SUPPLEMENTARY MATERIALS

A 46,XX Cell Lineage with Maternal Uniparental Isodisomy for All Chromosomes in a Female with a Silver-Russell Syndrome-like Phenotype and a 45,X Turner Cell Lineage Accompanied by Biparentally Derived Autosomes

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SUPPLEMENTARY METHODS

Primers

The primers for bisulfite-PCR assays utilized for genetic screenings for Silver-Russell syndrome are shown in supplementary table 1, those for bio-COBRA (combined bisulfite restriction analysis) assays for multiple DMRs (differentially methylated regions) are shown in supplementary table 2, and those for Y chromosome analysis are shown in supplementary table 3. The primers for genomewide microsatellite analysis were based on ABI PRISM Linkage Mapping Set v2.5-MD10 (Applied Biosystems, Foster City, California, USA), and loci with high heterozygosities in the Japanese population were examined.[1] The probe-primer mixtures for quantitative real-time reverse transcriptase PCR analysis were as follows (assay IDs): Hs01005963_m1 for *IGF2*, Hs00256090_m1 for *SNRPN*, Hs00414677_m1 for *ZAC1*, Hs00399294_g1 for *H19*, Hs00292028_m1 for *MEG3*, Hs00169368_m1 for *PHLDA2*, and Hs00175938_m1 for *CDKN1C* (Applied Biosystems); the TATA box binding protein (*TBP*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were used as internal controls, using the Human TBP Endogenous Control and Human GAPDH Endogenous Control, respectively (Applied Biosystems).

Supplementary Table 1. Bisulfite-PCR primers utilized for genetic screenings for Silver-Russell syndrome

DMR	Nucleotide positions*	Forward primer sequence (5' → 3') / Reverse primer sequence (5' → 3')	AT / PS
H19-DMR (A)	1976042–1976360 (–)	AACCCCTTCCTACCACCATC	60
		GGGTTTGGGAGAGTTTGTGA	317
MEST-DMR (A) (methylated allele)	129919201–129919491 (+)	TAGTTGCGTTTCGTAAGGTAGTGTC	58
		ACACAATCCTCCGCTCGCCTA	291
MEST-DMR (A) (unmethylated allele)	129919254–129919492 (+)	GTTTGGTGTGGTGTTGTTTTGTGTGGG	60
		CACACAATCCTCCACTCACCTACA	239

*Nucleotide positions are based on the human reference sequence assembly (NCBI Build 36.1); The (+) and the (–) symbols after nucleotide positions indicate the DNA strand utilized to design PCR primers.

AT: Annealing temperature (°C), PS: product size (bp).

The primer sequences and PCR conditions for *H19*-DMR (A) and the *MEST*-DMR (A) (methylated allele) have been reported previously[2, 3], whereas those for the *MEST*-DMR (A) (unmethylated allele) are designed in this study.

Supplementary Table 2. Bisulfite-PCR primers and restriction enzymes utilized for bio-COBRA assays

