Increased recurrence risk in Phelan-McDermid (22q13.3 deletion) syndrome: the importance of FISH demonstrated by a case series of five families

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Supplement 1. Clinical description and molecular and cytogenetic results of the families

Family 1

This family consists of two siblings with a 2.61Mb deletion of 22q13.32q13.33 (Figure S1) and a 14.06 Mb duplication of 13q32.3q34 due to a paternal translocation t(13;22)(q32.3;q13.32) (Figure S2) and two healthy, non-consanguineous parents. The eldest sibling, a boy, was born at a gestational age of 38 weeks, with a birth weight of 2400 g. The neonatal period was uncomplicated, but he later showed global developmental delay. He stood unsupported at the age of two years and walked independently at the age of three years. At the age of three years and nine months, he was still non-verbal but able to understand commands. He had the habit of chewing on non-edible substances. At physical examination, he had a supranasal iris defect of the right eye, a coarse face, anteverted nares, bushy eyebrows, hypertelorism and brachydactyly. A cerebral MRI showed paucity of the white matter in both cerebral hemispheres, with mild prominence of the ventricles and mild thinning of the corpus callosum.

The second child in this family is a girl, born at a gestational age of 40 weeks, with a birth weight of 3100 g. At the age of seven months she had partial head control, but did not have the ability to reach objects. She had a flat nasal bridge, low set ears, hypertelorism, sparse scalp hair and brachydactyly. No specific behavioural changes were noted by the parents.

Molecular and cytogenetic results

Index	Karyotype	46,XY
	MLPA subtelomeres	del 22q13.3
	FISH[6]	ish del(22)(q13.3)(ARSA-)
	SNP array[1]	arr[hg19] 13q32.3q34(101,105,237-115,169,878)x3, 22q13.32q13.33(48,691,434-51,304,566)x1
Sibling	FISH[6]	ish del(22)(q13.3)(ARSA-)
Father	Karyotype	46,XY,t(13;22)(q32.3;q13.32)
	FISH	confirmation, see Figure S2. B

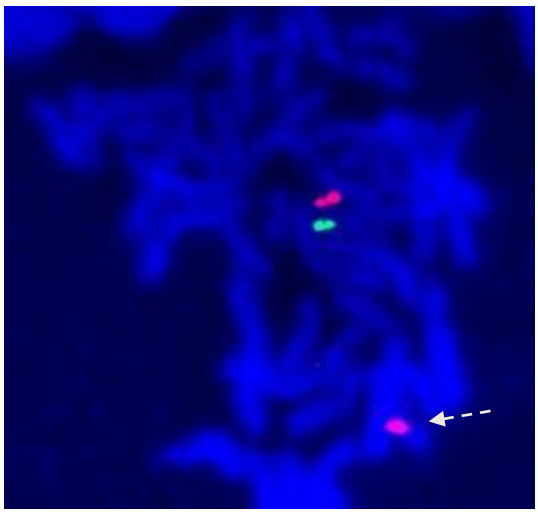

Fig. S1 *Fluorescent in situ hybridization (FISH) on metaphases of cultured lymphocytes of the index. The LSI TUPLE1 probe (22q11.2) is shown in red and the LSI ARSA probe (22q13.3) in green (Vysis®, Abbott Molecular, USA). The arrow indicates the absence of 22q13.3 on the derivative chromosome 22.*

