Cytogenetic and Genome Research

Original Article

Cytogenet Genome Res 2020;160:688–697
DOI: 10.1159/000512206

Received: August 26, 2020
Accepted: October 7, 2020
Published online: December 16, 2020

Two Novel Cases of Autosomal Translocations in the Horse: Warmblood Family Segregating t(4;30) and a Cloned Arabian with a de novo t(12;25)

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Keywords
Balanced-unbalanced autosomal chromosome rearrangements · Assisted reproductive technologies · Subfertility · Congenital abnormalities · Horses

Abstract
We report 2 novel autosomal translocations in the horse. In Case 1, a breeding stallion with a balanced t(4p;30) had produced normal foals and those with congenital abnormalities. Of his 9 phenotypically normal offspring, 4 had normal karyotypes, 4 had balanced t(4p;30), and 1 carried an unbalanced translocation with tertiary trisomy of 4p. We argue that unbalanced forms of t(4p;30) are more tolerated and result in viable congenital abnormalities, without causing embryonic death like all other known equine autosomal translocations. In Case 2, two stallions produced by somatic cell nuclear transfer from the same donor were karyotyped because of fertility issues. A balanced translocation t(12q;25) was found in one, but not in the other clone. The findings underscore the importance of routine cytogenetic screening of breeding animals and animals produced by assisted reproductive technologies. These cases will contribute to molecular studies of translocation breakpoints and their genetic consequences in the horse.

Introduction

Decades of clinical cytogenetic studies in humans and animals clearly demonstrate that numerical and structural chromosome changes cause embryonic loss, congenital abnormalities, and infertility [Lear and Bailey, 2008; Raudsepp and Chowdhary, 2016]. It is also well-documented that assisted reproductive technologies, including somatic cell nuclear transfer (SCNT), facilitate chromosomal instability and aberrations [Booth et al., 2003; Hanada et al., 2005; Raudsepp and Chowdhary, 2016]. At the same time, clinical cytogenetic data show disparity in the prevalence of certain types of chromosome changes among species. For example, viable X chromosome aneuploidies are frequently found in humans [Skuse et al., 2018] and horses [Lear and Bailey, 2008; Raudsepp et al., 2012], but are rare in cattle, pigs or dogs [Raudsepp and Chowdhary, 2016]. Likewise, reciprocal translocations are the most common structural rearrangements in humans [White and Jacobs, 1992; Wilch and Morton, 2018] and pigs [Donaldson et al., 2019], and robertsonian translocations in cattle, while translocations are rare in horses, dogs or cats [Raudsepp and Chowdhary, 2016; Szczerbal and Switonski, 2016]. Reasons for such species differences are attributed to differ-

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ences in the cytogenetic and molecular architecture and regulation of chromosomes [Raudsepp and Chowdhary, 2016; Donaldson et al., 2019], though little is known about the molecular factors facilitating chromosome rearrangements in different species [Weckselblatt and Rudd, 2015; Donaldson et al., 2019; Schluth-Bolard et al., 2019]. To improve this knowledge, it is important to continue the collection of cytogenetic case reports to inform about novel aberrations and the recurrence of the known ones.

Translocations are structural rearrangements that involve double-strand breaks in nonhomologous chromosomes and mistakes in their repair, so that nonhomologous chromosomes exchange parts or fuse, giving rise to reciprocal or nonreciprocal translocations, respectively [Morin et al., 2017]. Translocations can be genetically balanced with no DNA loss or gain in the genome. Carriers of such translocations appear phenotypically normal but have reduced fertility because of producing both genetically balanced and unbalanced gametes. The former can pass the translocation between generations, whereas fertilization of unbalanced gametes typically results in embryonic or fetal death [King et al., 1981; Ghosh et al., 2016; Raudsepp and Chowdhary, 2016]. On the other hand, carriers of unbalanced translocations have partial aneuploidy and show a range of developmental and reproductive disorders depending on the extent of the aneuploidy and the chromosome regions involved [Morin et al., 2017].

In the horse (*Equus caballus*, ECA), only a handful of distinct translocations have been reported and none as recurrent [Lear and Bailey, 2008; Durkin et al., 2011; Lear and Villagomez, 2011; Ghosh et al., 2016]. The majority are balanced reciprocal or nonreciprocal autosomal translocations, but in only 1 case transmission to the next generation has been verified [Durkin et al., 2011]. In addition, there are a few reports about balanced or unbalanced translocations between an autosome and the sex chromosomes [Power, 1987; Bugno-Poniewierska et al., 2018; Ruiz et al., 2019]. Even though recurrent patterns of the involvement of specific equine chromosomes in translocations have been noted [Ghosh et al., 2016], the number of reported cases is too limited for broader conclusions. To date, no translocations or other chromosome abnormalities have been associated with assisted reproductive technologies in horses.

Here, we report on 2 cases of novel autosomal translocations in the horse: one in a Warmblood family where the translocation is transmitted between generations; the other case involves 2 genetically identical cloned Arabian stallions of which one carries a translocation and the other has a normal karyotype. The 2 cases are characterized using conventional and molecular cytogenetic approaches and DNA genotyping.

