

Supplementary materials

Cytogenetic and molecular insight into the genetic background of disorders of sex development in seventeen cats, by Stachowiak et al.

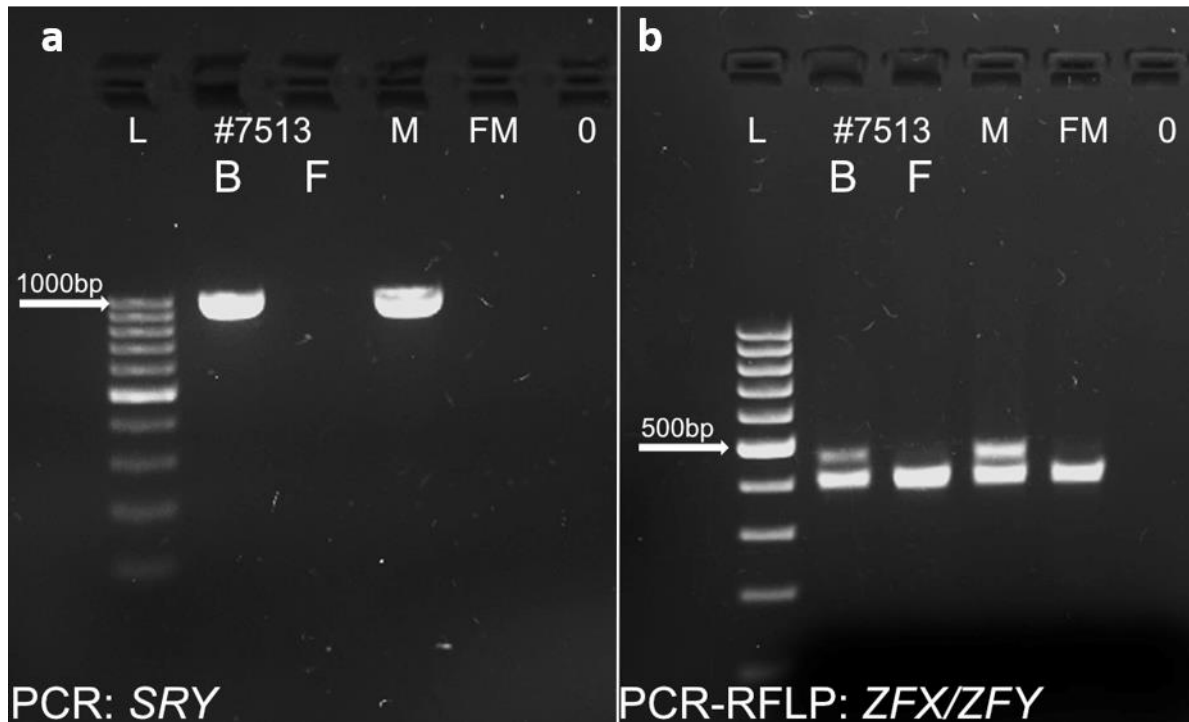


Figure S1. Detection of Y-linked genes in blood (B) and fibroblasts (F) of DSD case #7513. (a) *SRY* – 1022 bp band, and (b) *ZFY* – 448 bp band (391 bp band represents the X-linked *ZFX* gene). L – 100 – 1000 bp ladder, M – control male, FM – control female, 0 – negative control.

