Cytogenomic characterization of mosaic X-ring chromosomes in seventeen patients with Turner syndrome (TS)-42 years of experience at a single-site institution

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Supplementary data

Supplementary tables

Case	Short Stature	Cognitive Impairment/ Developmental Delay	High Arched Palate	Hearing Loss	Low Posterior Hairline	Widely spaced nipples	Neck Webbing	Seizures	Autoimmune disorders	Bicuspid Aortic Valve	Aortic Coarctation
RCX-1	X		X		X						
RCX-2	X			X	X	X	X	X			X
RCX-3	X		X	X					X	X	X
RCX-4		X									
RCX-5	X	X	X		X			X			
RCX-6	X	X			-		-				
RCX-7	X	X		-	X		X	X			
RCX-8	X	X	X	-				X		X	
RCX-9	X		X				-				
RCX-10	X										
RCX-11	X	X	X						X		
RCX-12	X		-	X		X	X		X	X	
RCX-13						X	-				
RCX-14	X	X	X	X		X	X				
RCX-15	X		-	-	X	X	-				
RCX-16	X			X							
RCX-17	X	X		-					X		

Supplementary Table 1. Selected clinical features were identified by comparing our cohort with mosaic RCX and selected RCX literature.

Abbreviations: SZ; seizures, DD, developmental delay, CI; cognitive impairment, +; present, -; absent.

Subject	Specimen type	Mosaicism total cells studied as %	Fraction	Secondary rings	Ring X size	Ring X size category	*XIST* status
RCX-1	P, Sk	1	Mi		U		U
RCX-2	Px2	1.48148	Mi		U		U
RCX-3	P	50	Se		U		U
RCX-4	P	50	Se		44.6 Mb	I	+
RCX-5	Px2	26.04	Mod	Fr	U		U
RCX-6	Px2	9	Mi		U		U
RCX-7	P	30	Mod		U		U
RCX-8	P	35	Se	SR	U		-
RCX-9	P	60	VS		52 Mb	I	+
RCX-10	P	10	Mod		U		+
RCX-11	P	50	Se		U		U
RCX-12	P	50	Se	SR	13.5 Mb	S	+
RCX-13	P	26.66	Mod		21.6 Mb	S	+
RCX-14	P	36.36	Se		19.9 Mb	S	+
RCX-15	P	48.48	Se		104 Mb	L	+
RCX-16	Px2	30	Mod	M	U		U
RCX-17	Px2	45.0	Se		105.4 Mb	L	+

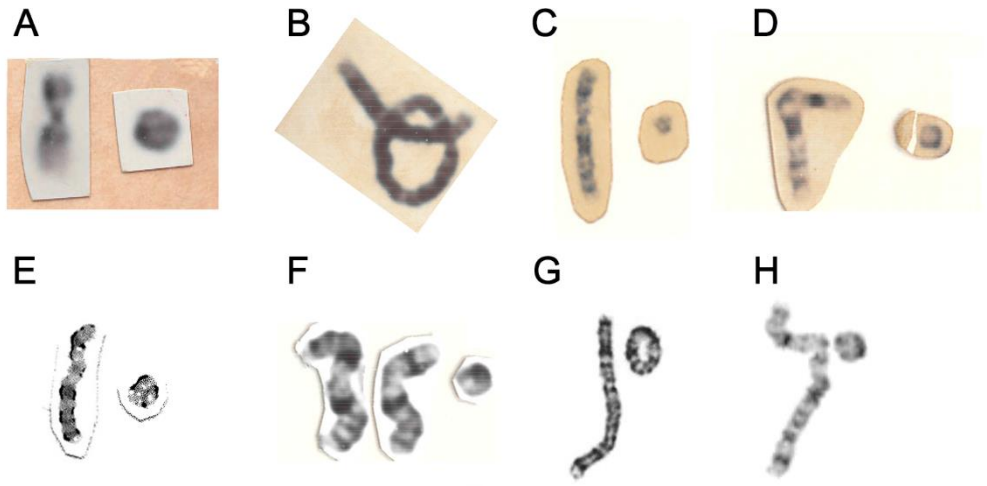
Supplementary Table 2. Cytogenetic and laboratory findings for this cohort with mosaic RCX.

Abbreviations: P; peripheral blood, Sk; skin, S; small, I; intermediate, L; large, U; unknown, +; present, -; absent, Mb; megabase, Fr; Fragments, SR; secondary rings; M; marker, VS; very severe, Se; severe, Mod; moderate, Mi; Mild; Levels were defined as mild <10%, moderate 10-30%, severe 30-50%, or very severe >50% based on recommendations from Martinez-Glez *et al*. [1].

Subject	International Standards for Human Cytogenomic Nomenclature
RCX-1	PBL: 45,X[50] Skin: 45,X[49]/46,X,r(X)[1/50] Research SNP array (fibroblasts): arr(X)x1
RCX-2	Initial study: 45,X[20] Subsequent study 1: 45,X[63]/?, ring?[2] Subsequent study 2: 45,X[37]/46,XX[13]
RCX-3	46,X,ring[15]/45,X[15]
RCX-4	46,X,r(X)[15]/45,X[15].ish DXZ1x2 Research SNP array: arr[GRCh37] Xp22.33p21.2(60,814_30,062,591)x1,Xp21.2q13.3(30,095,893-74,737,869)x1~2,Xq13.3q28(74,740,379_155,236,744)x1
RCX-5	Initial study: 45,X[10]/45,idem,+frag[13] Subsequent study 1: 45,X[25]/46,X,r(X)[25].ish X cenx1[50]/X cenx2[50] Subsequent study 2: arr Xp22.33q28(814-154,908,075)x1 (HumanQuad610; hg18) Research array: arr(X)x1 (850k v1.2; hg19)
RCX-6	Initial study: 45,X[17]/46,X,r(?X)[3] Subsequent study: 45,X[44]/46,X,r(?X)[6]
RCX-7	45,X[14]/46,X,r(X)(p?q?)[6].ish DXZ1x2
RCX-8	46,X,i(Xq)[13]/47,X,i(Xq),+r(X?p)[7].nuc ish (X cenx2)[68/100]/(X cenx3][30/100]
RCX-9	45,X[20]/46,X,r(X)(p22.13q13.3)[30].ish Xp21.2p21.3(DMDx2),Xq13.2(XISTx2) Research SNP array: arr[GRCh37] Xp22.33p22.11(60,814_21,901,059)x1,Xp22.11q13.3(21,901,903_73,569,476)x1~2,Xq13.3q28(74,074,277_155,236,747)x1
RCX-10	45,X[45]/46,X,r(X)(p?11.23q22.3)[5].ish r(X)(DMD-,XIST+),22q11.2(HIRAx2) Research array: arr(X)x1 (850k v1.3; hg19)
RCX-11	45,X[25]/46,X,r(X)(p?22.1q?21.3)[25]
RCX-12	45,X[9]/46,X,+r1[15]/46,X,+r2[6]. ish r(X)(DXZ1+,SRY-)/r(X)(DXZ1++,SRY-).nuc ish (X cenx2)[323/337]/(X cenx3][14/337] Research SNP array:arr[GRCh37] Xp22.33p11.1(60,814_58,483,247)x1,Xq11.1q13.3(61,719,290_75,249,257)x1~2,Xq13.3q28(75,265,698_155,236,747)x1
RCX-13	45,X[22]/46,X,r(X)(p11.22q13.3)[8].arr[hg19] Xp22.33p11.22(60,814-53,032,280)x1,Xp11.22q13.3(53,056,518-74,609,263)x1~2,Xq13.3q28(74,610,826-155,254,881)x1
RCX-14	45,X[33]/46,X,r(X)(p11.22q13.3)[12]. arr[hg19] Xp22.33p11.22(60,814-54,732,159)x1,Xp11.22q13.3(54,737,365-74,659,064)x1~2,Xq13.3q28(74,661,776-149,822,608)x1,Xq28(149,826,503-150,493,345)x2,Xq28(150,497,850-155,236,712)x1
RCX-15	45,X[17]/46,X,r(X)(p22.33q?22.3)[16] Research SNP array: arr[GRCh37] Xp22.33p22.2(60,814_11,424,850)x1,Xp22.2q23(11,433,808_115,478,048)x1~2,Xq23q28(115,478,048_155,236,747)x1
RCX-16	Initial study: 45,X[18]/45,X, X,+mar[2] Subsequent study: 45,X[29]/46,X,r(?X)(p?q?)[21]
RCX-17	Initial study: 45,X[12]/46,X,r(X)[8] Subsequent study: 45,X[16]/46,X,r(X)(p11.4q27.2)[15].ish r(X)(DXZ1+)[17/41].arr[GRCh37] Xp22.33p11.4(60,814_37,619,114)x1,Xp11.4q27.2(37,625,334_143,036,520)x1~2,Xq27.2q28(143,049,500_155,236,747)x1