DMR	Chromosome number Nucleotide positions*	Forward primer sequence (5' → 3') Reverse primer sequence (5' → 3')	AT PS	Enzyme† Frag. size
ARHI-DMR	Chromosome 1	GGTTTTAAGGAATAGAAGTTGTTGA	55	*Bst*UI
	68285331–68285550 (–)	AACCCAACAACTAACAATAAATATTTT	220	122/62/36
NAP1L5-DMR	Chromosome 4	GGGGTTTTTTAGTTATTTGATTAGT	55	*Taq*I
	89837763–89838003 (+)	AAAATCTCTCTAAACCAACTC	241	154/65/22
ZAC1-DMR	Chromosome 6	GGGGTAGTYGTGTTTATAGTTTAGTA	62	*Taq*I
	144370901–144371052 (–)	CRAACACCCAAACACCTACCCT	152	91/61
GRB10-DMR	Chromosome 7	GTTATATAATATTGTTTTATGGTTGG	57	*Taq*I
	50817378–50817623 (+)	GCTCTCCAAATACTCAAATAAACTCC	246	158/88
PEG10-DMR	Chromosome 7	GGTTTTTTTATTTGTTTTGGGGTATA	57	*Taq*I
	94123783–94123981 (–)	ATATAAAACCCCATCCTTCCTATCTT	199	106/93
MEST-DMR‡	Chromosome 7	TYGTTGTTGGTTAGTTTTGTAYGGTT	57	*Taq*I
	129919303–129919521 (+)	CCCAAAAACAACCCCAACTC	219	101/97/21
H19-DMR‡	Chromosome 11	GAGTTYGGGGGTTTTTGTATAGT	60	*Taq*I
	1977615–1977893 (–)	TAAATAATACCCRACCTAAAAATCTAA	279	142/137
IGF2-DMR2	Chromosome 11	ATTGTTGGTTATTTTTGGGGG	57	*Taq*I
	2,110,802–2,111,138 (+)	AACTCAAATCACTAATCAATCACAAAA	337	242/95
LIT1-DMR	Chromosome 11	TTTTGGTAGGATTTTGTTGAGGAGT	57	*Bst*UI
	2677736–2678042 (+)	CCTCACACCCAACCAATACCTC	307	255/52
IG-DMR-CG4	Chromosome 14	AATTATTTTTTGGATAAGAGAGTATA	57	*Bst*UI
	100345398–100345600 (+)	ATTACAAACCAAAATAAAATATAATAAATC	203	123/62/18
SNRPN-DMR	Chromosome 15	AGGGAGTTGGGATTTTTGTATTG	57	*Rsa*I
	22751048–22751345 (+)	CTCCCCAAACTATCTCTTAAAAAAAACC	240	205/35
PEG3-DMR	Chromosome 19	AAAAGGTATTAATTATTTATAGTTTGGT	57	*Taq*I
	62043541–62043862 (+)	AAAAATATCCACCCTAAACTAATAA	322	206/116
MCTS2-DMR	Chromosome 20	GTTAGAATTAATTTATTAGGGTG	57	*Taq*I
	29598611–29598909 (+)	AAATCCCCTACAAAAAAACC	299	172/127
NNAT-DMR	Chromosome 20	ATTTTTTTTGTATTTTTTTTATAGATAT	55	*Mlu*I
	35582379–35582576	ATTTTAAACCCAAATCCTCTACTTC	197	153/44
L3MBTL-DMR	Chromosome 20	GGTTTAGTTAATTTTTATAGATATTGATT	57	*Bst*UI
	41575924–41576143 (–)	ACCCTAAATATATCTTACTTTCCCC	220	163/57
NESP55-DMR	Chromosome 20	GTTTTTTTGGTTTTTTTGTTTTAT	57	*Taq*I
	56848649–56848844 (+)	AAACAACTCAAAATCTACCTCCTC	196	147/49
NESPAS-DMR	Chromosome 20	AATTTGTGGTATGAGGAAGAGTGAT	57	*Bst*UI
	56859212–56859446 (+)	TCAACCATTAAACAAAAATCATACC	235	130/105
XIST-DMR	Chromosome X	AAAATGTTTTAGAAAGAATTTTAAGTGTAG	57	*Taq*I
	72989197–72989403 (+)	AAATAAATTTTAAACCAACCAAATCAC	207	147/60

*Nucleotide positions are based on the human reference sequence assembly (NCBI Build 36.1); The (+) and the (–) symbols after nucleotide positions indicate the DNA strand utilized to design PCR primers.

†These enzymes digest methylated clones.

‡Note that the *MEST*-DMR examined with these primers is different from the *MEST*-DMR (A) examined with the primers shown in supplementary table 1; similarly, the *H19*-DMR examined with these primers contains the CTCF binding site 6 and is different from the *H19*-DMR (A) examined with the primers shown in supplementary table 1 that resides outside the CTCF binding sites.

AT: Annealing temperature (°C), PS: product size (bp); Y: C or T (pyrimidine) ; and R: A or G (purine).