Materials and Methods

Case Descriptions and Sampling

Case 1. A phenotypically normal 14-year-old Warmblood stallion (ID: H704; Table 1) was subjected to chromosome analysis due to a history of having foals with congenital abnormalities (Table 2). For example, breeding records of the past 5 years (2016–2020; Table 2) show that the stallion produced 49 pregnancies of which 61% resulted in live-born foals. However, 20% of the latter had congenital abnormalities such as contracted tendon and microphthalmia, and 30% of all live-born foals died or were euthanized due to multiple reasons. Consecutively, 9 of his 2014–2015 foals by different dams, 2 females and 7 males (Table 1), were also subjected to chromosome analysis. All foals were characterized as phenotypically normal, though one (ID: H716) was reported to have "shabby" hair, not thriving well, and was later euthanized.

Case 2. Two 4-year-old Arabian stallions, both produced by SCNT from the same sire (samples not available) were subjected to chromosome analysis because of observed abnormalities during reproductive work-up. One stallion (ID: H962) had small testes and azoospermia, the other (ID: H963) only had small testes (Table 1).

Blood samples from all horses were collected in EDTA- and sodium heparin-containing vacutainers (VACUTAINER™, Becton Dickinson). In addition, skin biopsies were obtained from the 2 Arabian clones.

Cell Cultures and Chromosome Preparations

Metaphase chromosome spreads for all horses were prepared from peripheral blood lymphocytes following standard protocols [Raudsepp and Chowdhary, 2008]. Briefly, 1 mL of sodium heparin-stabilized peripheral blood was grown for 72 h in 9 mL of culture medium RPMI-1640 supplemented with HEPES and Glutamax (Gibco), 10% fetal bovine serum (Atlanta Biologicals), 1× antibiotic-antimycotic (100×; Invitrogen), and 15 μg/mL pokeweed mitogen (Sigma Aldrich). Additionally, primary fibroblast cultures were obtained from skin biopsies of the 2 Arabian stallions following standard procedures [Vangipuram et al., 2013]. The cells were grown in alpha MEM with nucleosides and Glutamax (Gibco), supplemented with 20% fetal bovine serum, at 5% CO_2 to semi-confluency (approximately 60–70%). Both blood lymphocyte and fibroblast cultures were harvested with demecolcine solution (10 μg/mL; Sigma Aldrich), treated with Optimal Hypotonic Solution (Rainbow Scientific), and fixed in 3:1 methanol:acetic acid. The cells were dropped on clean, wet glass slides and checked under a phase contrast microscope (×200) for quality.

Karyotyping and Cytogenetic Analysis

Chromosomes were stained by GTG-banding [Seabright, 1971] for karyotyping. A minimum of 30 cells were captured and analyzed for each individual from blood lymphocytes and an additional 20 cells from primary fibroblasts of the 2 Arabian stallions.

Table 1. Composite information about the horses, phenotypes, genotypes, and karyotypes reported in this study

Horse ID	Sex	Relations	Karyotype	Phenotype	*SRY*	*AR*	ECA29 deletion
Case 1: Warmblood family							
H704	Male	Sire	64,XY,t(4;30)(p10;q10)	Sires foals with congenital defects	Positive	Positive	No D/D; likely heterozygote
H709	Male	Foal	64,XY	Normal	Positive	Positive	No D/D
H710	Male	Foal	64,XY	Normal	Positive	Positive	No D/D
H711	Female	Foal	64,XX	Normal	Negative	Positive	No D/D
H712	Male	Foal	64,XY,t(4;30)(p10;q10)	Normal	Positive	Positive	No D/D
H713	Male	Foal	64,XY,t(4;30)(p10;q10)	Normal	Positive	Positive	D/D
H714	Male	Foal	64,XY,t(4;30)(p10;q10)	Normal	Positive	Positive	D/D
H715	Male	Foal	64,XY,t(4;30)(p10;q10)	Normal	Positive	Positive	No D/D
H716	Male	Foal	64,XY,+4,der(4;30)(p10;q10)	"Shabby" hair, poor thriving	Positive	Positive	D/D
H717	Female	Foal	64,XX	Normal	Negative	Positive	No D/D
Case 2: Arabian clones							
H962	Male	Clone	64,XY	Small testes, azoospermia	Positive	Positive	D/D
H963	Male	Clone	64,XY,t(12;25)(q10;q10)	Small testes	Positive	Positive	D/D

SRY, sex determining region Y; *AR*, X-linked androgen receptor; D/D, homozygous deletion; no D/D, deletion heterozygotes or individuals with no deletion (Note: the PCR test cannot discriminate between the latter two).