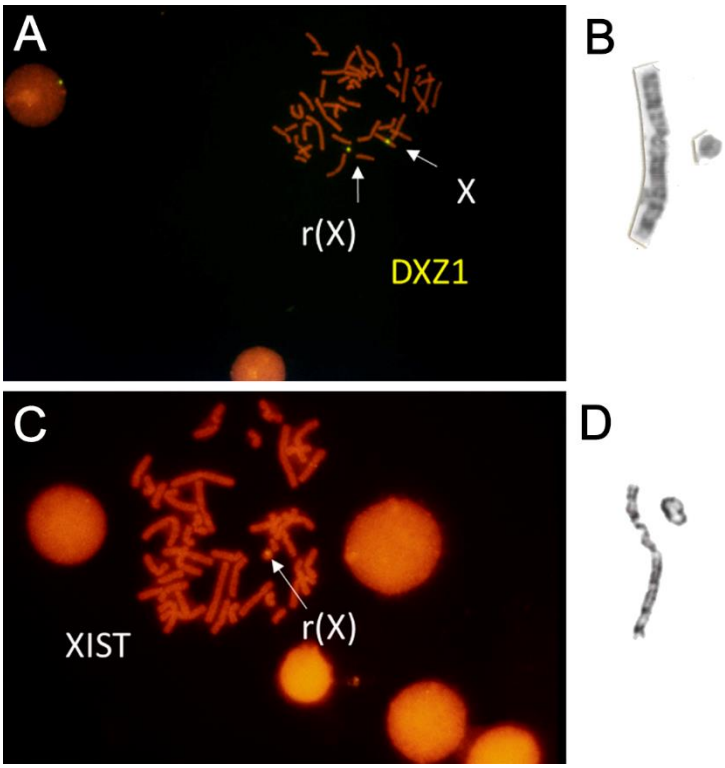
Supplementary Table 3. Cytogenetic nomenclature for this cohort with mosaic RCX.

Supplementary figures



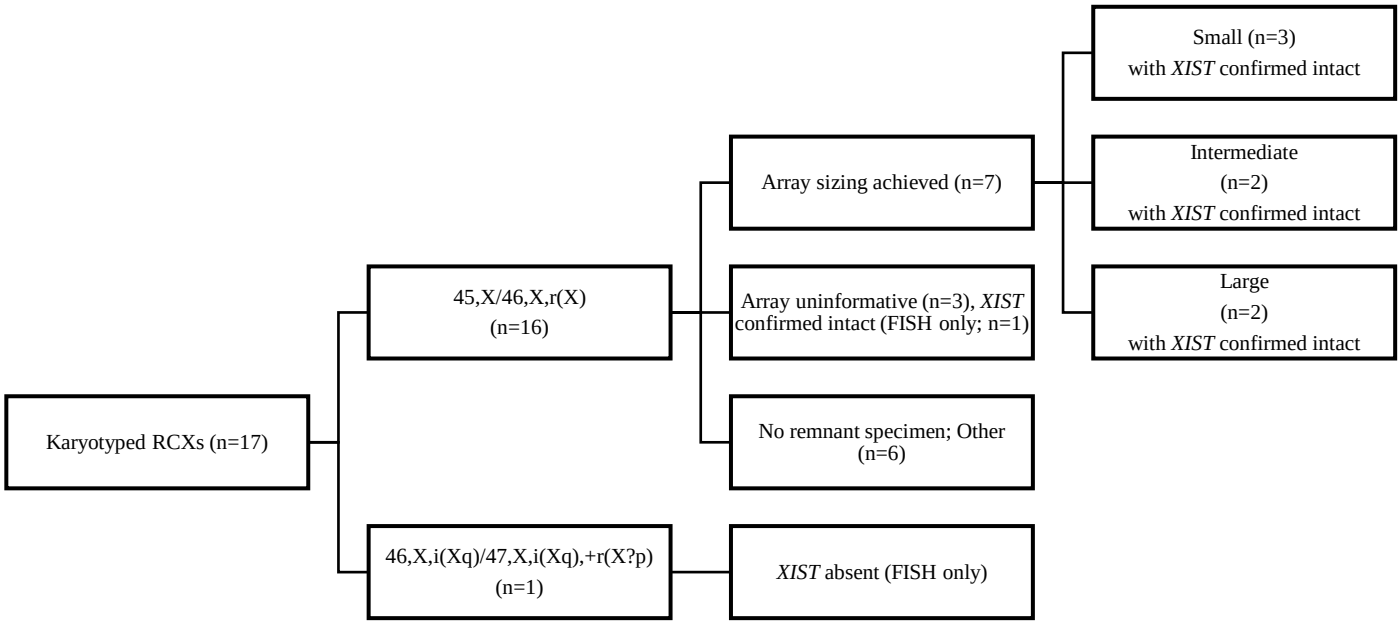
Supplemental Figure 1. Available partial G-banded karyotype images for eight subjects.

