演題名番号：

Title：

Secular decrease in chromosomal mosaicism in cultured and uncultured peripheral blood cells of six patients with mosaic Down syndrome

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We present secular changes in the mosaic ratio of six patients with mosaic DS.

Background

- As noted in a review paper (Papavassiliou 2014), a large-scale study revealed that approximately 1.1%–3.8% of all children with Down syndrome (DS) are born with mosaic DS (Hook 1999, Morris 2012).
- Down syndrome is diagnosed postnatally by chromosome G-banding analysis of peripheral blood cultures.
- Most patients do not undergo subsequent repeat analysis.

Patients

- Six mosaic DS patients aged between 3 and 23 years
- Six adult patients with standard trisomy 21 and one patient with translocated trisomy 21

Methods: Chromosome analyses

Low-invasive chromosomal analyses of 100 cells per patient from different tissues were performed (SRL, Inc. Japan).

(1) T lymphocyte chromosome analyses of cultured peripheral blood samples by PHA (phytohemagglutinin) stimulation, including (1-a) **G-banding** and (1-b) **chr21 FISH** analysis using chromosome 21q22.13-q22.2 probes (Vysis, Abbott Molecular, USA)
(2) **Chr21 FISH** analysis of **uncultured** blood cells, including various leukocytes, instead of bone marrow puncture
(3) **Chr21 FISH** analysis of epithelial cells from the **buccal mucosa**
(4) **Chr21 FISH** analysis of **fibroblasts** cultured from a tooth extraction.

Results

The six patients (A–F) with mosaic DS (five females and one male) were aged from 3 years and 3 months to 23 years (Table 1). Five patients (**B–F**) aged between 7 and 23 years showed **drastically decreased mosaic ratios** (1%–8%) by G-banding, and FISH analysis of the peripheral blood culture and of uncultured blood cells compared with the postnatal result (Table 1, red). Patient **A** showed **a gradual decrease in mosaicism** on G-banding and FISH of the peripheral blood culture and by FISH analysis of uncultured blood cells at 11 months, 2 years and 9 months and 3 years and 3 months of age compared with the postnatal result (Table 1, red). However, **none of the six patients** showed a decreased mosaic ratio in the buccal mucosa analysis or tooth-extraction-derived-fibroblast FISH analysis.

Table 1 Chromosomal analyses of the mosaic ratio of six patients with mosaic Down syndrome.

Patient	Sex	Age at examination	Material and Method †	Cell count number	Signal number by FISH one: two: three	Trisomy ratio (%)
(A)	F	shortly after birth	(1-a) blood G ‡§	30		63
		0y10 m	(1-a) blood G ‡	100		47
			(1-b) blood FISH	100	0:58:42	42
			(2) uncultured FISH	100	2:64:34	34
			(3) buccal mucosa FISH	100	0:60:40	40
		2y9 m	(1-a) blood G ‡	100		29
			(1-b) blood FISH	100	0:72:28	28
			(2) uncultured FISH	200	6:152:42	21
			(3) buccal mucosa FISH	100	2:46:52	52
		3y3 m	(1-a) blood G ‡	100		37
			(1-b) blood FISH	100	0:64:36	36
			(2) uncultured FISH	100	0:73:27	27
			(3) buccal mucosa FISH	100	0:66:34	34
(B)	M	shortly afterbirth	(1-a) blood G §	unknown		51
		7y1 m	(1-a) blood G	100		8
			(1-b) blood FISH	100	0:96:4	4
			(3) buccal mucosa FISH	100	0:40:60	60
		7y9 m	(2) uncultured FISH	100	1:94:5	5
		8y3 m	(4) fibroblast FISH	100	0:76:24	24
(C)	F	0y0m5d	(1-a) blood G §	20		75
			(1-b) blood FISH §	100	0:33:67	67
		10y8 m	(1-a) blood G	100		5
			(1-b) blood FISH	100	0:96:4	4
			(2) uncultured FISH	100	2:96:2	2
			(3) buccal mucosa FISH	100	0:35:65	65
(D)	F	shortly after birth	(1-b) blood FISH §	1000	0:601:399	40
		11y9 m	(1-a) blood G	100		7
			(1-b) blood FISH	100	0:95:5	5
			(2) uncultured FISH	100	1:93:6	6
			(3) buccal mucosa FISH	54	0:26:28	52
		12y4 m	(3) buccal mucosa FISH	100	1:54:45	45
(E)	F	0y11 m	(1-a) blood G §	unknown		12
			(5) skin fibro G §	unknown		10
		23y0 m	(1-a) blood G	100		6
			(1-b) blood FISH	100	0:97:3	3
			(2) uncultured FISH	100	1:98:1	1
			(3) buccal mucosa FISH	100	4:74:22	22
(F)	F	shortly after birth	(1-a) blood G §	20		65
		20y7 m	(1-a) blood G	100		6
		22y10 m	(1-b) blood FISH	100	0:97:3	3
			(2) uncultured FISH	100	6:88:6	6
			(3) buccal mucosa FISH	100	0:37:63	63
		22y11 m	(4) fibroblast FISH	100	0:37:63	63