Table 2. Summary of 2016–2020 breeding and foaling records of the Warmblood stallion from Case 1

Year	Semen collections	Pregnancies achieved	Lost embryos	Live foals	Phenotypes of live born
2020	6	4	3: no detailed information available	1	No information available on live foals
2019	8	6	1 mare died while pregnant	5	No information available on live foals
2018	6	6	2 embryos resorbed after confirmed pregnancy	4	No information available on live foals
2017	9	11	4: 2 embryos resorbed after confirmed pregnancy; 1 abortion; 1 stillborn	7	No information available on live foals
2016	15	22	9: 1 fetus aborted at 8.5 months gestation; 5 embryos resorbed after confirmed pregnancy; 1 pregnancy misdiagnosed; 2 pregnancies terminated after fetal sexing	13	4 live-born foals with flexural or angular limb deformities (3 euthanized, 1 died) 1 foal with bilateral microphthalmia (euthanized) 2 foals with difficult foaling (died after birth) 2 foals oxygen deprived (euthanized) 4 survived, no information available
Total	44	49	19	30	6: with congenital defects; 9: euthanized

Karyotyping and chromosome analysis were done with an Axioplan2 microscope (Carl Zeiss, Inc., Jena, Germany) and IKAROS (MetaSystems GmbH, Altlussheim, Germany) software. The chromosomes were identified and arranged into karyotypes according to the International System for Cytogenetic Nomenclature of the Domestic Horse [Bowling et al., 1997]. Chromosome aberrations were described according to the International System for Human Cytogenetic Nomenclature [ISCN, 1995].

Fluorescence in situ Hybridization

The rearrangements identified by conventional cytogenetic analysis were validated and breakpoints determined by 2-color FISH with probes listed in Table 3 following standard protocols [Raudsepp and Chowdhary, 2008]. The probes were labeled with biotin or digoxigenin by nick translation using Biotin or DIG Nick Translation Mix (Roche Diagnostics), respectively, and the manufacturer's protocol. Differently labeled probes were hy-

Table 3. Information about the BAC clones used for FISH to delineate the 2 translocations

Rearrangement	ECA	Cytoband	CHORI-241 BAC ID	BAC location in EquCab3
t(4p;30)	4	p16	120C15	4,506,617–4,699,244
	4	p13–p12	213K17	14,712,643–14,980,468
	4	q12	18K13	26,849,846–27,005,304
	4	q26–q27	59G9	102,644,729–102,824,499
	30	q12	63C17	801,540–971,456
	30	q15	324J16	28,615,569–28,834,006
t(12q;25)	12	p15	65F17	5,028–165,461
	12	q13	201L22	22,427,547–22,683,255
	12	q14	185A20	34,088,712–34,268,695
	25	q12	118D23	874,584–1,069,602
	25	q13	47C8	6,553,374–6,738,533
	25	q19	58P19	33,805,999–34,048,037

Previous cytogenetic mapping information was retrieved from Raudsepp et al. [2008].

bridized in pairs to metaphase chromosomes. Hybridization signals of biotin-labeled probes were detected with Alexa Fluor® 488 streptavidin conjugate (Molecular Probes, Life Technologies) and digoxigenin-labeled probes with anti-digoxigenin-rhodamine (Roche Diagnostics) or DyLight®594 anti-digoxigenin conjugate (Vector Laboratories). Chromosomes were counterstained with DAPI. At least 10 cells were captured and analyzed for each experiment using Isis V5.2 (MetaSystems GmbH) software.

DNA Isolation, Short Tandem Repeat Genotyping, and Analysis by PCR

Genomic DNA was isolated from EDTA-stabilized blood with QIAamp DNA Blood Mini Kit (Qiagen) and from primary fibroblasts with DNeasy Blood & Tissue Kit (Qiagen). The DNA was genotyped for 15 autosomal [Khanshour et al., 2013] and 8 X-linked short tandem repeats (STRs) [Anaya et al., 2017] using an ABI PRISM 377 (Applied Biosystems, Foster City, CA, USA) following previously described methods [Juras et al., 2003]. All animals were tested by PCR for the Y-linked *SRY* gene and for a 200-kb homozygous deletion in ECA29, a risk factor for disorders of sex development and reproduction [Ghosh et al., 2020]. The X-linked androgen receptor (*AR*) gene served as a positive control for all PCR amplifications. The primers were as follows: *SRY*-forward 5′-TGCATTCATGGTGTGGTCTC-3′, *SRY*-reverse 5′-ATGGCAATTTTTCGGCTTC-3′, 131 bp [Paria et al., 2011]; *AR*-forward 5′-AGCAGCAACAGGAGACCAGT-3′, *AR*-reverse 5′-GCTTAAGCCTGGGAAAGTG-3′, 294 bp; ECA29 deletion (3 primer pairs): E458-forward 5′-AAAGATGCCGGTTTAACCAA-3′, E458-reverse 5′-TGCAAACAGGCTTGTACTTGA-3′, 103 bp; E661-forward 5′-GCATTTTGTTCCATGTGTGC-3′, E661-reverse 5′-CACAGTCAAACCACCCACTG-3′, 365 bp; and E668-forward 5′-TGCTGTTGATCATCCTTATTATCC-3′, E668-reverse 5′-AAATGAAAATGAGAATAAAGGAAAGTG-3′, 179 bp [Ghosh et al., 2020].