The primer sequences have been designed by us, except for those for the following DMRs reported in the literature: the *ZAC1*-DMR,[4] the *MEST*-DMR, the *H19*-DMR,[5] the *LIT1*-DMR, the *SNRPN*-DMR,[6] and the *PEG3*-DMR.[7]

Supplementary Table 3. PCR primers and conditions utilized for sex chromosome analyses

Locus	Primer sequence (5' → 3')	AT	PS
PABY/*PABX*	GTACTACCTTTAGAAAACTAGTATTTTCCC (Y-specific)	54	950 (*PABY*)
	CTGCAGAAACAAGCTCATCAGCGTGACTAT (X-specific)		771 (*PABX*)
	GAATTCTTAACAGGACCCATTTAGGATTAA (common)		
SRY	GAATATTCCCGCTCTCCGGA	58	470
	GCTGGTGCTCCATTCTTGAGT		
ZFY/*ZFX*	CATCTTTACAAGCTTGTAGACACACT (Y-specific)	62	340 *(ZFY*)
	GAACACACTACTGAGCAAAATGTATA (X-specific)		488 (*ZFX*)
	ATTTGTTCTAAGTCGCCATATTCTCT (common)		
AMELY/*AMELX*	CTCTGATGGTTGGCCTCAAGCCTGT	62	618 (*AMELY*)
	CACTGTCCCTCATCCTAGAAACACA		804 (*AMELX*)
DYS14	GGGCCAATGTTGTATCCTTCTC	52	84
	GCCCATCGGTCACTTACACTTC		
DYZ3	TCCTTTTCCACAATAGACGTCA	58	174
	GGAAGTATCTTCCCTTAAAAGCTATG		

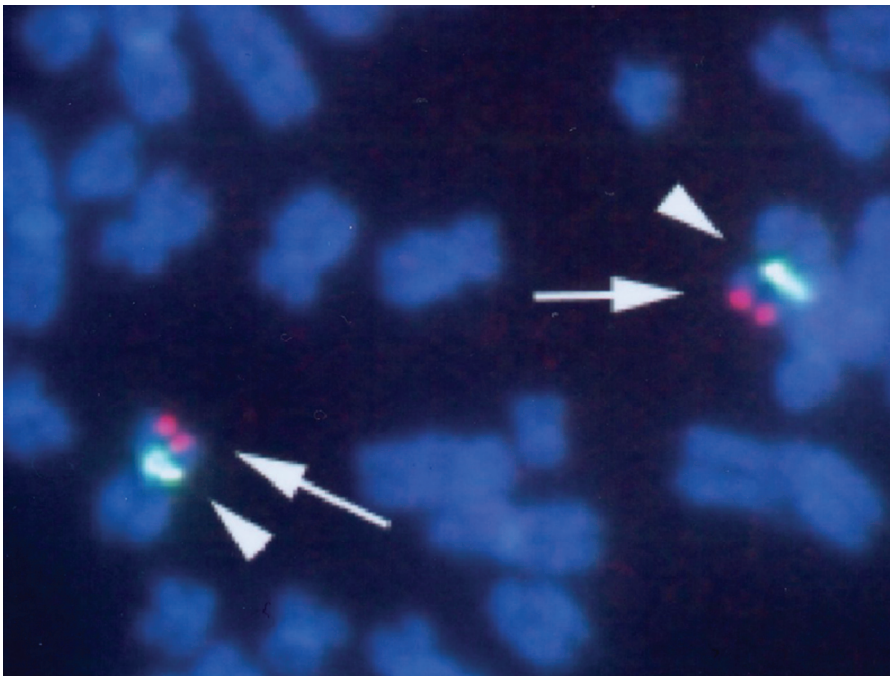
AT: annealing temperature (°C); and PS: product size (bp).