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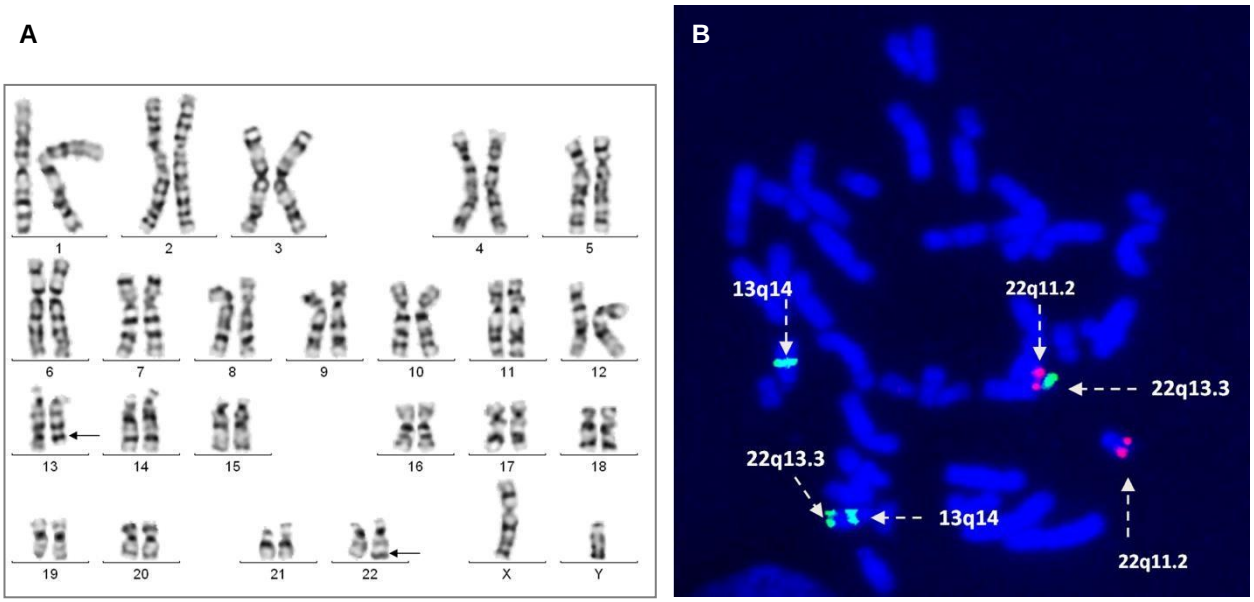

***Fig. S2 A** Karyotype of the father showing a translocation between chromosomes 22q13.3 and 13q32.3. **B** FISH on metaphases of cultured lymphocytes of the father, confirming the t(13;22). The LSI13 (13q14) and LSI ARSA (22q13.3) probes are shown in green and the LSI TUPLE1 (22q11.2) probe in red (AneuVysion®, Abbott Molecular, USA). The arrows indicate the specific probes for 13q14, 22q11.2 and 22q13.3, resulting in two green signals on the derivative chromosome 13 and the absence of a green signal on the derivative chromosome 22.*

Family 2

This family (Figure 1, main text) consists of two boys with a 0.28Mb deletion and duplication 22q13.33q13.33, respectively, due to a paternal translocation t(7;22)(p22.3;q13.33). The eldest boy, aged three years and nine months, was referred by his pediatrician for evaluation of his developmental delay, behavioral problems and feeding problems. He was born after an uneventful pregnancy at a gestational age of 39+2 weeks, a birth weight of 3725 g and had a good start. In the neonatal period, he barely cried and had feeding difficulties, probably due to abnormal mouth coordination. He rolled over at six months, sat independently at eight months, walked assisted at twelve months but independently at two-and-a-half years. Upon referral, he did not speak and understood up to eight words. He also had abnormal behavior like limited communication, hyperactivity, stereotypic movements and sleeping problems. For one year, he had had feeding problems with regurgitation, vomiting and gastro-oesophageal reflux. Physical examination showed hypotonia, areflexia and hyperlaxity of his joints. He also had recurrent upper airway infections and otitis media.

The second child in this family is his younger brother. He was studied at the age of two years and four months after the translocation was found in his father. Parents had noticed that he had problems with pronunciation and finishing words, but that he actively used about fifty words. He also understood commands. He had a low threshold for frustration, showed obsessive behavior in his actions and had a fascination for wheels. His motor skills seemed age-appropriate.

Molecular and cytogenetic results

Index	Karyotype	46,XY
	Array CGH[2]	arr[hg19] 7p22.3(53.984-229.993)x3, 22q13.33(50,896,795-51,178,404)x1
	FISH[6]	ish del(22)(q13.33q13.33)(RP11-164E23dim,CTA-799F10-)

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Sibling	FISH[6]	ish der(7)t(7;22)(P164D18-,CTA-799F10+,RP11-164E23+, RP11-93F2+)
Father	Karyotype	46,XY
	Array-CGH[2]	arr[hg19] 7p22.3 (53.984-229.993)x2, 22q13.33 (50,896,795-51,178,404)x2
	FISH[6]	ish t(7;22)(P164D18-,CTA-799F10+,RP11-164E23+; RP11-164E23dim,CTA-799F10-,P164D18+)

