytes after an extensive metaphase search. Their respective karyotypes were normal with the exception of two different sex chromosome constitutions (46,XX and 46,XY; online suppl. Fig. 2).

To further assess the distribution of the XY cell line in both twins, we also analyzed buccal smears using the same centromeric FISH probes. The results obtained from these ectodermic samples differed greatly from the mesodermic blood. Here, the phenotypically intersexual twin showed an almost 15 times higher ratio of XY cells (6.18% in contrast to 0.42%) than the phenotypically female twin (Table 1). We were able to confirm the Y-containing cell line in buccal DNA of the phenotypically intersexual twin by microarray analysis with an estimated ratio of about 10% (online suppl. Fig. 3).

Additionally, we compared DNA from buccal cells of both twins and DNA from the parents' blood cells in order to determine the twins' state of zygosity by microsatellite analysis (Table 2; online suppl. Fig. 4). The applied method usually detects DNA contamination larger than 5%. While the phenotypically female twin showed a common pattern of 1 or 2 STR alleles per marker in the buccal DNA, the same markers in the phenotypically intersexual twin revealed up to four different alleles. Twelve from 23 STR-markers showed additional alleles with much lower peaks, corresponding to the much lower percentage of XY cells in the analyzed tissue. Eight alleles were identified as paternally, 3 as maternally inherited. The allelic distribution of the twins in comparison to their parents allowed us to infer that the XX cell line is very likely monozygous, after substracting the markers that supposedly represent the XY cell line. As the XY cell line was detectable only through the ectodermic DNA of one child in the STR analysis, we were not able to prove its own state of zygosity, but we assume the male cell line to be identical in both twins.

We also applied a Deletion Insertion Polymorphism (DIP) analysis on the twins' blood and buccal DNA samples, as well as on DNA samples from parental blood (online suppl. Fig. 5). As shown in Table 3, we could confirm the monozygosity of the main, female cell line in both twins. Again, only in the buccal DNA of the phenotypically intersexual twin 12 of 34 analyzed DIP markers showed additional alleles of a much lower frequency. Six of these could be assigned to one of the parents, 1 paternal, 5 maternal.

We chose 2 markers, SRY and D114+, and tried to trace and quantify them in the twins' blood and buccal DNAs using droplet digital PCR (ddPCR; online suppl. Fig. 6) [Stahl et al., 2016]. We calculated the percentage of the male cell line in buccal DNA of the phenotypically intersexual twin at a level of 13.55% with the SRY marker and of 18.46% with the autosomal marker D114+. In its

Table 1. Percentage of XY cells on XX background in blood and buccal cells from both twins determined by chromosomal analysis, iFISH, and ddPCR

Method	Results	Percentage XY cells
Phenotypically intersexual twin		
Lymphocyte culture	chi 46,XX[51]/46,XY[1]	
iFISH on blood smear	nuc ish(DXZ1,DYZ3)×1[3]/(DXZ1×2)[655]	0.46%
iFISH on buccal smear	nuc ish(DXZ1,DYZ3)×1[64]/(DXZ1×2)[1036]	6.18%
ddPCR blood DNA	SRY/D114+	0.59%/1.23%
ddPCR buccal DNA	SRY/D114+	13.55%/18.46%
Phenotypically female twin		
Lymphocyte culture	chi 46,XX[170]/46,XY[1]	
iFISH on blood smear	nuc ish(DXZ1,DYZ3)×1[3]/(DXZ1×2)[624]	0.48%
iFISH on buccal smear	nuc ish(DXZ1,DYZ3)×1[5]/(DXZ1×2)[1195]	0.42%
ddPCR blood DNA	SRY/D114+	0.54%/0.98%
ddPCR buccal DNA	SRY/D114+	0%

Both twins show a similar percentage of chromosomally male cells in native blood smears. Depending on the method, a 13–23 times higher count of XY cells could be detected in the buccal smear of the phenotypically intersexual twin.