(A-H) Representative partial karyotypes of RCX-1 (A), RCX-2 (B), RCX-3 (C), RCX-5 (D), RCX-6 (E), RCX-8 (F), RCX-11 (G), and RCX-16 (H). Where available, the normal homolog X is depicted on the left, and the RCX is depicted on the right. While the ring sizes could not be definitively determined by karyotype alone, the level of mosaicism demonstrated a preponderance of monosomy X for RCX-1, RCX, 2, and RCX-6. Equal monosomy X and RCX numbers were identified for RCX-3, RCX-5, RCX-11, and RCX-16. RCX-8 displayed an unusual karyotype of an isochromosome X (on the left) with a secondary RCX (on the right). The original FISH images or images from genomic DNA methods predating FISH are unavailable for RCX-3, RCX-5, or RCX-8.



Supplemental Figure 2. Available images for the two subjects whose rings were clarified by FISH as X-chromosomal origin.

(A, B) Representative metaphase FISH images of RCX-7 (top) and RCX-10 (bottom), where FISH confirmed that the rings were X chromosomal in origin. The FISH probe DXZ1 demonstrated that the RC is X chromosomal in RCX-7 and that *XIST* is intact in RCX-10. The representative partial G-banded karyotypes of RCX-7 (C) and RCX-10 (D) are depicted on the right. The normal homolog X is on the left, and the RCX is on the right.



Supplemental Figure 3. Summary of subjects evaluated as part of this study and significant laboratory findings.

Case-level summaries

RCX-1

A ten-year-old White female, the second child born to her parents, presented at Pediatric Endocrinology to rule out TS. The subject's features included a high-arched palate, a low-set hairline, and a short fourth metacarpal. A family history of short stature was noted.

G-banded and Q-banded chromosomes were studied from peripheral blood. A fifty-cell study identified monosomy X with a modal number of 45 chromosomes. She had a sonogram with normal ovaries. Therefore, a subsequent study analyzed the findings in skin fibroblasts. A fifty-cell study was performed. One clone had 46 chromosomes per cell, minus a C-group chromosome, but with a large ring. QFG banding revealed the rare ring as an X chromosome. We combined all karyotype studies and estimated that the ring existed at 1% (mild). At that time, an approximation of breakpoints could not be determined. However, the ring appeared to be the same size as the normal X chromosome. A buccal skin study was attempted but not reported. This patient was initially reported in 1983 by this laboratory[2].

A follow-up research SNP array (Illumina 850k v1.3) was performed 42 years later on cultured skin fibroblasts, in which only monosomy X was identified, consistent with the original karyotypes, revealing that this line was more prevalent than the RCX. There was no additional information for this patient.

RCX-2

An 11-day-old White female child presented to JHH Pediatric Genetics for banding studies to rule out a chromosome 15 abnormality. Her clinical features included short stature, brachycephaly, webbed neck, small maxilla, low-set hairline, widely-spaced nipples, malformed ears, a submucosal cleft, cerebral

palsy, suspected Crouzon syndrome and concern for a chromosomal anomaly. She also displayed an absent corpus callosum and had a VP shunt.

G-banded karyotype studies performed on peripheral blood were performed twice in early life to obtain better banding. The initial study identified monosomy X in a modal number of 45 in the 20 cells examined. Analysis of 15 cells revealed a missing C-group chromosome. QFG- and RBA-banding studies identified a single X chromosome per cell. The follow-up study revealed a predominant finding of monosomy X with a modal number of 45, with a rare subset demonstrating two incomplete cells out of a total of 67 bearing a ring whose origin could not be definitively identified. However, it was speculated to be of X chromosome material, raising the possibility of mosaicism.

Fifteen years later, a 550 band-level karyotype from peripheral blood was repeated, identifying monosomy X in 37 cells with a modal number of 45 and a normal female chromosomal complement in the remaining 13 cells analyzed. Concerning all the cells studied combined, we estimated the level of mosaicism to be 1.48% (mild). At this time, internal laboratory notes indicated that the patient was developing sexually typically. Given a normal chromosomal complement, we hypothesized that a normal line might have existed initially in the early zygote and is now evolving.

She was the second child born to her family. The last available clinical note indicates that the patient sought care until 25 years of age and experienced TS and multiple medical problems: craniosynostosis, coarctation of the aorta with a left subclavian flap, and aortoplasty, status post repair. She was noted to have papilledema and 30% hearing loss in the right ear. She had a malrotation of the kidneys with renal and bladder dysfunction. Her height was 1.48 meters; her last available weight was 51.9 kg, and her last available BMI was 23.7. She underwent facial reconstruction. To our knowledge, a molecular diagnosis of Crouzon syndrome has yet to be confirmed. In addition to her seizures, she also had evidence of strokes (x2) with evidence of a small infarct in the thalamus. She experienced hypertension and ictal headaches and had experienced a possible cardiac arrest.

RCX-3

A 45-day-old White female child presented to JHH Pediatric Cardiology with clinical findings of short stature, coarctation of the aorta, and a small high-arched palate with concern for TS and potentially a second diagnosis.

A G-banded karyotype was performed on peripheral blood, identifying two cell lines in equal proportions in the thirty cells examined. The first line lost the X chromosome in a karyotype with a modal number of 45 chromosomes, and the second contained a small ring with a modal number of 46 whose origin could not be identified in the remaining 15 cells. Q-banding did not reveal any fluorescent material. The level of mosaicism was 50% (severe). Genomic DNA analysis studies using probes specific for the centromeres of the sex chromosomes were performed on a research basis for herself, her mother, and her father. These studies revealed that the ring chromosome was of X chromosomal material. Images from the original genomic DNA study are not available.

A last available clinical note indicated that the patient sought care at 34 years of age, and she displayed positive TS features, including hypothyroidism treated with methimazole and Hashimoto's thyroiditis. Her thyroid autoimmunity status was positive for TPO antibodies. While she underwent a recent cardiac MRI, there was potential mild localized narrowing at the coarctation repair site with no evidence of aortic root dilation. There was a possible heart murmur and a bicuspid aortic valve. The coarctation of the aorta was repaired in early life, followed by resection and end-to-end anastomosis cardiac catheterization. Re-coarctation of the aorta was noted with severe narrowing and a mild gradient, with an occluded right femoral artery and a very stenotic left femoral artery.

She was in the twelfth grade and was considering future careers. Her LFTS were normal (ALT/AST). Her bone density was normal. She was also noted to have long QT syndrome. Her last available height was 1.581 meters, her weight was 60.3 kg, and her BMI was 23.