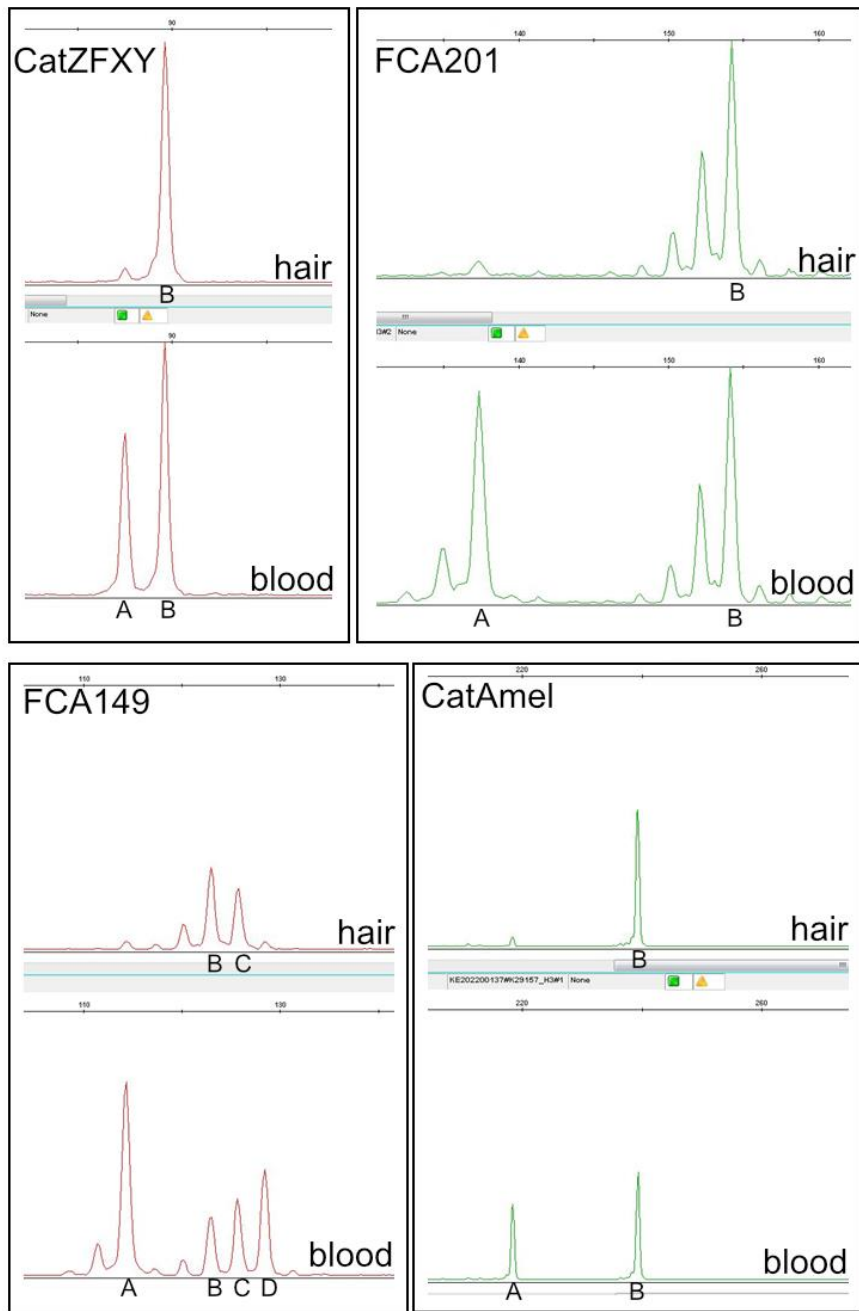


Figure S2. Analysis of STR markers, CatZFX, FCA201, FCA149, and CatAmel in DNA isolated from hair follicles and blood of DSD case #7513 showed additional alleles in DNA from blood that were not present in DNA from hair follicles, confirming the leukocyte chimerism. A,B,C,D – alleles.

Table S1. Control males analyzed in the study.

	Cat (lab ID)	Breed	SRY
1	#7361	Domestic shorthair	+
2	#7362	British	+
3	#7363	Maine coon	+
4	#7364	Domestic shorthair	+
5	#7365	Ragdoll	+
6	#7367	Domestic shorthair	+
7	#7371	British longhair	+
8	#7372	Maine coon	+
9	#7378	Domestic shorthair	+
10	#7383	Turkish angora	+
11	#7384	Turkish angora	+
12	#7387	Neva masquerade	+
13	#7388	Domestic shorthair	+
14	#7390	Domestic shorthair	+
15	#7392	British	+
16	#7393	British	+
17	#7394	Maine coon	+
18	#7395	Domestic shorthair	+
19	#7396	Domestic shorthair	+
20	#7399	Domestic shorthair	+
21	#7400	Domestic shorthair	+
22	#7405	Bengal	+
23	#7408	Maine coon	+
24	#7409	Maine coon	+
25	#7412	Domestic shorthair	+
26	#7417	Norwegian forest cat	+
27	#7418	British shorthair	+
28	#7421	Domestic shorthair	+
29	#7422	Domestic shorthair	+
30	#7424	Ragdoll	+
31	#7429	Domestic shorthair	+
32	#7432	Domestic shorthair	+
33	#7435	Domestic shorthair	+
34	#7376	Domestic shorthair	+
35	#7419	Domestic shorthair	+
36	#7427	British	+
37	#7434	Domestic shorthair	+
38	#7413	Domestic shorthair	+