Table 2. Microsatellite analysis on DNA from buccal cells from both twins and parental blood

Marker	Phenotypically female twin	Phenotypically intersexual twin	Mother	Father
Amelogenin	104, 104	104, 104, **109**	104, 104	104, **109**
D1S1656	231, 248	231, 248	248, 248	231, 248
D2S441	356, 356	356, 356	356, 360	356, 356
TPOX	280, 280	**268**, 280, 280	268, 280	268, 280
D2S1338	250, 254	250, 254, **258**	254, 262	250, **258**
D3S1358	124, 128	124, 128	124, 124	128, 128
FGA	345, 349	345, 349, **353**	345, 345	349, **353**
D5S818	130, 138	130, 138	130, 130	138, 138
CSF1PO	337, 345	337, **341**, 345	337, **341**	337, 345
D7S820	228, 232	228, 232	228, 232	228, 228
D8S1179	218, 222	218, 222, **226**	222, 222	218, **226**
D10S1248	302, 306	302, 306	306, 306	302, 310
TH01	161, 173	161, 173, **176**	173, 176	161, 176
D12S391	312, 316	312, 316, **328, 332**	312, **332**	316, **328**
vWA	142, 154	**138**, 142, 154	142, 154	**138**, 154
D13S317	183, 187	183, 187, **191**	183, 199	187, **191**
PENTA E	413, 428	413, 428	413, 413	428, 433
D16S539	278, 286	278, 286, **290**	278, 294	286, **290**
D18S51	158, 170	**153**, 158, 170	**153**, 170	158, 162
D19S433	188, 194	188, 194	188, 188	188, 194
PENTA D	403, 413	403, 413	403, 413	403, 403
D21S11	223, 229	223, 229	215, 223	229, 229
D22S1045	330, 333	330, 333	330, 333	318, 330

Note the STR-markers with three or four alleles in the phenotypically intersexual twin (bold), assigned – if possible – to their parental origin.

Table 3. DIP marker analysis on DNA from the twins' buccal cells and parental blood

Marker	Phenotypically female twin	Phenotypically intersexual twin	Mother	Father
AMX/Y	AMX, AMX	AMX, AMX, **AMY**	AMX, AMX	AMX, **AMY**
HLD106	D106+, D106+	D106+, D106+	D106+, D106+	D106+, D106+
HLD70	D70–, D70+	D70–, D70+	D70–, D70+	D70–, D70–
HLD84	D84+, D84+	**D84**–, D84+, D84+	**D84**–, D84+	D84+, D84+
HLD103	D103–, D103+	D103–, D103+	D103+, D103	D103–, D103+
HLD104	D104–, D104–	D104–, D104–	D104–, D104–	D104–, D104–
HLD116	D116–, D116–	D116–, D116–, **D116+**	D116–, **D116+**	D116–, D116–
HLD112	D112–, D112+	D112–, D112+	D112–, D112+	D112–, D112–
HLD307	D307–, D307+	D307–, D307+	D307–, D307+	D307+, D307+
HLD310	D310–, D310+	D310–, D310+	D310–, D310+	D310+, D310+
HLD110	D110–, D110+	D110–, D110+	D110+, D110+	D110–, D110-
HLD133	D133+, D133+	**D133**–, D133+	D133–, D133+	D133–, D133+
HLD79	D79+, D79+	D79+, D79+	D79+, D79+	D79+, D79+
HLD105	D105–, D105–	D105–, D105–, **D105+**	D105–, **D105+**	D105–, D105–
HLD140	D140–, D140–	D140–, D140–, **D140+**	D140–, D140+	D140–, D140+
HLD163	D163–, D163+	D163–, D163+	D163+, D163+	D163–, D163+
HLD91	D91–, D91+	D91–, D91+	D91–, D91+	D91–, D91+
HLD23	D23+, D23+	**D23**–, D23+, D23+	**D23**–, D23+	D23+, D23+
HLD88	D88–, D88–	D88–, D88–, **D88+**	D88–, D88+	D88–, D88+
HLD101	D101–, D101–	D101–, D101–	D101–, D101+	D101–, D101–
HLD67	D67–, D67+	D67–, D67+	D67+, D67+	D67–, D67+
HLD301	D301+, D301+	D301+, D301+	D301–, D301+	D301+, D301+
HLD53	D53–, D53+	D53–, D53+	D53–, D53–	D53–, D53+
HLD97	D97–, D97+	D97–, D97+	D97–, D97–	D97–, D97+
HLD152	D152–, D152+	D152–, D152+	D152+, D152+	D152–, D152+
HLD128	D128–, D128+	D128–, D128+	D128+, D128+	D128–, D128–
HLD134	D134+, D134+	**D134**–, D134+, D134+	D134–, D134+	D134–, D134+
HLD305	D305–, D305+	D305–, D305+	D305–, D305+	D305–, D305+
HLD48	D48–, D48+	D48–, D48+	D48–, D48+	D48–, D48+
HLD114	D114–, D114–	D114–, D114–, **D114+**	D114–, D114+	D114–, D114+
HLD304	D304–, D304–	D304–, D304–, **D304+**	D304–, D304+	D304–, D304+
HLD131	D131+, D131+	**D131-**, D131+, D131+	**D131**–, D131+	D131+, D131+
HLD38	D38–, D38–	D38–, D38–	D38–, D38–	D38–, D38+
HLD82	D82–, D82+	D82–, D82+	D82–, D82+	D82–, D82+