Results

Case 1: A Warmblood Family

Karyotyping of GTG-banded metaphases of the Warmblood stallion revealed a normal diploid number (2n = 64) and normal XY sex chromosomes. However, in all cells one homolog of ECA4 and ECA30 was missing. Instead, we observed the presence of 2 unpaired derivative chromosomes: a small submetacentric chromosome and a large acrocentric chromosome. Based on the GTG-banding pattern, the former was tentatively identified as a translocation between ECA4p and ECA30, and the latter as ECA4q (Fig. 1). The derivative chromosomes were definitely identified and the rearrangement precisely delineated by FISH using 6 BAC clones representing proximal and distal regions of ECA4p, 4q, and 30 (Fig. 1c; Table 3). This confirmed that the short arm of the small submetacentric derivative chromosome corresponded to ECA4p (Fig. 1e, f) and the long arm to ECA30; the large acrocentric derivative chromosome was ECA4q (Fig. 1d). Analysis by FISH indicated that the translocation was balanced and nonreciprocal, and involved a breakpoint at the ECA4 centromere and centric fusion of ECA4p and ECA30. The translocation was observed in all ~60 blood lymphocyte cells analyzed by GTG-banding and FISH, suggesting that the rearrangement was not mosaic. We concluded that the Warmblood breeding stallion with a history of having foals with congenital abnormalities, carried a constitutional balanced and nonreciprocal autosomal translocation with 2 derivative chromosomes. The karyotype was denoted as 64,XY,t(4;30)(p10;q10).