Table S2. PCR primers and assays used in the study.

Gene	Primer sequence 5' → 3'	Amplicon (bp)	Annealing temperature (°C)
X inactivation status			
<i>AR</i> (exon 1)	F: atccacgttgccctgct	209	60
	R: 6-FAM-cagtggcctccctgttct		
Detection of Y-linked genes or sequencing of candidate genes			
<i>SRY</i> (coding sequence) ⁴	F: aactttgctaccaccaacc	1022	58
	R: caatctgcggaaaactggat		
<i>ZFX/ZFY</i> ²⁰	F: ataatcacatggagagccaccagct	448	58
	R: gcacttctttggtatctgagaaagt		
<i>TETY1</i> ²¹	5' cagatgtgcaggggagcac 5' aatataatgtcctcaataaacgatg	150	58
<i>TETY2</i> ²¹	5' aatgaccagcttccattg 5' gctttgggcatcagtctagc	178	58
<i>CUL4BY</i> ²¹	5' ataaaccctgccactgga 5' tctccaagctgtatttaacagaga	106	58
<i>CYORF15</i> ²¹	5' tgtggttaggtgaaaacacacc 5' tgattcctatgaccaggcttc	100	58
<i>HSFY</i> ²¹	5' tggatcagcatgccattta 5' ctggctgacacagcaggata	211	58
<i>FLJ36031</i> ²¹	5' gcctgaggagaaggtgttgt 5' gcaccttctctggctgacc	128	58
<i>TAC3</i> (exon 3)	F: tttctaacaagcccacagca	424	62
	R: attcaccaagccatctccag		
<i>CYP11B1</i> ¹⁴	F: aggctcagggaaactttgtctg	413	60
	R: gtttcgaccaggagtagag		
<i>LHCGR</i> (exon 1)	F: ggttgaggcagagcacagt	500	59
	R: gggatggcagagacagtcc		
<i>LHCGR</i> (exon 2)	F: aactccggtgtgtctgatcc	441	62

	R: ctggggtttcttgaacacaa		
<i>LHCGR</i> (exon 3)	F: gagatggcagcacttgatga	491	61
	R: gctacttggcaggactccac		
<i>LHCGR</i> (exon 4)	F: gcttcatagagtggggtgga	510	62
	R: cctggtggctgacaagaaat		
<i>LHCGR</i> (exons 5 and 6)	F: gtcccagcccctagatcttt	679	62
	R: tgggtcagttggttgtgtg		
<i>LHCGR</i> (exon 7)	F: tgaatgtgcatacgccagt	454	62
	R: agaggaggctgtgagatga		
<i>LHCGR</i> (exon 8)	F: caatccccctttccctcta	333	62
	R: cagtgccccagttagtgtt		
<i>LHCGR</i> (exon 9)	F: attaggggaaggcaagatg	448	62
	R: tttcggaagaaacaggatg		
<i>LHCGR</i> (exon 10)	F: ggagcccacacaaaactt	441	62
	R: tttgcaaacatccctcaat		
<i>LHCGR</i> (exon 11)	F: actgaggctgttggcttt	873	62
	R: gccatcagctctggatttg		
<i>LHCGR</i> (exon 11)	F: acgctattcagctggaccaa	859	62
	R: gcaatggtcatggacaacac		
<i>SRY</i> and <i>AMELX/AMELY</i> copy number (ddPCR)			
<i>SRY</i> (coding sequence)	F: ttgcacggagagtcttacct	114	58
	R: tctcagaccacacatgaa		
	Probe: FAM-tgtgaaaccagaggaaaggg-BHQ1		
<i>SRY</i> (5'-flanking region)	F: gagacttaagcgtggcggat	100	58
	R: ccgaagcccaacctaccatt		
	Probe: FAM-gcagtgagccgagcatgaacct-BQH1		
<i>F2</i> (reference gene)	F: agcgagaaccaggacttcaa	98	58
	R: ccggacacataacaccacac		
	Probe: HEX-ctggagaagaacttctgccg-BHQ1		
<i>AMELX</i>	F: tcagtcccgttttctcagtt	107	58
	R: agtcagagaggccgagcag		