Note the additional minor DIP markers in the phenotypically intersexual twin (bold), assigned – if possible – to their parental origin. The results of the twins' blood cells' DNA are not shown, as they were identical to the buccal DNA of the phenotypically female twin.

blood DNA, we found levels of 0.58% and 1.23%, respectively. In the blood DNA of the phenotypically female twin we could detect a level of 0.54% for SRY and 0.98% for D114+, while we were unable to trace them in the DNA sample gained from a single buccal swab (Table 1).

Discussion and Conclusion

During pregnancy, various possible explanations were considered to explain the twins' sex difference, from erroneous assessment of monochorionicity to congenital adrenal hyperplasia with individual expression, somatic mutation or Turner mosaicism. Surprisingly, the diagnosis of 46,XX/46,XY chimerism was revealed in both children, but with a very different percentage in ectodermal tissue.

When chimerism is detected in the hematopoietic system of monochorionic diamniotic twins, it is usually caused by a twin-to-twin blood transfusion during pregnancy and an exchange of hematopoietic stem cell through vascular anastomoses. Artificial reproductive treatments seem to enhance the formation of monochorionic dizygotic twins and hence hematopoietic chimerism, as it is rather frequent in this constellation [Peters et al., 2017]. Very few twin cases are published that show chimerism

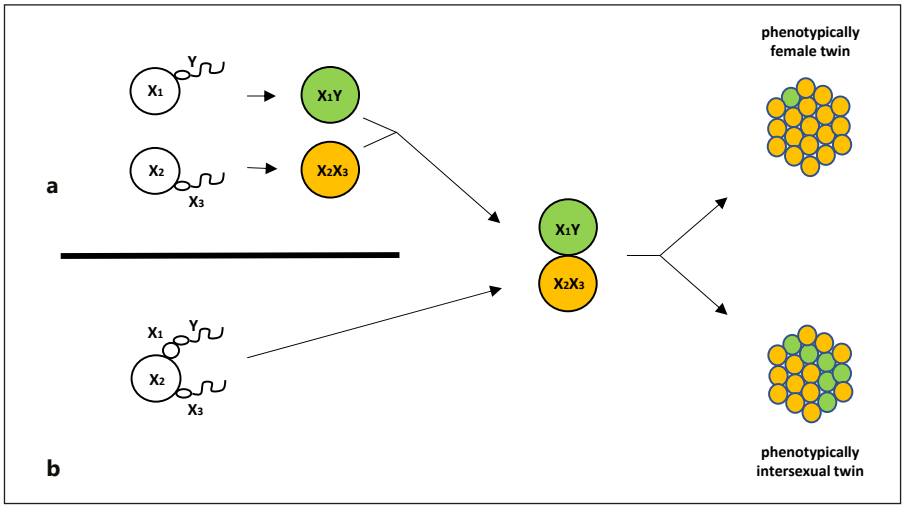

Fig. 1. Origin of the tetragametic chimerism. In this case, two possible mechanisms can be envisioned: either two independent zygotes fused (**a**) or double fertilization of ovum and polar body 2 occurred (**b**).

in cells other than in hematopoietic tissues. These findings, commonly made in buccal smears, were often assumed to have arisen from bone marrow stem cells which are able to differentiate into mature cells of various organs as confirmed by human allogenic transplant studies [Bogdanova et al., 2010; Fumoto et al., 2014].

With an incidence of 1:4,500 to 1:5,500 [Sax, 2002; Lee et al., 2016], ambiguous or intersexual genitalia can be caused by a range of genetic mutations affecting either primary gonadal differentiation or interfering with sex-specific steroid production or the sex steroid signal transferring cascades.