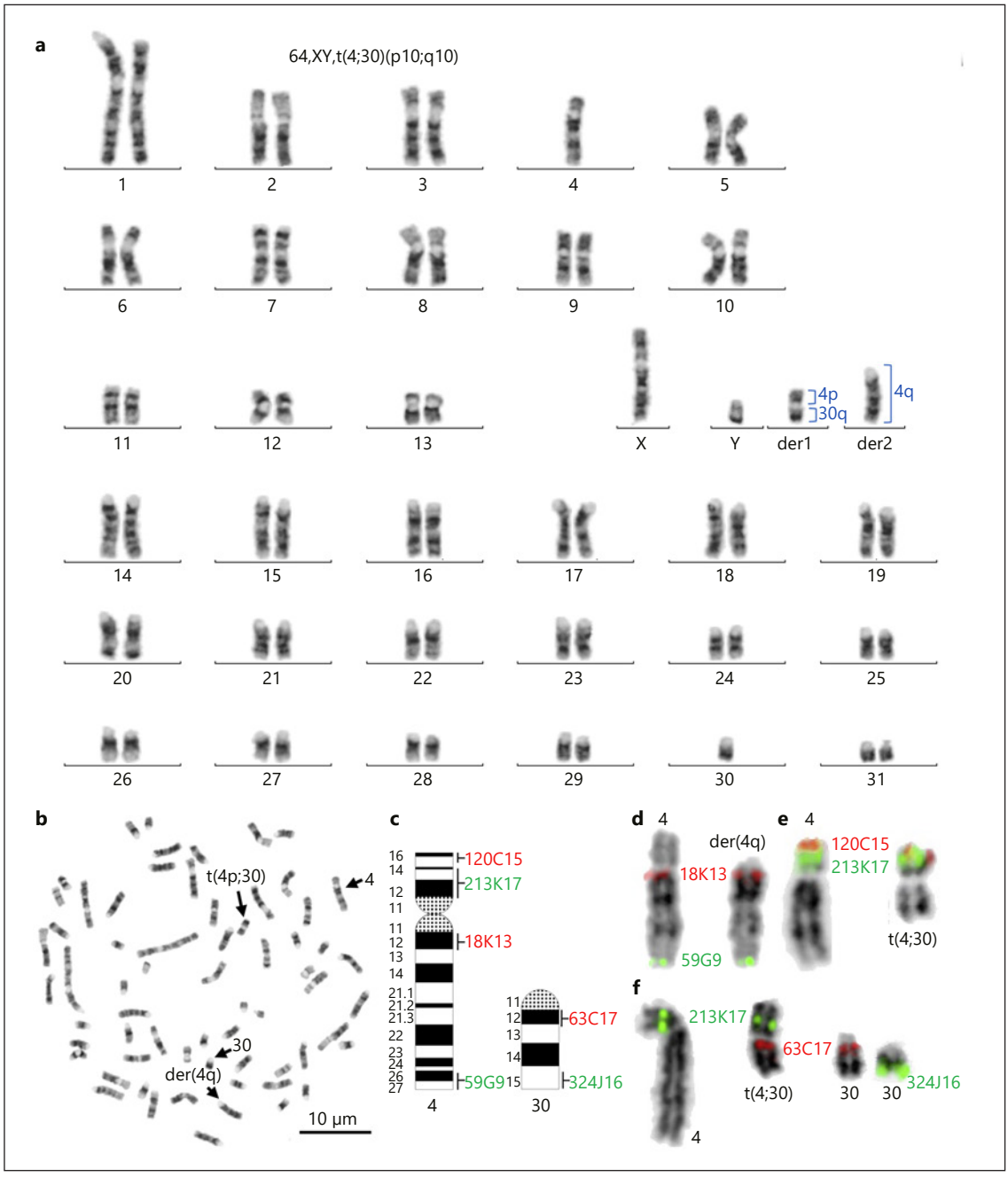

Fig. 1. Balanced autosomal translocation in a Warmblood breeding stallion (ID: H704). **a** GTG-banded karyotype 64,XY,t(4;30)(p10;q10),der(4q). **b** Corresponding metaphase spread where normal and derivative chromosomes 4 and 30 are shown by arrows. **c** Idiograms of ECA4 and ECA30 showing the location of FISH markers. Red and green font colors correspond to red and green fluorescence labels used. **d–f** Inverted DAPI images and FISH signals in ECA4, ECA30, t(4p;30q) and der(4q) after FISH experiments with pair-wise combinations of BAC clones listed in **c**.

Similarly, karyotyping and FISH analysis of 9 foals of this Warmblood stallion revealed that 4 foals had normal 64,XY male or 64,XX female karyotypes, whereas 4 foals carried the same balanced rearrangement as the stallion (Table 1). Notably, the foal described as having "shabby" hair and not thriving well (ID: H716; Table 1) had an unbalanced form of the rearrangement with tertiary trisomy ECA4p. The karyotype of this foal contained only one

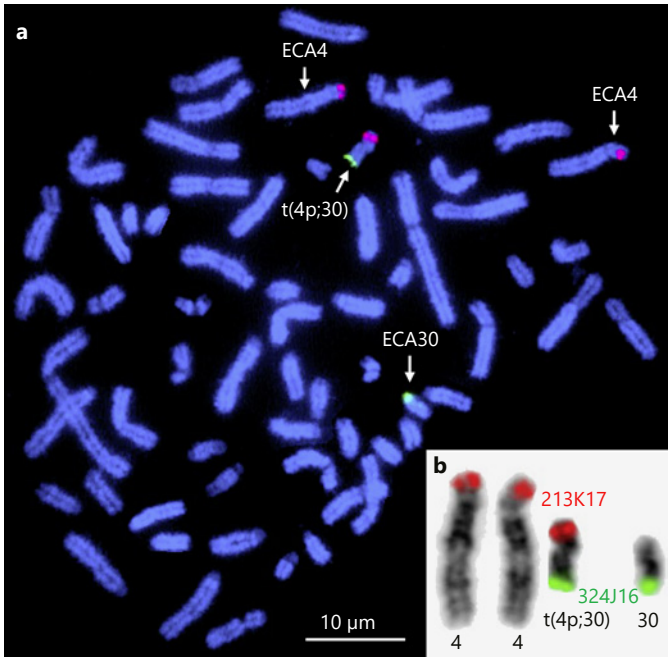

Fig. 2. Unbalanced autosomal translocation with tertiary trisomy 4p in a Warmblood foal. **a** A DAPI (blue) counterstained metaphase spread with FISH signals in ECA4p16 (BAC 213K17, red) and ECA30q15 (BAC 324J16, green) (see legend in Fig. 1c) showing the presence of 2 normal homologs of ECA4, one normal ECA30, and a derivative chromosome der(4p;30). **b** Inverted DAPI images merged with FISH signals of enlarged normal homologs of ECA4 (left), derivative chromosome der(4p;30) (middle), and normal ECA30 (right).

derivative chromosome, t(4p;30), and 2 normal homologs of ECA4 and was denoted as 64,XY,+4,der(4;30)(p10;q10) (Fig. 2).

In addition to cytogenetic analysis, the stallion and the foals were genotyped for genome-wide STR markers confirming the stallion as the sire of all 9 foals (online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000512206). Analysis by PCR showed that all XY individuals were positive and all XX individuals were negative for the *SRY* gene, which is normal. Finally, all horses were tested for a 200-kb homozygous deletion in ECA29 involving *AKR1C* genes – a risk factor for abnormal sex development and/or reproduction [Ghosh et al., 2020]. The results showed that 3 foals were homozygous for the deletion: 2 (ID: H713 and H714) carrying a balanced translocation and 1 (ID: H716, "shabby hair") with unbalanced translocation (Table 1). These findings indicate that the stallion must have been heterozygous for the deletion.