Supplementary Table 4. The results of microsatellite analysis

Locus	Mother	Patient	Brother	Locus	Mother	Patient	Brother
D1S238	295/309	309	295/305	*D13S170*	147/153	153/(163)	143/147
D1S207	148/166	166	164/166	*D13S153*	93/107	93/(97)	97/107
D1S218	275/277	(269)/275	269/275	*D14S72*	194/198	194/(198)	198/200
D1S2841	238/248	238/(248)	238/248	*D14S80*	98	98/(105)	98/103
D2S117	196/208	208/(210)	196/206	*D14S608*	204/214	204/(218)	214/218
D2S2330	170/172	170/(172)	172	*D14S588*	118/126	(114)/126	114/126
D2S2333	95/99	99/(103)	95/101	*D14S250*	161	161/(175)	161/175
D2S367	320/328	320/(328)	328	*D14S1010*	142/150	(148)/150	142/148
D3S1580	235	235	235/237	*D14S1007*	111	111/(119)	111/119
D3S1292	120/124	120/(128)	120/132	*D15S205*	161/163	163/(165)	157/163
D3S1566	165/167	167	167/175	*D15S1007*	88/90	88/(92)	90/92
D3S1263	195/201	195/(205)	201/215	*D15S128*	205/211	205	205/211
D4S1535	261	261	261/263	*D16S515*	336/338	(332)/338	332/336
D4S402	113/137	113/(131)	107/113	*D16S3091*	175/181	181/(185)	175/185
D4S1572	206/210	206	206	*D16S423*	136	136/(150)	136/150
D4S392	95/99	95/(101)	93/99	*D17S928*	82/86	86/(88)	86/88
D5S400	198/234	198/(234)	228/234	*D17S787*	145/155	155/(171)	145/163
D5S644	97/99	(95)/99	95/99	*D17S831*	116/118	(112)/118	112/118
D5S407	87/97	(83)/97	83/87	*D18S452*	125/129	125/(133)	125/131
D6S281	144/152	144/(152)	144/152	*D18S53*	170/172	170/(172)	170/172
D6S289	161	161	161	*D18S61*	229	(225)/229	225/229
D6S257	177	(175)/177	175/177	*D19S220*	279	(275)/279	275/279
D6S292	150/158	158/(168)	158/168	*D19S209*	244/252	(242)/244	242/244
D7S531	249	249/(251)	249/251	*D19S226*	252/254	(246)/254	246/254
D7S484	100/102	(98)/100	98/100	*D20S196*	263/288	263/(285)	263/285
D7S2846	176	176	176	*D20S117*	178/180	178/(180)	180
D7S519	264/266	(262)/264	262/264	*D20S186*	128/138	(116)/128	116/138
D7S672	132/148	(136)/148	132/136	*D21S266*	156/160	(156)/160	156/160
D7S669	124/130	124	124/130	*D21S1256*	102/110	(102)/110	102
D7S684	179	(167)/179	167/179	*D21S1252*	162	162/(168)	162/168
D7S1824	169/173	173/(185)	173/185	*D22S274*	284/292	292	292
D7S550	188	(186)/188	186/188	*D22S423*	298/308	298/(306)	298/306
D8S284	286/300	286	300/302	*D22S315*	195/197	(195)/197	195/197
D8S272	239	239	239/247	*SHOX**	153	153	151/153
D8S277	171/177	(167)/177	167/171	*DXYS85**	76	76	76
D8S264	143/145	145	143/145	*DXYS228**	199	199	199
D9S1677	240/242	242/(254)	242/254	*DXYS232**	170	170	170
D9S167	317/319	319	317/319	*DXYS10091**	214	214	214
D9S1817	288/296	296/(306)	288/306	*DXYS10083**	166/168	168	162/168
D9S157	227/239	227/(239)	239	*DXYS10086**	170/184	170	170/172
D10S537	155/157	(155)/157	155	*DXYS10096**	255/259	255	255
D10S249	131/133	(127)/131	127/133	*DXYS233**	164/168	168	168/170
D10S192	251/257	(253)/257	251/257	*DXS1047*	161	161	161
D11S2071	188	188	184/188	*DXS986*	246/258	246	246
D11S922	116/120	(90)/116	90/116	*DXS1060*	169/175	169	175
D11S1318	140	140	140	*DXS1226*	293/295	293	293
D11S4088	212/214	214	208/214	*DXS8051*	113	113	113
D11S988	117/125	(115)/117	117/127	*DXS1001*	200/204	204	200
D11S902	145/147	145	145	*DXS8055*	312/316	316	312
D11S904	198	(184)/198	184/198	*DXS1073*	308	308	308
D11S1918	186/194	(182)/186	182/194	*DXS8091*	86	86	86
D11S4083	179/193	193	179/191	*DXS990*	125/129	129	125
D11S4109	153/171	171	153/167	*DXS987*	214/222	222	222
D11S901	168	(166)/168	166/168	*DXS993*	270	270	270
D11S1356	191/217	(205)/217	191/205	*DXS1227*	81/83	83	83
D11S934	177/181	181	181	*DXS1068*	254/262	262	254
D11S912	96/100	100	100	*DXS1106*	130/132	130	132
D11S1304	175	175	175	*DXS8043*	149/155	155	155
D11S968	143	143	143	*DXS991*	328/332	332	328
D12S345	219/235	235	219/231	*DXS1214*	287/293	287	287
D12S1617	252/254	254/(262)	254/258	*DXYS227*†	124	124	124
D12S99	272	272/(274)	272/286	*DXYS154*†	244/248	244	244/246
D13S285	93/103	(95)/103	95/103	*DXYS225*†	210/214	214	214