The most common causes for non-sex-specific gonadal differentiation are forms of Turner mosaicism. These implicate a complete or partial loss of the Y chromosome due to one or more mitotic or meiotic errors. As a consequence, mosaicism of a cell line with monosomy X and one or more cell lines containing a common or derivative Y chromosome including the *SRY* gene are present. Based on the amount and distribution of *SRY*-carrying cells in the early gonad, tissue-specific testes differentiation may take place. This can result in a male, intersexual or female phenotype.

Very rarely, mosaicism or chimerism between otherwise normal XX and XY cell lines can be found. The individual phenotypical sex differentiation depends on the mosaic or chimeric distribution in the gonads. In the case of mosaicism, a 47,XXY conception is assumed, followed by early mitotic losses of the Y chromosome in one cell line and one X chromosome in another [Zech et al., 2008]. As the organism developed from one fertilized ovum, microsatellite analysis can only detect up to 2 alleles per locus. Contrary to that, chimeras will show markers with more than 2 alleles present. Phenotypically female, intersexual or male chimeras are described, sometimes only detected due to fertility problems or other health issues [van Bever et al., 2018; Hercent et al., 2019] or even because of abnormal results during parentage testing. Madan [2020] reviewed XX/XY chimeras and discussed 3 basic theoretical ways how they can arise: (1) Tetragametic chimeras formed by fusion of 2 zygotes (2 ova fertilized by 2 spermatozoa) or (2) by a fertilized ovum fused with a fertilized second polar body. In these cases, up to 4 different alleles per STR marker are detectable by microsatellite analysis based on 2 non-identical maternal and paternal haploid genomes, respectively. (3) Also, trigametic chimeras can be found. Here, a maximum of 3 alleles per marker can then be shown using microsatellite analysis. Some of them seem to involve parthenogenetic division of the female pronucleus and fertilization of the 2 daughter cells by 2 spermatozoa [Giltay et al., 1998; Strain et al., 1998; Kawamura et al., 2020]. Masset et al. [2021] proposed another theoretical model called heterogoneic division after triploid conception to explain other forms of trigametic chimeras, such as the case of sesquizygotic twinning described by Gabbett et al. [2019]. This theory starts with a triploid zygote, followed by a genome reduction to diploid cells via a tripolar spindle apparatus and uneven spindle apposition. Also, selection against one of the diploid cell lines has to be assumed to end up at a trigametic chimera with only 2 cell lines.