	Probe: HEX-tcctggtttaagccctgatg-BHQ1		
AMELY	F: caagatgtttctcagtcctgct	90	58
	R: gaggccaaataggagtgtgc		
	Probe: FAM-gttaaagcctgcattgtccc-BHQ1		

References for Table S2:

⁴ Szczerbal, I. *et al.* X monosomy in a virilized female cat. *Reprod. Domest. Anim.* **50**, 344–348 (2015).

¹⁴ Owens, S. L. *et al.* Congenital adrenal hyperplasia associated with mutation in an 11 β -hydroxylase-like gene in a cat. *J. Vet. Intern. Med.* **26**, 1221–1226 (2012).

²⁰ Senese, C., Penedo, M. C., Shiue, Y. L., Bowling, A. T. & Millon, L. V. A HaeIII PCR-RFLP in the ZFY/ZFX genes of horses. *Anim. Genet.* **30**, 390–391 (1999).

²¹ Murphy, W. J. *et al.* Novel gene acquisition on carnivore Y chromosomes. *PLoS Genet.* **2**, e43 (2006).

Table S3. Results of the molecular analysis of *SRY* gene in XY DSD and control cats.

	Cat (lab ID)	Breed	Genotype for <i>SRY</i> c.389G>C SNP (p.Arg130Thr)	<i>SRY</i> coding sequence copy number	<i>SRY</i> 5'-flanking region copy number
XY DSD cats					
1	#6533	Persian	gc	5	5
2	#6664	Domestic shorthair	gc	5	5
3	#6680	Domestic shorthair	gc	5	5
4	#6699	Domestic shorthair	gc	6	6
5	#6721	Crossbred (Persian x Domestic shorthair)	gc	5	5
6	#6856	Domestic shorthair	gg	5	5
7	#6973	Domestic shorthair	gg	5	5
8	#7036	British shorthair	gc	5	5
9	#7145	British shorthair	gc	5	5
10	#7254	Domestic shorthair	gc	5	5
11	#7302	Domestic shorthair	gg	5	5
12	#7320	British longhair	gc	5	5
13	#7451	Domestic shorthair	gg	4	4
14	#7455	Ragdoll	gc	5	5
Control cats					
1	#7361	Domestic shorthair	gg	5	5
2	#7362	British	gc	5	5
3	#7363	Maine coon	gc	5	5
4	#7364	Domestic shorthair	gg	5	5
5	#7365	Ragdoll	gc	5	5
6	#7367	Domestic shorthair	gg	5	5
7	#7371	British longhair	gc	5	5
8	#7372	Maine coon	gc	5	5
9	#7378	Domestic shorthair	gg	5	5