While she had PE tubes, hearing aids with a history of tympanoplasty, and cardiac monitoring, she was otherwise healthy. She underwent normal sexual (breast) development, with moderate axillary hair, adult pubic hair, and stage B5 PH5. She achieved menarche at 11 years of age. The LH/FSH levels were 12.7 and 54.4, respectively. She received estrogen replacement at sixteen to facilitate breast development. Her thyroid hormone level labs were normal, indicating her Synthroid dose was satisfactory. She did receive growth hormones until she reached age 15. The patient indicated that she was never sexually active. She elected to use OCPs for regular monthly periods. A last available note indicates she is still receiving care at age 34.

RCX-4

A one-year-old White female with features of failure to thrive, thin, low-set ears, and unusually spaced mandibles presented to Hopkins. She was noted to have cognitive impairment. Her family history indicated some level of cognitive impairment for her mother. The last follow-up was at two years of age. Her bone age was low.

Her G-banded chromosome analysis study of whole blood identified two lines among the 30 cells examined. 50% of her cells had monosomy X with a modal number of 45 chromosomes, and 50% showed an RCX with a modal number of 46 chromosomes (severe).

Metaphase FISH studies using an X centromere-specific probe (DXZ1) revealed two signals, indicating that the ring was formed from chromosome X material. The approximation of breakpoints could not be performed at that time. However, a substantial amount of X material was missing from the ring. Historical FISH images are not available.

Ten years later, a research SNP array (Illumina 850k v1.2) was performed on a remnant specimen, which was identified as: arr[GRCh37] Xp22.33p21.2(60,814_30,062,591)x1,Xp21.2q13.3(30,095,893-

74,737,869)x1~2,Xq13.3q28(74,740,379_155,236,744)x1. The first terminal deletion on the short arm was from band p22.33 to p21.2 from nucleotide 60,814 to 30,062,591 with a minimum size of 30 Mb. This region covers pseudoautosomal region 1 (PAR1), which contains the *SHOX* gene and several non-coding regulatory elements. Pathogenic variants in *SHOX* are associated with idiopathic growth retardation, short stature, and Leri-Weill dyschondrosteosis (OMIM *312865). The second terminal deletion on the long arm was from band Xq13.3 to Xq28 from nucleotides 74,740,379 to 155,236,744 with a minimum size of 80.5 Mb. The SNP array did not indicate mosaicism for these regions.

The interstitial mosaic deletion (RCX) region includes part of the short and long arms. It covers the centromeric region of chromosome X. The SNP array identified this region as starting from band Xp21.2 to Xq13.3 from nucleotides 30,095,893 to 74,737,869 with an approximate size of 44.6 Mb (including the centromere)(intermediate). Many genes are located within this region (n=359), including *XIST* (GeneScout). This gene is associated with several genetic elements essential for the inactivation of an X chromosome in females (OMIM *314670). The presence of the *XIST* locus in the ring implies it can be inactivated. The B-allele frequency indicated the presence of mosaicism in this region. No X-inactivation studies were performed clinically. There is no additional information available for this patient.

RCX-5

An eight-year-old White female presented to the Hopkins Endocrine Clinic to rule out TS (mosaic X or Y) following results from a prior study performed at an outside laboratory in which the patient was mosaic for TS: 10 out of 23 cells had monosomy X, and 13 out of 23 cells had monosomy X with a possible secondary fragment that was not further characterized in a modal number of 45 chromosomes. She presented with concern about dysmorphic features and developmental delay. She displayed short

stature, cognitive impairment, behavioral differences, a low-set hairline, and a high-arched palate. She also had respiratory concerns.

We performed chromosome studies (G banding and Q banding) on peripheral blood. Of the 50 cells analyzed, 50% of the cells lost the X chromosome, and 50% presumably had RCX in a modal number of 46 chromosomes. A single cell showed a seemingly male complement, which might have been due to contamination. Combining cells from all karyotype studies, the level of mosaicism was estimated to be 26.04% (moderate). We performed genomic DNA analysis on a research basis, revealing the absence of Y-centromeric DNA in the proband. Four years later, metaphase FISH studies using probes specific for the centromeres of the sex chromosomes were performed (DXZ1, DYZ1/DYZ3) (Vysis, Inc.). These studies revealed that the ring chromosome was of X chromosomal material. 50% of the nuclei had one hybridization signal, and 50% had two. Historical FISH and genomic DNA analysis images are not available.

When the subject was 31 years of age, an SNP array was performed on a lymphoblast cell line (Illumina, Inc. USA) using a HumanQuad610 BeadChip identified monosomy X: arr Xp22.33q28(814-154,908,075)x1 using the genome build hg18. A follow-up research SNP array (Illumina 850k v1.2) again identified monosomy with breakpoints at Xp22.33q28 at genomic coordinates 60,814-155,236,747 (hg19), suggesting that the levels of RCX were below the level of detection by the SNP array.

The last available note indicates that care was received at 33 years of age for low bone mineral density, a seizure disorder, and TS. She had a history of significant cognitive impairment, respiratory problems, dysphagia, and oropharyngeal dysfunction. She was born from a second normal pregnancy, full-term, spontaneous vaginal delivery. She experienced early life failure to thrive and seizures at two years of age. She lacked a history of cardiovascular abnormalities. Her last available height was 1.398 meters, her weight was 47.1 kg, and her BMI was 24.1. She experienced growth deficiency and was treated with growth hormone, but treatment was discontinued due to scoliosis. She displayed TSII breast development and pubic hair. Other documents indicate TSIII development. She indicated that she had

never been sexually active. She achieved menarche at 18 on Premarin and ProVera. The LH/FSH levels were 15.6 and 39.6, respectively. She was administered an estrogen replacement (Premarin) and was treated for hypogonadism (OCPs-Loestrin). Her LFTs were normal. She was noted to have low bone density. Other features included dysphagia, oropharyngeal dysfunction, sleep apnea, awake apneic spells, convulsions, GERD, and scoliosis/kyphosis/lordosis. She had OCD and displayed bruxism, yelling, and lip-smacking.

RCX-6

A fourteen-year-old Black female presented to the Pediatric Genetics Clinic to rule out mosaic monosomy X and RCX. A previous chromosome study was performed elsewhere and reported that 17 of the 20 cells had monosomy X, and the remaining three had a ring chromosome. She was noted to have short stature, cognitive impairment, cubitus valgus, hyperconvex nails, and clinodactyly. G-banded cytogenetic studies performed on peripheral blood identified a preponderance of monosomy X in 44 metaphases, and six cells presumably had an RCX in a modal number of 46 chromosomes. The level of mosaicism was estimated to be 9% (mild).