In our twin case, based on the presence of 2 maternal and 2 paternal genomes, the monozygotic origin of the XX cell line and the presence of the XY cells in both twins can be explained by tetragametic chimerism. The microsatellite analysis of the phenotypically intersexual twin

showed the presence of up to 4 different alleles of the molecular markers and thus confirmed a tetragametic state. The non-appearance of third or fourth alleles in the microsatellite analysis of the phenotypically female twin, despite the proven existence of the XY cell line by FISH analysis in buccal smear and blood cells, is most likely due to its very low ratio (as shown in Table 1). Parthenogenetic partition of the ovum can be excluded because of the additional maternal alleles present for the markers CSF-1PO, D12S391, and D18S51 (Table 2). The DIP marker analysis confirmed these results with 5 maternally inherited additional markers (D84–, D116+, D105+, D23–, and D131–; Table 3). Also, heterogoneic division after triploid conception is ruled out because of the additional maternal and paternal markers found in this case. As shown in Figure 1, both amalgamation of dizygous twins and secondary twinning, as well as fertilization of the ovum and the second polar body, can explain the tetragametic patterns that were found in STR and DIP analysis.

With ddPCR we were able to trace two of the DIP markers that represent the XY cell line, one Y-chromosomal (*SRY*) and one autosomal (D114+), in the mesodermic tissues of both twins and the ectodermic tissue of the phenotypically intersexual twin. The ddPCR calculations of the percentage of XY cells on the XX background were generally a bit higher, although comparable to the FISH results (Table 1), with the exception of the buccal DNA sample from the phenotypically female twin, where the detection of the XY cell line failed. We assume that the DNA, extracted from a single buccal swab, did not contain sufficient male cells due to the mosaic distribution in its buccal tissue. The FISH analysis, performed on cells from another swab, showed only 5 of 1,200 analyzed nuclei with a male sex chromosome constitution. With respect to screening for low-level chimerism, molecular cytogenetics shows some advantage due to the possibility to analyze individual cells.

Monozygotic twinning happens at a very early stage in fetal development with a frequency of 1:250 pregnancies. In approximately 30% of monozygous twins, twinning takes place during the first 3 days of pregnancy and results in a dichorionic/diamniotic fetal development. If twinning occurs between day 3 and 8, it results in a monochorionic/diamniotic pregnancy (~70%). Twinning between day 8 and 13 leads to monochorionic/monoamniotic twins (~1%), and after the 13th day, to Siamese twins (1:70,000 births) [Entezami et al., 2002; McNamara et al., 2016]. Hence, if we assume an originally dizygotic twin conception and embryo amalgamation in our case, the fusion should have happened before "monozygotic" twinning between day 3 and 8 (Fig. 1a). One argument against the theory of embryo amalgamation is the existence of the zona pellucida. It is formed around the developing ovum prior to fertilization and is maintained until nidation on the 6th/7th day. It seems rather unlikely that 2 normal zygotes with intact zonae pellucidae are able to fuse before nidation. For a possible amalgamation, the 2 zygotes would then have to nidate at the same spot. Thus, the temporal and spatial window for a theoretical fusion-fission event that would result in a monochorionic, diamniotic twin pregnancy is very narrow. On the other hand, double fertilization occurs rather frequently, usually leading to triploid zygotes and early pregnancy loss. Given that the female meiosis 2 resulted in a fertilizable polar body 2 (Fig. 1b), no fusion event is necessary to explain a chimeric, tetragametic embryo after double fertilization [McNamara et al., 2016]. Common monozygotic twinning could then lead to the results we found in this twin case. Therefore, it seems the most likely explanation in our case. Rodriguez-Buritica et al. [2015] described a genetically comparable chimeric case of sex-discordant monochorionic twins, but with a much higher male cell count.