10	#7383	Turkish angora	gc	5	5
11	#7384	Turkish angora	gc	5	5
12	#7387	Neva masquerade	gc	5	5
13	#7388	Domestic shorthair	gg	5	5
14	#7390	Domestic shorthair	gg	5	5
15	#7392	British	gc	5	5
16	#7393	British	gc	5	5
17	#7394	Maine coon	gc	5	5
18	#7395	Domestic shorthair	gc	6	6
19	#7396	Domestic shorthair	gg	5	5
20	#7399	Domestic shorthair	gc	5	5
21	#7400	Domestic shorthair	gg	5	5
22	#7405	Bengal	gc	5	5
23	#7408	Maine coon	gc	5	5
24	#7409	Maine coon	gc	5	5
25	#7412	Domestic shorthair	gc	5	5
26	#7417	Norwegian forest cat	gg	5	5
27	#7418	British shorthair	gc	5	5
28	#7421	Domestic shorthair	gc	5	5
29	#7422	Domestic shorthair	gc	5	5
30	#7424	Ragdoll	gc	5	5
31	#7429	Domestic shorthair	gg	5	5
32	#7432	Domestic shorthair	gg	5	5
33	#7435	Domestic shorthair	gg	5	5
34	#7376	Domestic shorthair	gg	5	5
35	#7419	Domestic shorthair	gg	5	5
36	#7427	British	gc	5	5
37	#7434	Domestic shorthair	gg	5	5
38	#7413	Domestic shorthair	gg	5	5

Table S4. Genotypes for polymorphisms in *LHCGR* gene analyzed in XY DSD versus control cats.

	Cat (lab ID)	g.70455430C>G (5'-flanking, - 23bp upstream to TSS)	g.70455376delC (5'UTR, exon 1)	g.70455334C>T p.Ala7Thr (exon 1)	g.70400960C>T p.Ala690Thr (exon 11)
XY DSD cats					
1	#6533	CC	ins/ins	CC	CC
2	#6664	CC	ins/ins	CC	CT
3	#6680	CG	ins/del	CC	CT
4	#6699	CG	ins/del	CC	CC
5	#6721	CC	ins/del	CC	CC
6	#6856	CC	ins/ins	CC	CC
7	#6973	CC	ins/ins	CC	CC
8	#7036	CC	ins/ins	CC	CC
9	#7145	CC	ins/ins	CC	CC
10	#7254	CC	ins/ins	CC	CC
11	#7302	GG	del/del	CC	CC
12	#7320	CC	ins/ins	CC	CC
13	#7451	CG	ins/del	CC	CC
14	#7455	CC	del/del	CC	CC
Control cats					
1	#7361	CC	ins/ins	CC	CC
2	#7362	CC	ins/ins	CC	CC
3	#7363	CC	ins/ins	CC	CC
4	#7364	CC	ins/ins	CC	CT
5	#7365	CC	ins/ins	CC	CC
6	#7367	CG	ins/del	CC	CC
7	#7371	CC	ins/ins	CC	CC
8	#7372	CG	ins/del	CC	CC
9	#7378	CC	ins/ins	CC	CC
10	#7383	CC	ins/ins	CC	CC
11	#7384	CC	ins/ins	CC	CT
12	#7387	CC	ins/ins	CC	CC
13	#7388	CC	ins/del	CC	CT
14	#7390	CG	ins/del	CC	CC
15	#7392	CC	ins/ins	CC	CC
16	#7393	CC	ins/ins	CC	CC
17	#7394	CC	ins/ins	CC	CC
18	#7395	CC	ins/ins	CC	CT
19	#7396	CC	ins/ins	CC	CC
20	#7399	CG	ins/del	CC	CC
21	#7400	CC	ins/ins	CC	TT
22	#7405	CC	ins/ins	CC	CT
23	#7408	CC	ins/ins	CC	CT
24	#7409	CC	ins/ins	CC	CT

25	#7412	CC	ins/ins	CC	CT
26	#7417	CC	ins/ins	CC	CC
27	#7418	CC	ins/ins	CC	CT
28	#7421	CC	ins/ins	CC	CC
29	#7422	CG	ins/del	CC	CC
30	#7424	CC	ins/del	CC	CC
31	#7429	CC	ins/ins	CC	CC
32	#7432	CC	ins/ins	CC	CC
33	#7435	CG	ins/del	CC	CC
34	#7376	CC	ins/ins	CC	CC
35	#7419	CC	ins/ins	CT	CT
36	#7427	CC	ins/ins	CC	CC
37	#7434	CG	ins/del	CC	CC
38	#7413	CC	ins/ins	CC	CT