Case 2: Cloned Arabian Horses

Genotyping with genome-wide STRs showed that the 2 Arabian stallions were genetically identical, thus confirming them to be clones derived by SCNT from the same donor. Analysis of GTG-banded metaphase spreads from blood lymphocytes revealed that both had a normal 2n = 64 diploid number and XY sex chromosomes. A PCR test with the *SRY* gene was positive in both stallions, as is normal for males. Karyotyping, however, revealed that one of the clones (ID: H962) had a normal 64,XY male karyotype, while the karyotype of the other clone (ID: H963) was abnormal. It was missing a homolog of ECA12 and ECA25 and contained 2 derivative chromosomes – a medium-sized submetacentric and a small acrocentric (Fig. 3). Analysis by 2-color FISH using combinations of ECA12 and ECA25 markers (Fig. 3c; Table 3) confirmed and refined these observations, revealing that the submetacentric derivative chromosome resulted from centric fusion of ECA12q and ECA25q, and the small acrocentric derivative chromosome was ECA12p (Fig. 3d–h). Analysis by FISH also confirmed that the other clone (ID: H962) had a normal karyotype with no rearrangements. Karyotyping and FISH results from primary skin fibroblasts of the 2 stallions were the same as from blood lymphocytes, suggesting that the rearrangement observed in clone H963 was constitutional. We concluded that despite expectations to be genetically identical, the 2 Arabian clones were chromosomally different: one with normal 64,XY male karyotype, another with a constitutional balanced nonreciprocal translocation 64,XY,t(12;25)(q10;q10). Analysis by PCR showed that both clones were homozygous for the 200-kb deletion in ECA29 (Table 1).

Discussion

Here, we characterized 2 novel cases of balanced nonreciprocal autosomal translocations in the horse, bringing the tally of all autosomal translocations in this species to 11 (online suppl. Table 2). However, on the background of previous reports, the 2 cases presented in this study are unique and notable in specific ways.

The unusual feature of Case 1 was that the translocation t(4;30) carrier stallion was identified not by subfertility, but due to having live-born foals with congenital abnormalities, such as microphthalmia and flexural deformities, also known as contracted tendons [Caldwell, 2017]. In contrast, all other equine cases of balanced autosomal translocations have been identified due to subfertility caused by recurrent early embryonic loss (online