At seven years of age, she was the shortest in her class and was not receiving growth hormone therapy but did well in school. A physical examination revealed her weight and head circumferences at the 75th percentile. She was typically presenting and had a typical neck and hairline. There was no noted cardiac history. She was at Tanner stage II and presented axillary hair and no signs of breast development. She had a slight cubitus valgus, hyperconvex nails, and mild fifth finger clinodactyly that was bilateral. A last available clinical note indicates that she received care at 40. She achieved menarche in her 20s with the use of oral contraceptive pills. She received estrogen replacement. A cardiac MRI performed earlier in life indicated a normal thoracic aorta without coarctation, dissection, or aneurysm. Imaging revealed a

very small uterus and two fallopian tubes. Her last available height was 1.372 meters, her weight was 66.7 kg, and her BMI was 35.44.

RCX-7

A twenty-year-old Black female presented to the Adult Endocrinology Clinic. She displayed short stature, cognitive impairment, seizures, and mobility issues and was non-verbal. She also had a webbed neck and a low-set hairline. G-banded chromosome analysis performed on peripheral blood revealed the presence of two cell lines. The first showed a preponderance of monosomy X in 14 cells examined, and the remaining six cells had an RCX in a modal number of 46 chromosomes. We could not yet approximate breakpoints, as the ring was too small to assess accurately. The level of mosaicism estimated was 30% (moderate).

Using probes complementary to the centromeres of chromosomes X and Y, metaphase FISH identified that the ring chromosome contained the centromere of X (Oncor DXZ1 and DYZ1/DYZ3). A last available note indicated that she received care at 49 years old for a stroke, experienced decline due to multiple comorbid conditions, and was receiving palliative care. A pelvic ultrasound earlier in life indicated a small uterus, and no ovaries were identified. Later in life, her right ovary was not visualized, while her left was small and lacked follicles. Her height was 1.448 meters, weight was 50.9 kg, and BMI was 24.29. She underwent breast development but did not experience menarche. She lacked cardiovascular anomalies or hearing loss. She had type II diabetes. She had hypothyroidism and hypoglycemia. Her development of secondary sexual characteristics was noted at TS III/TS IV, with pubic hair TS V. She was not sexually active and achieved menarche at 13 years of age. Her follicle-stimulating hormone levels were 35.6. She received estrogen replacement (Premarin). She had elevated alkaline phosphatase levels and displayed low bone density. She was receiving palliative care. Other comorbidities included stroke, worsening mental status, coronary heart disease, congestive heart failure,

ischemic cardiomyopathy, and gout. She had left hemiplegia and hemiparesis following a cerebrovascular accident. She also had nerve palsy thrombocytosis and suffered from an acute kidney injury, cavernoma, microalbuminuria, heart failure, and hypertension.

RCX-8

A two-year-old White female presented to JHH Genetics with a severe r(Xp) phenotype. She had short stature, developmental delay, seizures, mobility issues, was non-verbal, and dysmorphic features. She had lymphedema and a high-arched palate. G-banded karyotyping was performed at a 400 band level and confirmed the presence of two cell lines. The first cell line showed an isochromosome composed of Xq in a modal number of 46 chromosomes present in 14 cells examined. The remaining seven cells had the same isochromosome, and an additional ring was formed from the Xp material. The level of mosaicism in this study was estimated to be 35% (severe).

Interphase FISH using a probe (DXZ1) hybridized to the centromeres of the X chromosomes, and 68% of the nuclei had two X signals, and 30% had three signals of X (Oncor). We used whole-chromosome paint FISH probes on a research basis (cosmid). Of the 50 cells analyzed, 50% had 46 chromosomes with a normal X chromosome and an isochromosome X. The remaining 50% had 47 chromosomes with a normal X chromosome, an isochromosome X, and an additional RCX chromosome. The isochromosome appeared monocentric. These results indicate the presence of small markers that seemed to be rings with a modal number of 46 chromosomes. After testing for *XIST* via cosmid, all 15 cells examined had three *XIST* signals, including those with the RCX chromosome. We observed two signals on the isochromosome and one on the normal X chromosome. No *XIST* signal was observed on the ring chromosome in 7 of the 15 cells studied. As the small ring lacked *XIST*, this abnormality was sufficient for her severe phenotype. FISH studies determined that one ring was likely monocentric and the larger was likely dicentric based on probes using SRY/CEPX. Chromosome and X-painting FISH studies

were carried out on both of her parents, and the results indicated that the ring rose *de novo*. Historical FISH images are not available.

The last available clinical note indicates that the patient was seen at 18 years of age and experienced seizures, developmental delay, type 1 membranoproliferative glomerulonephritis, and associated

hypertension. She had a height of 1.248 meters, was 48 kg in weight, and had a BMI of 30.8. She had a bicuspid aortic valve, ophthalmologic features, and hypothyroidism without hearing loss. She displayed precocious development of secondary sexual characteristics. An ultrasound at age one indicated that her uterus was within normal limits and that both ovaries contained follicles. She lacked bone density concerns. She also had scoliosis, hypotonia, and chronic kidney disease and was wheelchair-bound.

RCX-9

A seven-year-old White female presented at the JHH Endocrine Clinic. She presented with short stature, a high-arched palate, and type II diabetes. She had an FSH of 7.1, E2 <20, and an LH <0.2 at nine years. G-banded karyotype studies performed in blood identified similar levels for the two cell lines. The first line was monosomic for the X chromosome and was observed in 20 out of the 50 cells analyzed. The remaining 30 cells displayed an RCX with breakpoints at Xp22.12 and Xq13.3, representing 60% mosaicism (very severe). Metaphase FISH studies (Oncor) revealed that the *DMD* and *XIST* loci were intact with an expected copy number of 2, indicating that terminal losses were distal to Xp22.12 and Xq13.3.

Seventeen years later, a research SNP array (Illumina 850k v1.3) was performed on the remaining specimen which was identified as: arr[GRCh37] Xp22.33p22.11(60,814_21,901,059)x1,Xp22.11q13.3 (21,901,903_73,569,476)x1~2,Xq13.3q28(74,074,277_155,236,747)x1. The first terminal deletion on the short arm was from band Xp22.33 to Xp22.11 from nucleotides 60,814 to 21,901,059 with a minimum

size of 21.8 Mb. This region covers pseudoautosomal region 1 (PAR1), which contains the *SHOX* gene and several non-coding regulatory elements. Pathogenic variants in *SHOX* are associated with idiopathic growth retardation, short stature, and Leri-Weill dyschondrosteosis (OMIM *312865). The second terminal deletion on the long arm is from band Xq13.3 to Xq28 from nucleotides 74,074,277 to 155,236,747 with a minimum size of 81.2 Mb. The SNP array did not indicate mosaicism for these regions.