Family 3

This family consists of two siblings with a 2.06 Mb deletion of 22q13.32q13.33 due to a maternal mosaic deletion detectable in 20% of the mother's urinary cells (Figure S3). The eldest child, a boy, aged three years and three months was referred by his pediatrician for counseling about his recent diagnosis of PMS. He was the first child of two healthy parents, born after a normal pregnancy at a gestational age of 40+4 weeks. He was delivered by caesarean section due to abnormal position and fetal distress but had Apgar scores of 9 and 10. His birth weight and length were 3550 g and 51 cm, respectively. The neonatal period was uneventful except for difficulties with breast feeding. Apart from allergies for cats, dogs and antibiotics, the boy is healthy. He has frequent regurgitation of food without vomiting but eats well. He has a high threshold for pain and mild hypotonia. Motor milestones were obtained at four to five months for rolling, six months for crawling, and sixteen months for walking independently. He had used two words around the age of ten months, but lost them. From the age of twenty-two months he received physiotherapy and speech therapy because of sensomotor developmental and language delays. In addition, he showed autistic-like features including hyperactivity and some rituals, like tapping objects. Upon referral, he showed broad eyebrows, long eyelashes, a high palate and dysplastic toenails.

His younger sister had also been tested by the pediatrician because of parental concerns. She was born spontaneously after a normal pregnancy at a gestational age of 40+1 weeks with normal Apgar scores. Her birth weight and length were 3430 g and 51 cm, respectively. The neonatal period was uneventful and apart from strabismus, she had no medical problems. She obtained her motor milestones for rolling over at five months and walking independently at eleven to twelve months. She also had a high threshold for pain and hyperactive behavior.

Molecular and cytogenetic results

Index	Array CGH[2]	arr[hg19] 22q13.32q13.33(49,161,984-51,219,150)x1
	SNP array[3]	maternal origin of aberrant chromosome
Sibling	Array CGH[2]	arr[hg19] 22q13.32q13.33(49,161,984-51,219,150)x1
	SNP array[3]	maternal origin of aberrant chromosome
	FISH[6]	ish del(22)(q13.33q13.33)(RP11-164E23-, CTA-799F10-)
	FISH[6]	nuc ish del(22)(q13q13)(ARSA-)
Mother	Array CGH[2]	arr[hg19] 22q13.32q13.33(49,161,984-51,219,150)x2
	SNP array[3]	maternal origin of aberrant chromosome
	FISH[6]	ish 22q13.33(RP11-164E23,CTA-799F10)x2 ish 22q13.33(ARSAx2), buccal cells ish 22q13.33(ARSAx1)[20/10], urinary cells