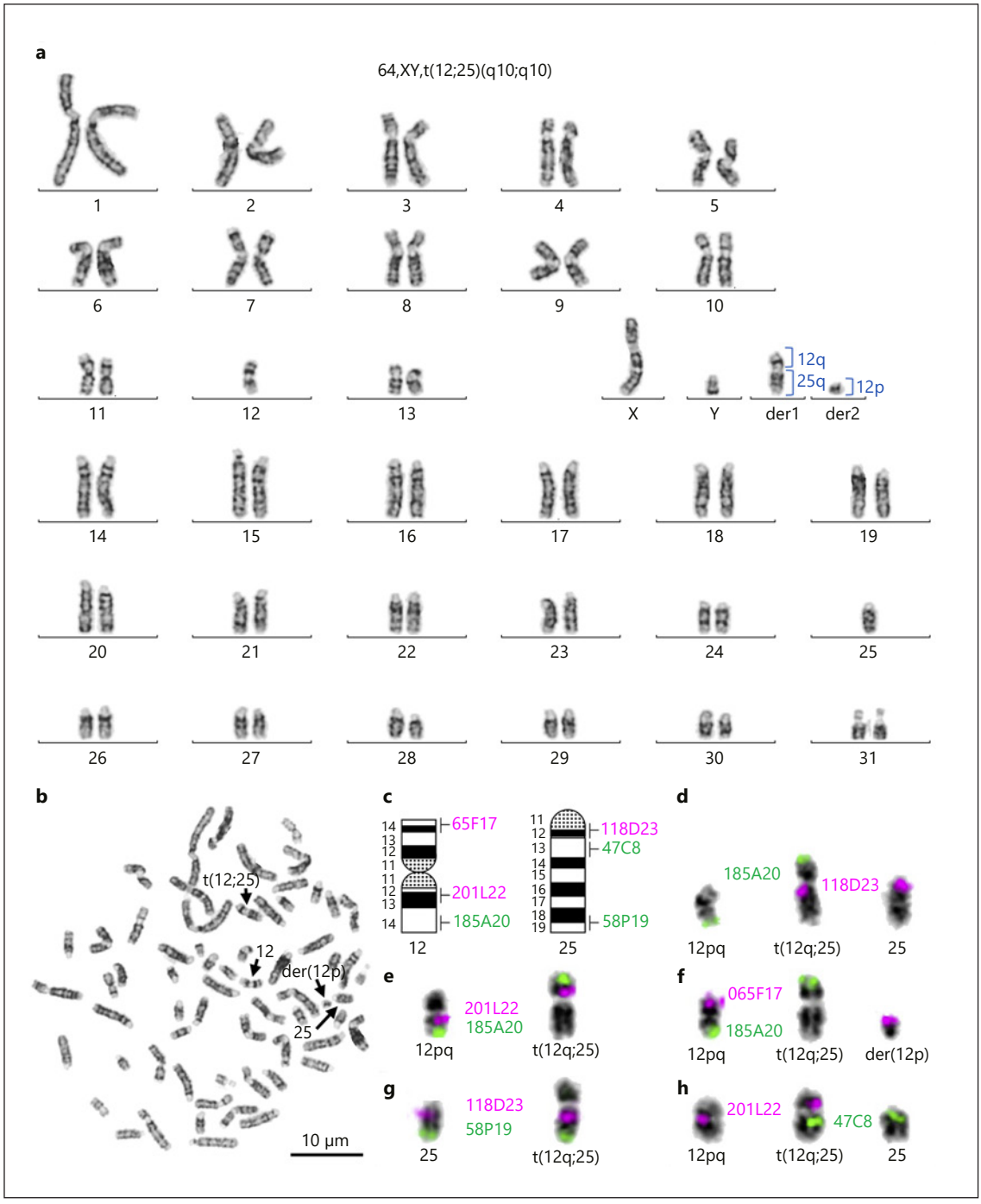

Fig. 3. Balanced autosomal translocation in a cloned Arabian stallion (ID: H963). **a** GTG-banded karyotype 64,XY,t(12;25)(q10;q10),der(12p). **b** Corresponding metaphase spread where normal and derivative chromosomes 12 and 25 are shown by arrows. **c** Idiograms of ECA12 and ECA25 showing the location of FISH markers. Purple and green font colors correspond to purple and green fluorescence labels used. **d–h** Inverted DAPI images and FISH signals in ECA12, ECA25, t(12q;25q) and der(12p) after FISH experiments with pair-wise combinations of BAC clones listed in **c**.

suppl. Table 2) [Lear and Bailey, 2008; Durkin et al., 2011; Lear and Villagomez, 2011; Raudsepp and Chowdhary, 2016]. The latter happens when a genetically unbalanced gamete is involved in fertilization, causing genetic imbalance and early death of the embryo [Durkin et al., 2011]. Genetic causes of equine microphthalmia and contracted tendons are not known, but research in humans shows that congenital microphthalmia is a multigenic condition with complex mendelian inheritance [Riera et al., 2017; Slavotinek, 2019], while syndromic clubfoot, the human analog to equine contracted tendons, is a complex trait with yet unknown genetics [Sadler et al., 2019]. Thus, it is unlikely that a translocation between ECA4p and ECA30 specifically affects genes critically involved in the 2 congenital disorders. A more plausible explanation is that, in contrast to other equine autosomal translocations, unbalanced forms of t(4;30) are better tolerated, and the resulting developmental abnormalities are compatible with life. A proof to this was identifying unbalanced t(4;30) with tertiary trisomy 4p in one of the foals (H716; Table 1) with a relatively mild effect on the phenotype ("shabby" hair and poor thriving). The reason may be the small size of 4p (approximately 25 Mb) and low gene density (approximately 1.1 coding genes/Mb) (https://www.ncbi.nlm.nih.gov/genome), making trisomy for this region compatible with almost normal development. However, if meiotic segregation results in more extensive genetic imbalance, the offspring will have more severe congenital defects – in this case microphthalmia and flexural deformities. Certainly, these speculations need to be confirmed by chromosome analysis of the developmentally abnormal offspring sired by the stallion. Nevertheless, our results strongly suggest that translocation t(4;30) in this Warmblood family has a milder genetic impact compared to previously reported autosomal translocations in the horse. The results also indicate that some familial cases of equine congenital abnormalities may be caused by cytogenetically detectable unbalanced translocations.

The findings of Case 2 are equally curious. Here, SCNT was used with the intention to generate identical genetic copies of an elite high-performance animal but instead produced 2 sexually underdeveloped subfertile or infertile clones of which one also carried a chromosomal abnormality. Because the other clone (H962; Table 1) was chromosomally normal, we assumed that the somatic cell donor animal had a normal karyotype, and the translocation t(12;25) occurred de novo in clone H963. Since we detected the translocation in both blood lymphocytes and skin fibroblasts, the rearrangement must have happened during the early cleavage divisions, right after SCNT. Though, a possibility of transmission from the donor horse due to somatic mosaicism cannot be ruled out. It was, however, a surprise that the clone with the normal karyotype, and not the one with the translocation, had azoospermia, particularly because recently a balanced Y-autosome translocation was associated with azoospermia in a Friesian stallion [Ruiz et al., 2019] (online suppl. Table 2). Overall, discovering a chromosomal aberration in a cloned animal was not unexpected because SCNT has been associated with chromosomal and genome instability [Hanada et al., 2005; Hamada et al., 2012; Raudsepp and Chowdhary, 2016]. It is just that there have been no reports about clinical cytogenetic evaluation of equine clones, this despite the fact that almost 300 cloned horses have been produced worldwide [Gambini and Maserati, 2017]. Our findings clearly show that cytogenetic evaluation of the somatic cells of donor horses for SCNT and the produced clones is warranted.