The interstitial mosaic deletion included parts of the short and long arms and covers the centromeric region of chromosome X. The SNP array identified this region as starting from band Xp22.11 to Xq13.3 from nucleotides 21,901,903 to 73,569,476 with an approximate size of 51.7 Mb (including the centromere) (intermediate). At least 389 genes were in this region, including XIST (GeneScout). The presence of the *XIST* locus in the ring implies it can be inactivated. The B-allele frequency indicated the presence of mosaicism for this region.

A last available note indicates that she received care at 18 years of age and was in the 12th grade with educational aspirations for her future. Her height was 1.481 meters, weight was 81.6 kg, and BMI was 37.2. She had adult breasts, stage B5 PH5. She received estrogen replacement (Estradiol and Alesse). She received OCPs for her hypogonadism. Additionally, she experienced depression and anxiety.

RCX-10

A twenty-eight-year-old Black female presented to Hopkins Genetics Clinic for testing to rule out chromosome 22 abnormalities. She presented with short stature. We performed conventional G-banded chromosome analysis on peripheral blood. This study identified mosaicism for two cell lines. The predominant line displayed monosomy X with a modal number of 45 chromosomes (90%), while the remaining five cells displayed an RCX with a normal number of chromosomes or 10% mosaicism (moderate). The approximate breakpoints identified cytobands Xp11.23 and Xq22.3.

Metaphase FISH demonstrated loss of DMD and the presence of *XIST* (Oncor); we identified a normal copy number for chromosome 22 (HIRAx2)(Vysis, Inc.). These studies indicated monosomy for Xp distal to Xp11.23 and Xq distal to Xq22.3.

A last available note indicates that she received care at 42 years of age and had hypertension. A follow-up research SNP array (Illumina 850k v1.3) was performed on the remnant specimen, which identified only the monosomy X, consistent with the original karyotypes, revealing a preponderance for this line over the RCX. She received estrogen replacement treatment, and she experienced low bone density and hypertension. Her last available height was 1.651 meters, her weight was 71.2 kg, and her BMI was 26.1. Additionally, she experienced myocardial infarction, a psychotic episode, and an altered mental status.

RCX-11

A fourteen-year-old White female presented to Hopkins Endocrinology for a diagnosis of TS. She experienced growth deficiency, short stature, and developmental delay. She also had a high-arched palate and hyperconvex nails. It was reported that while the patient had prior cytogenetic studies performed elsewhere, her parents desired a repeat study.

The G-banded karyotype performed on peripheral blood identified two cell lines at equal levels. The first was monosomy X in a modal number of 45 chromosomes observed in 25 cells, and the second line contained an RCX with approximate breakpoints at cytobands Xp22.1 and Xq21.3 observed in the remaining 25 cells, or 50% mosaicism (severe). These findings suggest a terminal loss of Xp and Xq, respectively. The results confirmed the previous cytogenetic results.

The last available note indicates that the patient received care at 31 years. Her height was 1.448 meters, weight was 68 kg, and BMI was 32.4. She experienced ophthalmologic features and achieved menarche at 11 years. She also had celiac disease, schizophrenia, psychosis, and Hashimoto’s disease.

RCX-12

A two-day-old White female child from an outside hospital presented with concerns about hypoglycemia and TS. G-banded chromosome analysis of peripheral blood revealed a mosaic karyotype in the thirty metaphases studied. Nine cells lost the RCX; the remaining 15 cells had an X chromosome and a very small marker chromosome of unidentified origin. The remaining six cells examined had one X and a larger marker than the one in the previous line. However, both markers appeared to be ring chromosomes according to karyotypic analysis. Mosaicism for the ring was estimated to be 50% (severe).

Metaphase FISH studies using centromeric X and SRY on Y showed a lack of SRY in the 20 cells (Vysis, Inc.). Both rings showed hybridization with the centromeric X probe, indicating that both were RCX chromosomes. The larger RCX presented two signals for the X centromere, while the smaller RCX only showed one signal, indicating a dicentric ring in the former and a monocentric ring in the latter. Additionally, interphase FISH revealed that 36% (122/337) of the nuclei examined had one X, 60% (201/337) had one X and a monocentric ring, and 4% (14/337) had one X and a dicentric RCX. AneuVysion FISH (Vysis, Inc.) cocktail containing probes 13, X, and Y identified 36.9% (41/111) of the nuclei examined had one X and two chromosomes 13, 53.2% (59/111) of nuclei examined had two X chromosomes and two chromosomes 13, and 9.9% (11/111) of the nuclei examined had three X chromosomes and two chromosomes 13.

The last available note indicates that the patient received care at 14 years of age to aid in future fertility interests and was being treated for hyperthyroidism and primary gonadal failure. She presented with short stature, hypoglycemia, webbed neck, widely spaced nipples, and dysmorphic features. She had follicles noted on her right side and underwent cryopreservation via a unilateral oophorectomy. She also experienced anxiety and ADHD. Regarding her cardiac presentation, she was doing well, with continued evidence of a mild dilation of the aortic root and ascending aorta and a bicuspid aortic valve with no

obstruction and subclinical/mild aortic regurgitation. She was the product of a full-term pregnancy; at birth, it was noted that she had a cystic hygroma. Her renal presentation included a bilateral duplicated collecting system. She also had growth hormone deficiency that was being treated and experienced hearing loss. Her last available height was 1.548 meters, her weight was 57.2 kg, and her BMI was 23.06. She displayed secondary sexual characteristics TS III and TS IV and experienced hypertension. She also experienced Grave's disease, ADHD, anxiety, and a learning disorder.

Fifteen years later, a research SNP array (Illumina 850k v1.3) was used to identify: arr[GRCh37] Xp22.33p11.1(60,814_58,483,247)x1,Xq11.1q13.3(61,719,290_75,249,257)x1~2,Xq13.3q28(75,265,698_155,236,747)x1. The first terminal deletion on the short arm was from band Xp22.33 to Xp11.1 from nucleotides 60,814 to 58,483,247 with a minimum size of 58.4 Mb. This region covers the pseudoautosomal region 1 (PAR1), which contains the *SHOX* gene and several non-coding regulatory elements. Pathogenic variants in *SHOX* are associated with idiopathic growth retardation, short stature, and Leri-Weill dyschondrosteosis (OMIM *312865). The second terminal deletion on the long arm is from band Xq13.3 to Xq28 from nucleotides 75,265,698 to 155,236,747 with a minimum size of 80 Mb. The SNP array did not indicate mosaicism for these regions.

The interstitial mosaic deletion included part of the long arm of chromosome X. The SNP array identified this region as starting from band Xq11.1 to Xq13.3 from nucleotides 61,719,290 to 75,249,257 with an approximate size of 13.5 Mb (small). Due to an absence of probe coverage for the centromere, we approximated the mosaic deletion using informative SNPs. However, metaphase FISH confirmed the involvement of the centromere. At least 104 genes, including *XIST* (GeneScout), are in this region. The presence of the *XIST* locus in the ring implies it can be inactivated. However, there are published reports of a lack of expression of *XIST* from small RCX chromosomes containing the *XIST* locus, thus resulting in a more severe phenotype. The B-allele frequency indicated the presence of mosaicism in this region.

