

Karyotypes, confined blood chimerism, and confusion: a case of genetic sex mislabelling and its potential consequences

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ABSTRACT

Disorders of sex development (DSD) encompass a range of conditions, and the management of infants with DSD can be extremely complex. However, the misdiagnosis of a normal infant as a case of DSD may lead to unfortunate long-term consequences for the individual and the family. We report a case of confined blood chimerism masking as 46 XY gonadal dysgenesis in a female from a twin pair with discordant genders, which led to incorrect sex determination at birth. The potentially serious consequences of a wrong DSD diagnosis are discussed, including the removal of normal ovaries. This case emphasises the importance of confirming a blood karyotype where there is discordance with the clinical phenotype and, where possible, identifying whether functional gonadal tissue is present.

Introduction

Disorders of sex development (DSD) encompass a range of conditions, but they have been defined as congenital conditions where the development of chromosomal, gonadal or anatomical sex is atypical.² The management of infants with DSD can be extremely complex, requiring the long-term involvement of a multidisciplinary team working alongside the family.⁷ However, in some cases a normal infant may be wrongly labelled as having DSD, which may lead to unfortunate life-long consequences for the individual and the family. Here, we present a case of confined blood chimerism masking as 46 XY gonadal dysgenesis, which led to incorrect sex determination at birth. We discuss the potentially serious ramifications of a wrong DSD diagnosis, including the surgical removal of normal ovaries.

Case Report

A newborn twin (Twin II) was referred to the Department of Paediatric Endocrinology

at Starship Children's Hospital (Auckland, New Zealand). The child was born at term weighing 3070 g. The twin pregnancy involved complications, with an anatomy scan at 18 weeks of gestation showing dysmorphic features in one twin, including a nasal malformation and severe hypertelorism. Amniocentesis was subsequently performed, revealing a 46 XY karyotype for both fetuses. No information was recorded as to whether this was a monochorionic or dichorionic twin pregnancy.

At birth, Twin I presented as a phenotypically normal boy, while Twin II appeared as a phenotypically normal female. Examination of the female twin showed entirely normal female external genitalia. In particular, there was no evidence of virilisation, with no labial fusion or cliteromegaly. No gonads were palpable. An urgent blood lymphocyte karyotype confirmed a 46 XY karyotype. There was no evidence of mosaicism.

Further studies were performed to examine testicular and hypothalamic-

Table 1: Biochemical parameters in the female twin at age 2 months.

	Results	Normal range
Sodium (mmol/l)	136	133–146
Potassium (mmol/l)	4.8	3.7–5.2
Cholesterol (mmol/l)	3.7	<5.0
17-OHP (nmol/l)	2.0	<4.0
DHEAS (μ mol/l)	<0.04	0.06–6.70
Renin (mU/l)	287	<780
ACTH (pmol/l)	11.9	2.0–11.0

17-OHP, 17-hydroxyprogesterone;
ACTH, adrenocorticotrophic hormone; DHEAS,
dehydroepiandrosterone sulphate

Table 2: Results from stimulation tests in the female twin at ages 2 months and 9 years. The normal ranges are provided in square brackets.

Age	Stimulation test	Parameter	Pre-stimulation	Post-stimulation
2 months	hCG	Testosterone (nmol/l)	0.4 [2-8]	0.3 [>8.0]
	GnRH	LH (IU/l)	<0.8	1.8#
		FSH (IU/l)	10.8	42.1#
9 years	GnRH	LH (IU/l)	<0.8 [<2.0]	33.5*
		FSH (IU/l)	1.9 [<2.0]	19.2*

FSH, follicle stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone.

From approximately 1 week to 6 months of age, GnRH stimulation tests have led to pubertal responses (>5.0 IU/l) from approximately 1 week to 6 months of age.

* Normal prepubertal baseline values usually are <5.0 IU/l, with post-stimulation LH levels \geq 10.0 IU/l providing strong evidence of precocious puberty in females.

pituitary function in Twin II. Assessment of adrenal androgens (Table 1) excluded congenital adrenal hyperplasia. A human chorionic gonadotropin (hCG) stimulation test confirmed the lack of functional testis, with low basal testosterone concentrations that did not rise with hCG stimulation (Table 2). A gonadotropin-releasing hormone (GnRH) stimulation test demonstrated a normal follicle stimulating hormone (FSH) rise but a blunted luteinising hormone (LH) peak (Table 2). This was interpreted as likely to be normal, although a mild defect in hypothalamic-pituitary function could not be excluded. Ultrasound scans demonstrated the presence of a uterus and fallopian tubes, but gonads were not visible.

The presence of a uterus in an individual with a 46 XY karyotype is consistent with a failure of testes development, and a diagnosis of XY gonadal dysgenesis was made. The family were counselled that raising the child as female was appropriate, and as the child had no functioning gonads (and would

therefore be infertile), the child would need pubertal induction and subsequently life-long sex steroid replacement. Early childhood surgery to remove the presumed streak gonads was recommended, due to the risk of malignancy. However, the parents decided against gonadectomy and asked for no further follow-up until it was time for pubertal induction.

At the age of nine years, Twin II represented with a six-month history of breast development. On examination substantial Tanner Stage 3 breast development and Tanner Stage 2 pubic hair were noted. The potential of an oestrogen-secreting gonadoblastoma was considered a possible cause for this unexpected feminisation.