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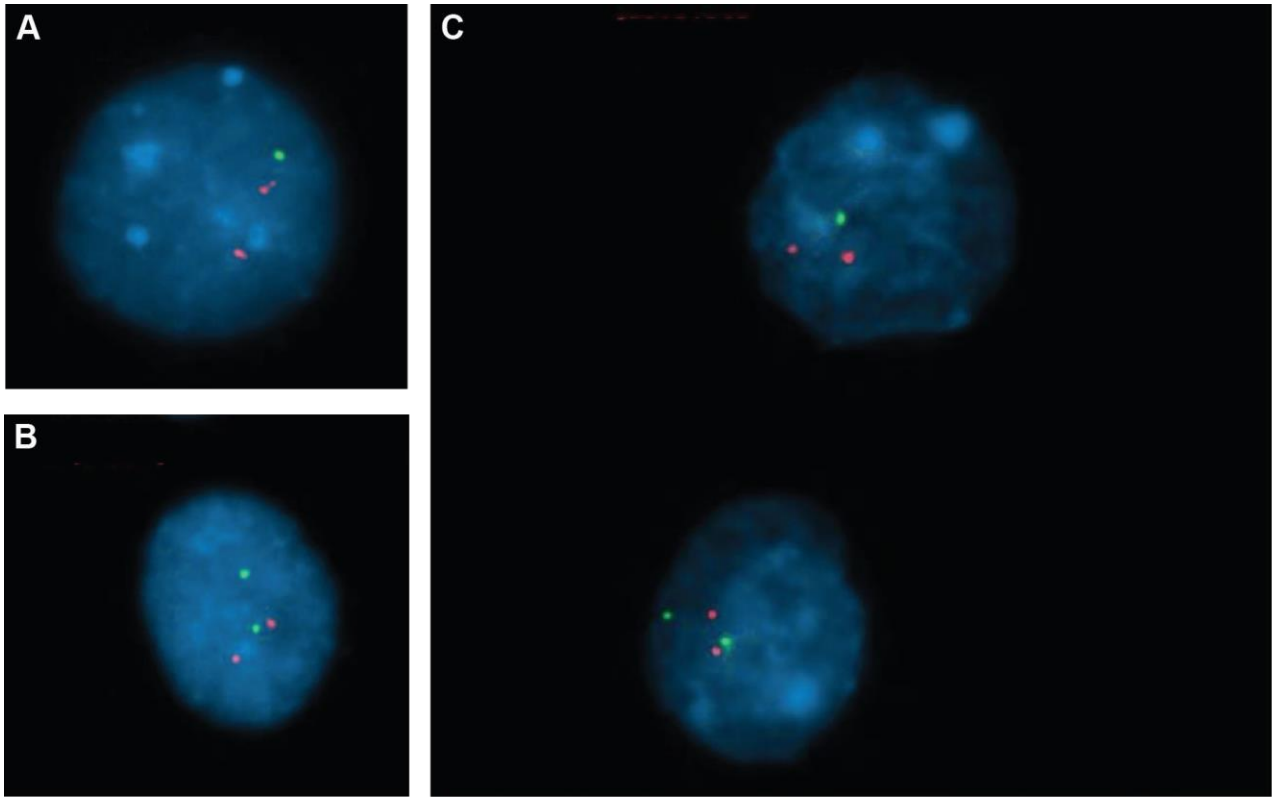

***Fig. S3 A** Fluorescent in situ hybridization (FISH) on metaphases of interphase nuclei of cultured lymphocytes of the index, **B** buccal cells of the mother and, **C** urinary cells of the mother. FISH was performed using the LSI TUPLE1 (22q11.2) probe in red and LSI ARSA (22q13.3) probe in green (Vysis®, Abbott Molecular, USA). The deletion was found in 20 of 100 nuclei of urinary cells, but not in buccal cells of the mother.*

Family 4

This family consists of two siblings with a 9.23 Mb deletion of 22q13.2q13.33 due to a maternal mosaic deletion detectable in 5% of the mother's buccal cells, due to a mosaic ring chromosome 22 (Fig. S4). The second child of the two healthy parents, a girl aged six weeks, had been born prematurely at a gestational age of 33+1 weeks by caesarean section due to intrauterine growth retardation and maternal Hemolysis-Elevated-Liver enzymes-Low platelets syndrome (HELLP). Her Apgar scores were 5/9/9, birth weight was 1000 g and head circumference was 31.2 cm. The girl had several dysmorphic features, including periorbital fullness, slightly upslanted palpebral fissures, down-turned corners of the mouth and prominent antihelix and antitragus of the ears. The parents' first child, a boy, was born prematurely at a gestational age of 31+1 by Caesarean section because of fetal distress. His birth weight was 1020 g (10th centile). He died eighteen hours postnatally of severe infantile respiratory distress syndrome.

Molecular and cytogenetic results

Index	SNP array[4]	arr[hg19] 7q34(141,937,563-142,486,549)x3 mat, 7q34(142,568,934-142,706,385)x3 mat, 22q13.2q13.33(42,078,534-51,092,628)x1
	Karyotype	46,XX,r(22)(p11q13.2)
Sibling	SNP array[4]	arr[hg19] 22q13.2q13.33(42,078,534-51,092,628)x1, fibroblasts

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Mother	SNP array[4]	arr[hg19] 7q34(141,927,973-142,486,784)x3, 7q34(142,572,022-142,706,788)x3
	FISH[6]	nuc ish 22q13.33(ARSAx1[5]/ARSAx2[102], buccal cells ish 22q13.33(ARSAx2)[30], cultured blood lymphocytes
	SNP array[4]	arr[hg19] 7q34(141,937,563-142,115,393)x3, 7q34(142,565,665-142,716,852)x3, DNA from urinary cells