RCX-13

A 1-month-old White female presented to JHH with a hypoplastic left heart and congenital anomalies. A SNP array (Illumina 850 k v1.0) performed on peripheral blood identified an abnormal result indicating the presence of two cell lines: arr[hg19] Xp22.33p11.22(60,814-53,032,280)x1, Xp11.22q13.3(53,056,518-74,609,263)x1~2,Xq13.3q28(74,610,826-155,254,881)x1. The first terminal deletion on the short arm was from band Xp22.33 to Xp11.22 from nucleotides 60,814 to 53,032,280 with a minimum size of 53 Mb. This region covers pseudoautosomal region 1 (PAR1), which contains the *SHOX* gene and several non-coding regulatory elements. Pathogenic variants in *SHOX* are associated with idiopathic growth retardation, short stature, and Leri-Weill dyschondrosteosis (OMIM *312865). The second terminal deletion on the long arm was from band Xq13.3 to Xq28 from nucleotides 74,610,826 to 155,254,881 with a minimum size of 80.6 Mb. The SNP array did not indicate mosaicism for these regions.

The interstitial mosaic deletion included part of the short and long arms and covered the centromeric region of chromosome X. The SNP array identified this region as starting from band Xp11.22 to Xq13.3 from nucleotides 53,056,518 to 74,609,263 with an approximate size of 21.6 Mb (including the centromere)(small). At least 153 genes, including *XIST* (GeneScout), are in this region. The presence of the *XIST* locus in the ring implies it can be inactivated. However, there are literature reports of a lack of expression of *XIST* from small RCX chromosomes containing the *XIST* locus, thus resulting in a more severe phenotype. The B-allele frequency indicated the presence of mosaicism in this region.

A subsequent thirty-cell karyotype study at a 650 banding level confirmed these findings, with monosomy noted in twenty-two cells and the ring present in eight cells. The level of mosaicism was estimated to be 26.66% (moderate).

NIPS testing performed prenatally by an outside laboratory indicated an increased risk for TS. Hypoplastic left heart syndrome and left pulmonary artery/left lung hypoplasia were identified prenatally.

She had a history of intracranial bleeding and complicated feeding issues (s/p GT and Nissen), including chylothorax and severe GERD. Specifically, she had a left frontoparietal hematoma and underwent left diaphragm plication for left hemidiaphragm paresis. She experienced worsening hypoxemia, poor weight gain, and dysrhythmias. She was noted to have hyperconvex nails and widely spaced nipples. She also displayed micrognathia.

She was not a candidate for further cardiac surgical repair due to hypoplastic left heart syndrome and severe hypoplasia of the left ventricle with a PFO and PDA. She received palliative care and passed away at ten months of age. She was the first child born to her parents. The family history included a strong family history of miscarriages on the maternal side, for which the cause was not identified. Her height was 0.636 meters, and her weight was 5.62 kg. She also experienced a renal malformation.

RCX-14

A three-week-old White female presented to JHH NICU, and a postnatal G-banded karyotype was used to confirm the SNP array findings consistent with TS. Her clinical features included a pelvic kidney, excess nuchal skin, cystic hygroma in utero, and widely spaced nipples. She experienced growth deficiency, short stature, developmental delay, dysmorphic features, a webbed neck, and a high-arched palate. She also had bilateral hearing loss and hypertension. Other features included obstructive sleep apnea, severe laryngomalacia, staring spells, ADHD, feeding difficulties, hypotonia, GERD, poor dentition, and sensory integration disorder.

We performed a G-banded chromosome analysis on peripheral blood at the 500-band level. The subject's mosaic karyotype identified a preponderance of monosomy X in 33 cells and an RCX in the remaining cells with breakpoints at Xp11.22 and Xq13.3. The level of mosaicism was estimated to be 36.36% (severe). The SNP array previously identified: arr[hg19] Xp22.33p11.22(60,814-54,732,159)

x1,Xp11.22q13.3(54,737,365-74,659,064)x1~2,Xq13.3q28(74,661,776- 149,822,608)x1,Xq28 (149,826,503-150,493,345)x2,Xq28(150,497,850-155,236,712)x1 (Illumina 850k v1.1).

The first terminal deletion on the short arm was from band Xp22.33 to Xp11.22 from nucleotides 60,814 to 54,732,159 with a minimum size of 54.7 Mb. This region covers pseudoautosomal region 1 (PAR1), which contains the *SHOX* gene and several non-coding regulatory elements. Pathogenic variants in *SHOX* are associated with idiopathic growth retardation, short stature, and Leri-Weill dyschondrosteosis (OMIM *312865). An interstitial deletion was found on the long arm from band Xq13.3 to Xq28 from nucleotides 74,661,776 to 149,822,608 with a minimum size of 75.2 Mb. The second terminal deletion on the long arm is from band Xq28 to Xq28 from nucleotide 150,497,850 to 155,236,712 with a minimum size of 4.7 Mb. The SNP array did not indicate mosaicism for these regions.

The interstitial mosaic deletion included part of the short and long arms and covered the centromeric region of chromosome X. The SNP array identified this region as starting from band Xp11.22 to Xq13.3 from nucleotides 54,737,365 to 74,659,064 with an approximate size of 19.9 Mb (including the centromere)(small). At least 133 genes, including *XIST* (GeneScout), are in this region. The presence of the *XIST* locus in the ring implies it can be inactivated.

The significance of this gene has been associated with several genetic elements essential for the inactivation of an X chromosome in females (OMIM *314670). However, there are published reports of a lack of expression of *XIST* from small RCX chromosomes containing the *XIST* locus, thus resulting in a more severe phenotype. The B-allele frequency indicated the presence of mosaicism in this region. Additionally, an interstitial segment with a copy number of two was found on the long arm from Xq28 to Xq28 from nucleotides 149,826,503 to 150,493,345, which was inferred to represent a 0.7 Mb duplication present on the single X present in the cells examined.

A last available note indicates that she received care at 18 months of age. She was the second child born to her parents. Her last available height was 0.76 meters, and her weight was 9.42 kg. She is still receiving care at the age of 7.

RCX-15

A twelve-year-old White female presented with concern for ovarian failure and a diagnosis of TS. She had growth deficiency, short stature, lymphedema, widely spaced nipples, and a low-set hairline. Peripheral blood was studied, and a thirty-three-cell G-banded chromosome analysis identified the presence of two cell lines at equivalent levels. Seventeen cells had monosomy X; the remaining 16 had an RCX with approximate breakpoints at Xp22.33 and Xq22.3. The level of mosaicism was estimated to be 48.48% (severe).