Worth mentioning is also the fact that both cloned stallions carried a homozygous deletion (D/D) in ECA29. This approximately 200-kb deletion at 29.7–29.9 Mb involves *AKR1C* genes which function as ketosteroid reductases in steroid hormone biosynthesis, including androgens and estrogens [Ghosh et al., 2020]. Mutations in *AKR1C* genes are associated with a spectrum of human disorders of sex development such as cryptorchidism, undervirilized external genitalia, dihydrotestosterone deficiency, and sex reversal [Baetens et al., 2019]. A recent study in horses shows that 80% of D/D individuals are developmentally or reproductively abnormal due to which the homozygous deletion is considered as a risk factor for equine disorders of sex development and reproductive disorders [Ghosh et al., 2020]. The observed abnormalities in the reproductive phenotype of the cloned horses are consistent with this. Consequently, the somatic cell donor must also have had the D/D genotype, though we have no information about his reproductive profile or why SCNT was used for his reproduction. Further, the homozygous deletion was also present in 3 foals in Case 1, two carrying the balanced translocation t(4;30) and one the unbalanced form, thus having simultaneously 2 risk factors for reduced fertility or infertility. A possibility to follow-up with the development and reproductive performance of all offspring in Case 1 (Table 1) would provide a unique opportunity for better understanding the phenotypic effect of the ECA29 deletion in horses.

Altogether, including the 2 new cases described here, just 15 unique translocations have been reported in horses – 11 autosomal and 4 involving autosomes and the sex chromosomes (online suppl. Table 2). In contrast, hun-

dreds of distinct translocations have been discovered in humans, and the incidence of balanced translocations alone is 1 per 500 in the general population [Morin et al., 2017; Wilch and Morton, 2018]. Likewise, to date almost 200 different translocations have been described in pigs, and the incidence of reciprocal translocations is estimated 1 in 200 live births [Donaldson et al., 2019]. Translocations, particularly robertsonian type centric fusions, are also a recognized concern in cattle [De Lorenzi et al., 2012]. Thus, compared to humans, pigs, and cattle, translocation frequency in horses seems to be low, and it is not well understood what causes such differences in the predisposition to structural chromosome rearrangements between species. Though, it may be that because of the prevalence of balanced translocations (online suppl. Table 2), which usually have no phenotypic effect other than subfertility [Lear and Bailey, 2008; Lear et al., 2008; Raudsepp and Chowdhary, 2016], many remain undetected in horses where only selected individuals are used for breeding. At the same time, balanced translocations are of recognized financial concern for elite breeding animals because, as shown by Case 1 in this study and by a previous report about a Thoroughbred breeding stallion [Durkin et al., 2011], they can easily be transmitted causing similar problems and economic loss in the next generation. As a proof of principle, a third such case was recently identified by the Texas A&M Molecular Cytogenetic lab by karyotyping 2 offspring of a Thoroughbred broodmare known to carry t(2;3) [Lear et al., 2014]: the same translocation was found in one of the foals (our unpublished data).

In humans and pigs, where translocation frequency is high, research is ongoing to explore the molecular landscape of breakpoints and study the genetic consequences of different translocations. It appears that translocation breakpoints are not randomly distributed across chromosomes, but occur preferentially in regions with open chromatin (G-negative bands), higher gene density, and common fragile sites, and are demarcated by repetitive elements such as LINEs, SINEs, endogenous retroviral elements, and sequences rich in CCCTC-binding factor/cohesin binding sites [Weckselblatt and Rudd, 2015; Lin et al., 2018; Donaldson et al., 2019]. Direct sequencing or long molecule optical mapping of translocation breakpoints in humans also reveal how translocations disrupt specific genes or generate fusion genes [Lin et al., 2018; Wilch and Morton, 2018; Wang et al., 2020].

No such studies have yet been conducted in horses. However, it is intriguing that only 14 autosomes (out of 31) and the sex chromosomes have been involved in the 15 translocations known in horses (online suppl. Table 2), whereas certain chromosomes are clearly more often involved than others. These are ECA1 (5 times), ECA16 (4 times), ECA4, ECA13, and ECAX (3 times each), and ECA30 (2 times) (online suppl. Table 2). The remaining chromosomes (ECA2, 3, 5, 10, 12, 17, 21, 22, 25, and Y) have been involved once each. While this information is limited, it is at least a start for learning more about the molecular landscape and consequences of translocations in the horse. Continuing collection of clinical and cytogenetic data on equine chromosome rearrangements and securing high quality samples (DNA, tissues, cell lines) for long-read sequencing and optical mapping would be the keys for any further progress in this field.

Acknowledgments

The authors thank the owners and breeders of the horses reported in this study for allowing the collection of original and additional samples.

Statement of Ethics

Procurement of blood and skin biopsies followed the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training. These protocols were approved as AUP #2018-0342 CA at Texas A&M University, TX, USA.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

Funding Sources

The study was funded by Texas A&M Molecular Cytogenetics service.

Author Contributions

C.F.C., O.P., J.D., D.D.V., D.E.K., and C.C.L. provided the samples and clinical data. S.G., M.J.J., C.C., M.N.M., and T.R. performed cell cultures, PCR, cytogenetic and FISH analyses. R.J. conducted STR genotyping. T.R. supervised the work and provided funding. S.G. and T.R. wrote the manuscript draft. All authors participated in revisions and editing of the final version.

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