Sex differences between chimeric twins are most likely caused by unequal distribution of XX and XY cells in their gonads. The gonads derive from the primordial germ cells that begin to form at the yolk sac in the primitive streak around the third week of gestation. In the sixth week of gestation the primordial germ cells migrate and divide during embryogenesis to form the genital ridge and later differentiate into functional gonads. The first sign of testicular determination is the tight packing of germ cells and somatic (early Sertoli) cells to form seminiferous cords. The differentiation of the Leydig cells is dependent on the formation of the Sertoli cells and seminiferous tubules [Ostrer, 2001]. The differentiation of germ cells into spermatogonia is linked to contact with Sertoli cells, which usually consist only of XY cells [Palmer and Burgoyne, 1991]. It is thought that the degree of testicularisation of the developing gonad is determined by its ratio of XY versus XX germ line cells. Thus, the development of the external and internal genitalia of those individuals affected by chimerism of cell lines with different sex chromosome constitutions varies widely, appearing either inconspicuously male or female or showing different degrees of ambiguous genitalia [Choi et al., 2019]. Due to their mutual state of XX/XY chimerism, both twins in this case are to be considered genetically intersexual, even though one appears female at first glance.

An elevated, though nonspecific risk for gonadoblastoma and dysgerminoma has been discussed for patients with ovotesticular tissue or mixed gonadal dysgenesis. The ongoing medical care of the twins includes regular monitoring of the gonads. The testicle in the child with the ambiguous genitalia still looks inconspicuous; no gonad on the left side is seen. Due to the young age of the two children, no prognostic statement regarding gender identity, gender role or sexual orientation can be made. In the case of the twin with the female external genitalia without any detectable testosterone, a female development was assumed. In fact, both her gonads, looking like ovaries, were confirmed during recent sonographic analysis.

In Germany, any sex reassignment surgery without the individual's consent, except for life threatening situations, is forbidden by law (§ 1631e BGB) in order to support healthy sexual and gender identity development. The family was attended to by pediatric endocrinologists, psychologists, and social workers during the first 18 months after birth, and this will be continued with longer intervals during childhood. In addition to information about their children's variance of sex development, they also received peer counseling. The family first decided to register both children as female, although the German law offers "diverse" or "without sex assignment" as options to "female" or "male." Having had time to observe their children's behavior and their individual differences they are now considering to give both kids a second and more gender-neutral name.

Acknowledgments

The authors thank the family for sharing their story with the scientific community and Karen Stout-Weider for proof-reading the manuscript.

Statement of Ethics

A written informed consent was obtained from the parents for publication of the details of their medical case. Ethical approval was not required for this study in accordance with local/national guidelines.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

The authors received no funding relevant to their study.

Author Contributions

Rainer Wimmer: main author, interpretation of chromosomal, FISH and STR analysis. Uta Neumann: endocrinology, DSD counseling. André Weber: chromosomal and FISH analysis. Melanie Isau: STR analysis. Heike Renner-Luetzkendorf: prenatal ultrasound analysis. Petra Zschieschang: genetic counseling. Oliver Wachter: deletion insertion polymorphism analysis, droplet digital PCR. Kaimo Hirv: deletion insertion polymorphism analysis, droplet digital PCR. Markus Stumm: case coordination, scientific head of department.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its supplementary material files. Further inquiries can be directed to the corresponding author.

References

Bogdanova N, Siebers U, Kelsch R, Markoff A, Röpke A, Exeler R. Blood chimerism in a girl with Down syndrome and possible freemartin effect leading to aplasia of the Müllerian derivatives. Hum Reprod. 2010;25(5):1339–43.

Choi EJ, Kim SR, Kim YJ, Kang SM, Kim GY, Kim JH. Clinical and genetic analysis of an infertile male with 46,XX/46,XY chimerism. Andrologia. 2019;51(3):e13215.

Entezami M, Albig M, Gasiorek-Wiens A, Becker R. Sonographische Fehlbildungsdiagnostik. Stuttgart, New York: Georg Thieme Verlag; 2002.

Fumoto S, Hosoi K, Ohnishi H, Hoshina H, Yan K, Saji H. Chimerism of buccal membrane cells in a monochorionic dizygotic twin. Pediatrics. 2014;133(4):1097–100.

Gabbett MT, Laporte J, Sekar R, Nandini A, McGrath P, Sapkota Y, et al. Molecular support for heterogonesis resulting in sesquizygotic twinning. N Engl J Med. 2019;380:842–9.