A research SNP array (Illumina 850k v1.3) identified: arr[GRCh37] Xp22.33p22.2(60,814_11,424,850)x1,Xp22.2q23(11,433,808_115,478,048)x1~2,Xq23q28(115,478,048_155,236,747)x1. The first terminal deletion on the short arm was from band Xp22.33 to Xp22.2 from nucleotide 60,814 to 11,424,850 with a minimum size of 11.4 Mb. This region covers pseudoautosomal region 1 (PAR1), which contains the *SHOX* gene and several non-coding regulatory elements. Pathogenic variants in *SHOX* are associated with idiopathic growth retardation, short stature, and Leri-Weill dyschondrosteosis (OMIM *312865). The second terminal deletion on the long arm was from band Xq23 to Xq28 from nucleotides 115,478,048 to 155,236,747 with a minimum size of 39.8 Mb. The SNP array did not indicate mosaicism for these regions.

The interstitial mosaic deletion included part of the short and long arms and covered the centromeric region of chromosome X. The SNP array identified this region as starting from band Xp22.2 to Xq23 from nucleotides 11,433,808 to 115,478,048 with an approximate size of 104 Mb (including the centromere) (large). At least 674 genes, including XIST (GeneScout), are in this region. This gene is

associated with several genetic elements essential for the inactivation of an X chromosome in females (OMIM *314670). The B-allele frequency indicated the presence of mosaicism in this region.

A last available note indicated that she was a high school sophomore (aged 16) with a history of bilateral pseudo-papilledema and well-controlled esophoria/esotropia. Her laboratory results showed a decreased but detectable AMH level, which fluctuated between <0.03 and 0.21. Her most recent AMH level was 0.11 at age 16. Her EKG and Echo results were within normal limits. She was treated by endocrinology for growth hormone needs. She was born prematurely with puffy hands, which prompted cytogenetic testing. She achieved the development of secondary sexual characteristics without assistance, though her periods were slightly fewer and inconsistent. She received OCPs as estrogen replacement. A renal ultrasound was normal. Given her mosaic TS, she was noted to have a mild presentation of her condition. Her height was at the 3rd percentile. The last available height was 1.49 meters, weight was 50.8 kg, and BMI was 22.9. Other features included ITP, congenital peripheral AVM, strabismus, and pseudotumor cerebri with papilledema. She also displayed widely spaced nipples.

RCX-16

An initial bone marrow karyotype was performed on a White female at 60 years of age to rule out AML. She presented with short stature, cognitive impairment, and bilateral hearing loss. A 20-cell G-banded karyotype study revealed monosomy X in 18 cells, and the two remaining cells showed the same event by adding a marker of unknown origin. The event might have represented TS or a clonal abnormality in neoplasms. The results of the concurrent FISH study for myelodysplastic syndromes/acute myeloid leukemia (MDS/AML) were unremarkable (5p15.2 (D5S23, D5S721), 5q31 (EGR1), 7cen (D7Z1), 7q31 (D7S522), 8cen (D8Z2), 11q23 (MLL) and 20q12 (D20S108)) for the 200 interphase nuclei examined. It was indicated that the patient was aware of her diagnosis since her teens.

We performed a constitutional karyotype at the 500 band level on peripheral blood six years later and found monosomy X in 29 cells and an RCX with uncertain breakpoints at similar levels. The level of mosaicism was estimated to be 30% (moderate).

A last available note indicated that she last received care at 66. This subject is deceased. Her history included coronary heart disease, cardiomyopathy, type II diabetes, hyper and hypotension, hyperlipidemia, hypothyroidism, gout, anemia, pancytopenia, thrombocytosis due to chronic liver disease, cirrhosis, age-related low bone mineral density, and chronic renal insufficiency. Toward the end of her life, she experienced generalized weakness, altered mental status, and hypotension. She also had diabetic nephropathy, retinopathy, and glaucoma. Her last available height was 1.422 meters, her weight was 55.8 kg, and her BMI was 28.9.

We attempted a research SNP array (Illumina 850k v1.3) on remnant fixed cells. However, the data were not acceptable for analysis.

RCX-17

G-banded chromosome analysis of an eight-year-old Black female at the 500 band level identified a mosaic karyotype with monosomy X in 12 cells, and the remaining eight cells showed an RCX; these results came from an outside laboratory. The approximation of breakpoints could not be carried out in that study. A repeat chromosome analysis was performed on cultured lymphocytes at the 550 band level, examining 31 metaphases. Sixteen cells displayed monosomy X, and the remaining 15 cells exhibited an RCX with breakpoints at Xp11.4 and Xq27.2. Her overall mosaicism was estimated to be 45% (severe).

Metaphase FISH using centromeric X and SRY identified two signals for the X centromere, with no hybridization for SRY in the 41 metaphases analyzed. These results supported the presence of a mosaic RCX (Vysis, Inc.). A SNP array (Illumina 850k v1.2) identified

arr[GRCh37]Xp22.33p11.4(60,814_37,619,114)
x1,Xp11.4q27.2(37,625,334_143,036,520)x1~2,Xq27.2q28(143,049,500_155,236,747)x1.

The first terminal deletion on the short arm was from band Xp22.33 to Xp11.4 from nucleotide 60,814 to 37,619,114 with a minimum size of 37.6 Mb. This region covers pseudoautosomal region 1 (PAR1), which contains the *SHOX* gene and several non-coding regulatory elements. Pathogenic variants in *SHOX* are associated with idiopathic growth retardation, short stature, and Leri-Weill dyschondrosteosis (OMIM *312865). The second terminal deletion on the long arm was from band Xq27.2 to Xq28 from nucleotides 143,049,500 to 155,236,747 with a minimum size of 12.1 Mb. The SNP array did not indicate mosaicism for these regions.

The interstitial mosaic deletion included part of the short and long arms and covered the centromeric region of chromosome X. The SNP array identified this region as starting from band Xp11.4 to Xq27.2 from nucleotides 37,625,334 to 143,036,520 with an approximate size of 105.4 Mb (including the centromere)(large). There are at least 726 genes located within this region, including *XIST* (GeneScout). This gene has been associated with several genetic elements essential for the inactivation of an X chromosome in females (OMIM *314670). The B-allele frequency indicated the presence of mosaicism in this region.

At ten years of age, she had a medical history of hypoglycemia, type I diabetes, celiac disease, short stature, and secondary sexual development. She had a history of developmental delay, vitamin D deficiency, ADHD, and sleep apnea and was being monitored for growth hormone therapy due to her growth deficiency. Her last available height was 1.279 meters, her weight was 29.9, and her BMI was 18.28.

572 Supplemental References

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