Tumour markers were negative. Blood samples were taken for a repeat chromosomal analysis, confirming a 46 XY karyotype. Fluorescent in situ hybridisation (FISH) with the SRY probe also yielded positive results. A GnRH stimulation test showed findings consistent with

puberty (Table 2), and the bone age was two years advanced. An MRI scan revealed the presence of a normal vagina and a normal-sized anteverted uterus with a thin endometrium. Unexpectedly, both ovaries were also identified and reported to have normal morphology, with 2–3 simple cysts noted bilaterally.

Twin confined blood chimerism was considered as a possible cause for these findings, and skin fibroblast analysis in Twin II was subsequently performed. This demonstrated a 46 XX karyotype confirming she was female. Lymphocyte DNA analysis demonstrated her blood genotype was identical to her twin brother, indicating that confined blood chimerism had occurred, presumably due to a unilateral blood trans-fusion from her twin brother.

Discussion

This case highlights the importance of fully investigating patients where the phenotype is discordant with the karyotype. The assumption that blood lymphocytes will provide a karyotype that reflects all body tissues is not always correct. Mosaic and chimeric karyotypes are well documented, are not always apparent on a blood karyotype alone, and are not uncommon.

A chimera is an organism that contains cells from more than one distinct zygote.^{4,8} This differs from mosaicism in which genetically different cell lines originate from a single zygote lineage.^{3,6} In the majority of cases, chimerism is seen after allogeneic bone marrow transplantation, in which bone marrow-derived cells belonging to the recipient are eradicated and substituted with donor cells.¹

However, there are cases reporting confined blood chimerism occurring in dizygotic twins. This is thought to occur via an exchange of haematopoietic stem cells *in utero*, via inter-twin placental vascular anastomoses.^{3,6} It has been suggested that confined blood chimerism in dizygotic twins has a higher incidence than previously thought, with an occurrence rate

of 8% amongst twin pairs and 21% in triplets.⁹ However, very few cases have been reported in the literature, particularly regarding confined blood chimerism in dizygotic twins of opposite genders.^{6,8}

In the present case, the occurrence of confined blood chimerism in Twin II led to the misdiagnosis of XY gonadal dysgenesis. Indeed, this misdiagnosis was felt to be so secure that gonadectomy in infancy was recommended to the parents. Tragically, the surgical removal of normal ovaries in such a situation has been reported previously, with permanent infertility and life-long sex steroid requirements in adult life.⁸ Fortunately, in this case the parents decided against the recommended procedure.

A fundamental learning point from this case is the need to clearly define whether functioning gonadal tissue exists. When this child initially presented, an inhibin B assay was unavailable. Inhibin B is found at low but measurable levels in infant girls, and might have resulted in the accurate recognition of functioning ovarian tissue, thus avoiding the misdiagnosis of XY gonadal dysgenesis.⁵ We would recommend that in assessing gonadal function, stimulation tests (such as using luteinising hormone or hCG) as well as assessment of inhibin B levels should be used. Where possible, pelvic MRI scans and/or laparoscopy should also be performed, as pelvic ultrasound scans are less sensitive, especially at identifying gonadal tissue.

This case emphasises the importance of confirming a blood karyotype where there is discordance with the clinical phenotype. In particular, where XY gonadal dysgenesis is being considered, assessments of ovarian function are mandatory. If there is suspicion of a chimera, fibroblast chromosomal analysis in addition to a blood lymphocyte karyotype should be performed. Notably, confined blood chimerism in twins is not uncommon, especially when they are monozygotic, and this diagnosis should be considered in DSD cases where there is a twin pregnancy and discordant twin genders.

Competing interests: Nil**Author information:**

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REFERENCES:

1. Assaf SA, Randolph LM, Benirschke K, Wu S, Samadi R, Chmait RH (2010) Discordant blood chimerism in dizygotic monozygotic laser-treated twin-twin transfusion syndrome. *Obstet Gynecol* 116 Suppl 2:483-485.
2. Barbaro M, Wedell A, Nordenström A (2011) Disorders of sex development. *Semin Fetal Neonatal Med* 16:119-127.
3. Bourthoumieu S, Esclaïre F, Yardin C (2006) Chimerism in twins: caution is needed in interpretation of karyotypes. *Am J Med Genet A* 140:533-535.
4. Chen K, Chmait RH, Vanderbilt D, Wu S, Randolph L (2013) Chimerism in monozygotic dizygotic twins: case study and review. *Am J Med Genet A* 161A:1817-1824.
5. Crofton PM, Evans AEM, Groome NP, Taylor MRH, Holland CV, Kelnar CJH (2002) Dimeric inhibins in girls from birth to adulthood: relationship with age, pubertal stage, FSH and oestradiol. *Clin Endocrinol (Oxf)* 56:223-230.
6. Hawcutt D, Hammond B, Sibbring J, Gokhale D, Ellis I, Bricker L, Subhedar N (2011) Twin-twin confusion syndrome: blood chimerism in opposite sex dizygotic twins. *J Obstet Gynaecol* 31:446-448.
7. Hughes IA, Houk C, Ahmed SF, Lee PA, LWPES Consensus Group, ESPE Consensus Group (2006) Consensus statement on management of intersex disorders. *Arch Dis Child* 91:554-563.
8. Johannsen TH, Lundsteen C, Visfeldt J, Schwartz M, Petersen BL, Byskov AG, Muller J (2003) Erroneous genetic sex determination of a newborn twin girl due to chimerism caused by foetal blood transfusion. A case report. *Horm Res* 60:148-151.
9. van Dijk BA, Boomsma DI, de Man AJ (1996) Blood group chimerism in human multiple births is not rare. *Am J Med Genet* 61:264-268.