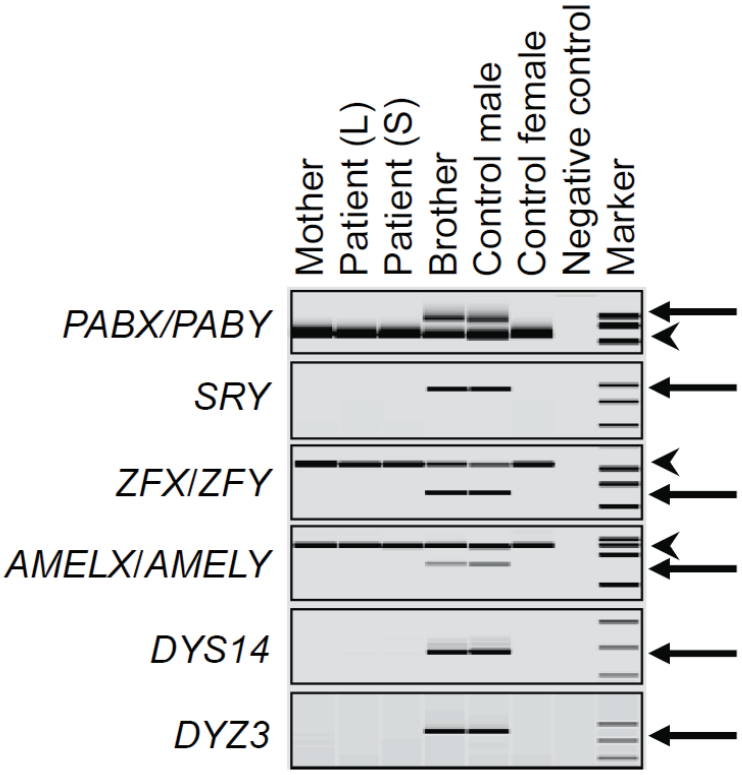
The Arabic numbers represent the sizes of the PCR products in bp.
The numbers in parentheses of the patient are minor peaks of non-maternal (paternal) origin.
*Loci on the short arm pseudoautosomal region, and †those on the long arm pseudoautosomal region.

Supplementary Figure 1. FISH analysis of the *H19*-DMR



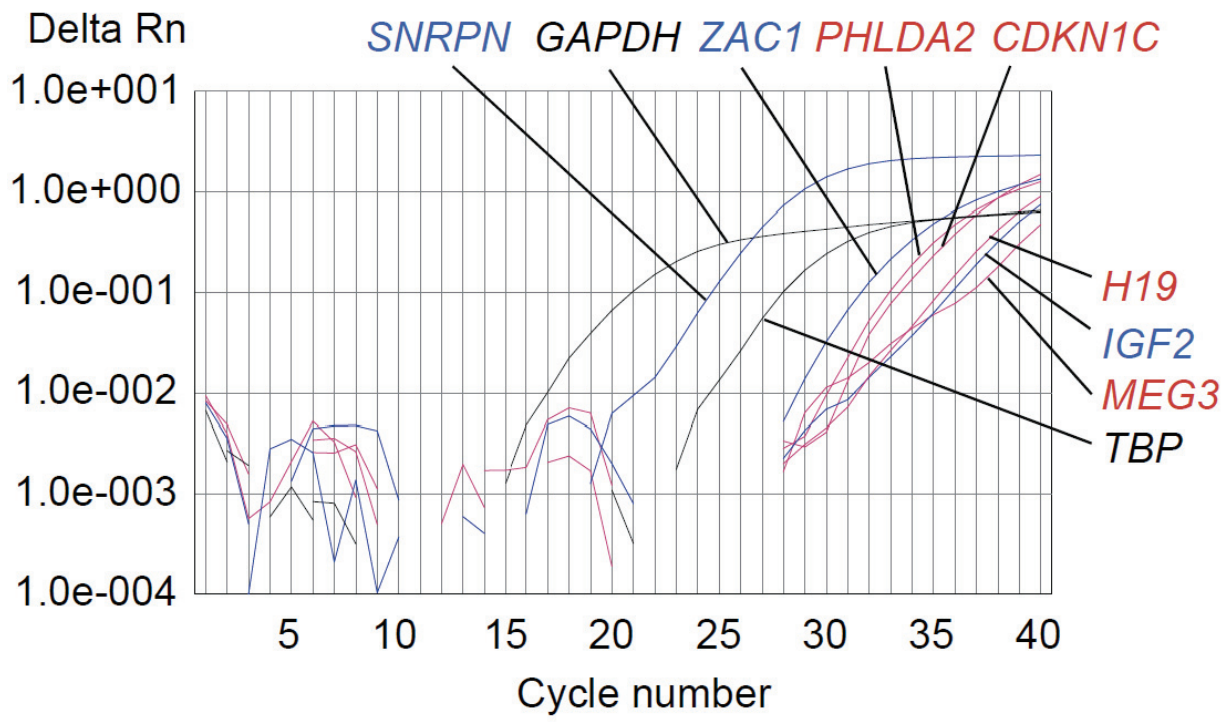
The RP5-998N23 has labeled with digoxigenin and detected by rhodamine anti-digoxigenin (red signals), and the control CEP 11 probe has been identified according to the manufacture's protocol (green signals).