Giltay JC, Brunt T, Beemer FA, Wit JM, van Amstel HK, Pearson PL. Polymorphic detection of a parthenogenetic maternal and double paternal contribution to a 46,XX/46,XY hermaphrodite. Am J Hum Genet. 1998;62(4):937–40.

Hercent A, Amar E, Valent A, Belloc S, Ferraretto X, Hermieu JF, et al. Various genital and reproductive phenotypes in 46,XX/46,XY chimeras. Sex Dev. 2019;13(5-6):271–7.

Johannsen TH, Main KM, Ljubicic ML, Jensen TK, Andersen HR, Andersen MS, et al. Sex differences in reproductive hormones during mini-puberty in infants with normal and disordered sex development. J Clin Endocrinol Metab. 2018;103(8):3028–37.

Kawamura R, Kato T, Miyai S, Suzuki F, Naru Y, Kato M, et al. A case of a parthenogenetic 46,XX/46,XY chimera presenting ambiguous genitalia. J Hum Genet. 2020;65(8):705–9.

Lee PA, Nordenstrom A, Houk CP, Ahmed SF, Auchus R, Baratz A, et al. Global disorders of sex development update since 2006: perceptions, approach and care. Horm Res Paediatr. 2016;85(3):158–80.
Madan K. Natural human chimeras: a review. Eur J Med Genet. 2020;63(9):103971.
Masset H, Tšuiko O, Vermeesch JR. Genome-wide abnormalities in embryos: origins and clinical consequences. Prenat Diagn. 2021;41(5):554–63.
McNamara HC, Kane SC, Craig JM, Short RV, Umstad MP. A review of the mechanisms and evidence for typical and atypical twinning. Am J Obstet Gynecol. 2016;214(2):172–91.
Ostrer H. Sex determination: lessons from families and embryos. Clin Genet. 2001;59(4):207–15.
Palmer SJ, Burgoyne PS. In situ analysis of fetal, prepuberal and adult XX--XY chimaeric mouse testes: Sertoli cells are predominantly, but not exclusively, XY. Development. 1991;112(1):265–8.
Peters HE, König TE, Verhoeven MO, Schats R, Mijatovic V, Ket JCF. Unusual twinning resulting in chimerism: a systematic review on monochorionic dizygotic twins. Twin Res Hum Genet. 2017;20(2):161–8.
Rodriguez-Buritica D, Rojnueangnit K, Messiaen LM, Mikhail FM, Robin NH. Sex-discordant monochorionic twins with blood and tissue chimerism. Am J Med Genet A. 2015;167A(4):872–7.
Sax L. How common is intersex? A response to Anne Fausto-Sterling. J Sex Res. 2002;39(3):174–8.
Stahl T, Rothe C, Böhme MU, Kohl A, Kröger N, Fehse B. Digital PCR panel for sensitive hematopoietic chimerism quantification after allogeneic stem cell transplantation. Int J Mol Sci. 2016;17(9):1515.
Strain L, Dean JC, Hamilton MP, Bonthron DT. A true hermaphrodite chimera resulting from embryo amalgamation after in vitro fertilization. N Engl J Med. 1998;338(3):166–9.
Tate JW, O'Hara BF, Wei S. 46,XX/46,XY chimerism & human sexual development. OBM Genet. 2022;6(2):1–12.
van Bever Y, Wolffenbuttel KP, Brüggenwirth HT, Blom E, de Klein A, Eussen BHJ, et al. Multiparameter investigation of a 46,XX/46,XY tetragametic chimeric phenotypical male patient with bilateral scrotal ovotestes and ovulatory activity. Sex Dev. 2018;12(1-3):145–54.
Zech NH, Wisser J, Natalucci G, Riegel M, Baumer A, Schinzel A. Monochorionic-diamniotic twins discordant in gender from a naturally conceived pregnancy through postzygotic sex chromosome loss in a 47,XXY zygote. Prenat Diagn. 2008;28(8):759–63.