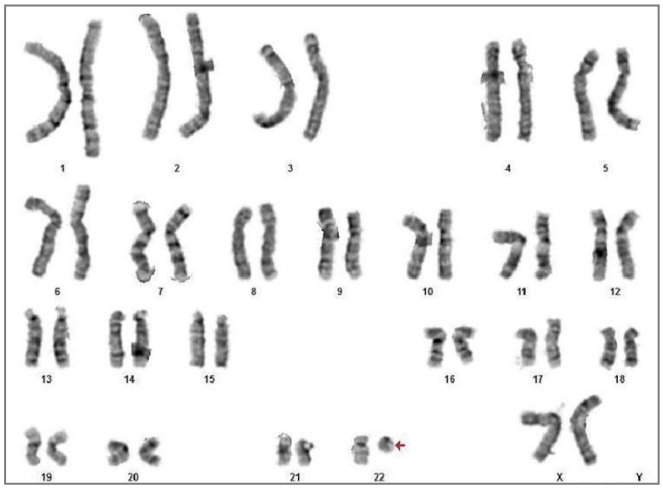

Fig. S4 *Karyotype of the proband showing a ring chromosomes 22 resulting in a deletion22q13.2q13.33 of 9.23 Mb (SNP array)*

Family 5

This family consists of a boy with a 2.98 Mb deletion of 22q13.31q13.33 due to a maternal mosaic ring chromosome 22 detectable in 3% of the mothers peripheral lymphocytes. The boy was referred to the clinic by his pediatrician at the age of two years and three months for analysis of his developmental delay. He was born after an uneventful pregnancy at a gestational age of 38+4 weeks, with a birth weight of 2910 g, length of 51 cm and Apgar scores of 10/10. He could roll over at six months, sit at twelve months and was not able to walk independently at referral. He showed limited interaction with his environment and had used two words in the past but then lost them. He only produced some sounds. His medical history indicated plagiocephaly for which he received physiotherapy, divergent strabism which was surgically corrected and recurrent ear infections for which he received grommets. He also had several mild dysmorphic features, including long eyelashes, broad eyebrows, large ears (p97), a small mandible and dysplastic fingernails and toenails. His mother was healthy, except for bronchial hyperreactivity, and had a normal development and education. She only had micrognathia and a high palate. The boy's older brother was also healthy and had a normal development.

Molecular and cytogenetic results

Index	Karyotype	46,XY,r(22)(p11;q13.31)
	FISH[6]	ish r(22)(TUPLE1+, N85A3-, P-99K24-)
	Array CGH[5]	arr[hg19] 22q13.31q13.33(47,981,336-51,304,566)x1
Sibling	SNP array[4]	arr[hg19] 22q13.2q13.33(42,078,534-51,092,628)x1, fibroblasts

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Mother	SNP array[4]	arr[hg19] 7q34(141,927,973-142,486,784)x3, 7q34(142,572,022-142,706,788)x3
	FISH[6]	nuc ish 22q13.33(ARSAx1[5]/ARSAx2[102], buccal cells ish 22q13.33(ARSAx2)[30], cultured blood lymphocytes
	SNP array[4]	arr[hg19] 7q34(141,937,563-142,115,393)x3, 7q34(142,565,665-142,716,852)x3, DNA from urinary cells

[1] *Human cytoSNP-850K-8v1.0, Illumina inc, San Diego, USA*
[2] *Agilent 180K custom HD-DGH, Agilent Technologies, Santa Clara, USA*
[3] *Illumina Omni Express 12-V1.0, Illumina inc, San Diego, USA*
[4] *Affymetrix Cytoscan HD,Thermo Fisher Scientific inc, Santa Clara, USA*
[5] #22 15K BAC array, *Agilent Technologies, Santa Clara, USA*