Supplementary Figure 2. PCR analysis of Y-chromosomal loci



L: leukocytes; and S: salivary cells. No Y-specific bands (arrows) are identified whereas X-specific bands (arrowheads) are detected in both leukocytes and salivary cells of the patient.

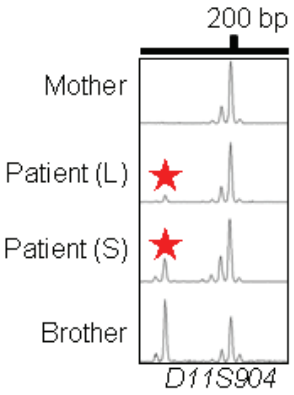
Supplementary Figure 3. Quantitative RT-PCR plot in a control subject



Paternally and maternally expressed genes are shown in blue and red, respectively.

SUPPLEMENTARY NOTE

Calculation of the ratio of cells with maternal uniparental isodisomy for all chromosomes (upid(AC)mat) in leukocytes and buccal epithelial cells



In this figure, two peaks are identified in the brother, and the area under curve (AUC) is larger for the short 184 bp peak than for the long 198 bp peak. This unequal amplification is consistent with short products being more easily amplified than long products. In the patient, the AUC ratio between the minor 184 bp peak of non-maternal origin and the major 198 bp peak of maternal origin is obtained as 0.09:1.0 for leukocytes (L) and 0.27:1.0 for salivary cells (S), after compensation of the unequal amplification between the two peaks, using the data in the brother.

Here, let "X" represent the frequency of the 46,XX upid(AC)mat cells in leukocytes (thus, (1 – X) denotes the frequency of 45,X cells in leukocytes). Then, the non-maternally (paternally) derived 184 bp peak is generated by one paternally derived chromosome in the 45,X cells, i.e., (1 – X), and the maternally derived 198 bp peak is formed by the products from two maternally derived homologous chromosomes in the 46,XX upid(AC)mat cells and one maternally derived chromosome in the 45,X cells, i.e., (2X + (1 – X)) = (X + 1). Thus, the AUC ratio between the two peaks is represented as (1 – X):(X + 1) = 0.09:1.0, and "X" is obtained as 0.835 (83.5%). Similarly, when "Y" represents the frequency of the 46,XX upid(AC)mat cells in salivary cells, "Y" is obtained as 0.574 (57.4%). Furthermore, when "Z" represents the frequency of the 46,XX upid(AC)mat cells in buccal epithelium cells, "Z" is obtained as 0.183 (18.3%) on the basis of the assumption that salivary cells comprises 40% of buccal epithelium cells and 60% of leukocytes.

We performed such calculations for all the informative loci, and the mean frequency is determined as 84% in leukocytes, 56% in saliva cells, and 18% in epithelial buccal cells, as described in the main text.

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