[6] *FISH probes:*

LSI TUPLE 1 / LSI ARSA dual probe	*22q11.2 / 22q13.3*	*Vysis, Abbott Molecular, USA*
LSI13	*13q14*	*AneuVysion, Abbott Molecular, USA*
P164D18	*7p22.3*	*Own BAC collection*
RP11-93F2	*7q36.3 (control probe)*	*Own BAC collection*
CTA-799F10	*22q13.33*	*Own BAC collection*
RP11-164E23	*22q13.33*	*Own BAC collection*
TUPLE1 / N85A3 dual probe	*22q11.2 / 22q13.33*	*CytoCell, Oxford Gene Technology, UK*
P99K24	*22q13.33*	*Own BAC collection*
D22Z1	*22 centromere*	*Own BAC collection*

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Supplement 2. De novo translocations and mosaicism reported in literature

Translocation	**Chromosomes**	**Karyotype**
Bonaglia 2001	t(12;22)	der(22)t(12;22)(q24.1;q13.3)
M. Phelan 2001[a]	t(9;22) t(22;acro)	der(22)t(9;22)(p21;q13.3) der(22)t(22;acro)(q13.33;p12)
Luciani 2003	t(22;acro) t(22;acro)	der(22)r(22;acro)(q13;p11) der(22)r(22;acro)(q13;p11)
Manning 2004	t(12;22) t(14;22)	der(22)t(12;22)(p13.31;q13.2), *case 10* der(22)t(14;22)(q32.31;q13.33), *case 11*
Bisgaard 2009	t(13;22)	dup(13)(q22qter),del(22)(q13.2qter)
Misceo 2010	t(X;22)	t(X;22)(q21.33;q13.33)
Trabacca 2011	t(2;22)	der(22)t(2;22)p(25.3;q13.31)(22qter-,2pter-)
Artigalas 2012	t(16;22)	t(16;22)(p11.2;q13)
Soorya 2013	t(22;??) t(22;??)	partner chromosome not reported partner chromosome not reported
Mosaicism	**% mos, tissue**	**Karyotype**
Terminal deletion		
Riegel 2000	65, fibroblast metaphases 60, fibroblast interphases	46,XX[7] / 46,XX,del(22)(q13)[13] 46,XX[10] / 46,XX,del(22)(q13)[15]
Phelan 2001[a]	70, blood >24, blood	46,XY[21] / 46,XY,del(22)(q13)[9] r(22)(p11.2q13.3)[38] / 46,XX,del(22)(q13.3)[12]
Phelan 2001[b]	26, cord blood interphases	46,XX[74] / 46,XX,del(22)(q13)[26]
Wilson et al 2003	90, lymphocytes	46,XY[2] / 46,XY,del(22)(q13)[18], *case 43*
Lindqvuist 2005	84, metaphases	46,XY / 46,XY,del(22)(q13.31), *case 4*
Bonaglia 2009	45, blood, saliva, EBV lines metaphases 70-80, blood, saliva, EBV lines metaphases	46,XX [55] / 46,XX,del(22)(pter-q13.2::)[45], *case 1* 46,XX [27] / 46,XX,del(22)(pter-q13.2::)[73], *case 2*
Ring chromosome		
Woods 1994	3, blood lymphocytes and 18, fibroblasts	46,XY[146] / r(22)[5] and 46,XY[49] / r(22)[11], *case 1*
Chen 2003*	>82, cord blood lymphocytes >66, hepatocytes	45,XX,-22[7] / r(22)(p13q13.31)[82] / 46,XX,idic r(22)(p13q13.31;p13q13.31)[11] 45,XX,-22[9] / r(22)(p13q13.31)[23] / 46,XX,idic r(22)(p13q13.31;p13q13.31)[3]
Koc 2009*	>98, blood	45,XX,-22[2] / r(22)(p11q13.2)[124] / 46,XX,idic r(22)(p11q13.2;p11q13.2)[1]
Bonaglia 2011	30, blood lymphocytes	del(22)(q13.31) mosaic
Canonero 2012	18, not reported	46,XX[66] / 46,XX;r(22)[34] and 22q13.3(ARSA x 2)[344] / del(22)(q13.3q13.3)(ARSA-)[75]
Kashevarova 2018	8, blood lymphocytes and 24, fibroblasts	46XX,r(22) / 45,XX,-22

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**Although there seems to be a high level of mosaicism, we cannot exclude the possibility that the loss and appearance of an isodicentric chromosome 22 are culture artifacts*

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