Abbreviations: F female, M male
†(1-a) blood G: G-banding of peripheral blood culture by PHA stimulation
(1-b) blood FISH: chromosome 21 FISH of peripheral blood culture by PHA stimulation
(2) uncultured FISH: chromosome 21 FISH of uncultured blood cells
(3) buccal mucosa FISH: chromosome 21 FISH of epithelial cells of the buccal mucosa
(4) fibroblast FISH: chromosome 21 FISH of fibroblast culture from a tooth extraction
(5) skin fibro G: G-banding of skin fibroblast culture performed at another hospital
‡ inv(9)(p12q13)
§ Analysis at another hospital

Table 2 Chromosomal analyses of the trisomy ratio of eight patients, seven with standard trisomy 21 and one translocated trisomy 21 (patient H).

Patient	Sex	Age at examination	Material and Method †	Cell count number	Signal number by FISH one: two: three	Trisomy ratio (%)
(G)	M	16y9 m	(1-a) blood G	100		100
			(1-b) blood FISH	100	0:0:100	100
			(2) uncultured FISH	200	0:6:94	94
			(3) buccal mucosa FISH	100	1:11:88	88
(H)	M	17y5 m	(1-a) blood G ‡	100		100
			(1-b) blood FISH	100	0:0:100	100
			(2) uncultured FISH	100	0:10:90	90
			(3) buccal mucosa FISH	100	0:21:79	79
(I)	M	19y8 m	(1-a) blood G	100		100
			(1-b) blood FISH	100	0: 0:100	100
			(2) uncultured FISH	100	0:10:90	90
			(3) buccal mucosa FISH	100	0:16:84	84
(J)	F	28y10 m	(1-a) blood G	100		99
			(1-b) blood FISH	100	0: 0:100	100
			(3) buccal mucosa FISH	100	0:2:98	98
(K)	M	32y2 m	(1-a) blood G	100		97
			(1-b) blood FISH	100	0:1:99	99
			(2) uncultured FISH	100	0:8:92	92
			(3) buccal mucosa FISH	100	0:6:94	94
(L)	F	32y5 m	(1-a) blood G	100		99
			(1-b) blood FISH	100	0:0:100	100
			(3) buccal mucosa FISH	100	0:10:90	90
(M)	M	38y4 m	(1-a) blood G	100		99
			(1-b) blood FISH	100	0:1:99	99
			(2) uncultured FISH	100	5:20:75	75
			(3) buccal mucosa FISH	100	0:38:62	62
(N)	M	38y6 m	(1-a) blood G	100		99
			(1-b) blood FISH	150	0:1:149	99.3
			(3) buccal mucosa FISH	100	0:7:93	93

Abbreviations: F female, M male
† (1-a) blood G: G-banding of peripheral blood culture by PHA stimulation
(1-b) blood FISH: chromosome 21 FISH of peripheral blood culture by PHA stimulation
(2) uncultured FISH: chromosome 21 FISH of uncultured blood cells
(3) buccal mucosa FISH: chromosome 21 FISH of epithelial cells of the buccal mucosa
‡ 46,XY,inv(1)(p13q21)pat,i(21)(q10)

Discussion

Previously, two reports documented a decreased mosaic ratio in mosaic DS patients during early childhood, mainly in peripheral blood cultures (Taylor 1968, Wilson 1980). We studied longitudinal changes in the mosaic ratio of six mosaic DS patients. No decrease was observed in the epithelial cells of the buccal mucosa or cultured fibroblasts. The observed secular decrease in the mosaic ratio in cultured and uncultured peripheral blood cells did **not** appear to be a mechanism of trisomy rescue because six adult patients with standard trisomy 21 and one patient with translocated trisomy 21 showed **no decrease** in trisomy cell number in cultured and uncultured peripheral blood cells over time (Table 2, green). Within the bone marrow, hematopoietic stem cells exist in a hypoxic microenvironment (niche) and are in a quiescent (G0), undifferentiated state (Spencer 2014). In this microenvironment, trisomy cells are considered more detrimental to viability than normal cells, which may explain the secular decrease in chromosomal mosaicism with mosaic DS in this study.

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ETHICS APPROVAL
This study has received ethical approval from Tokyo Kasei University Ethics Committee (Ita h-29-5) and the Tokyo Metropolitan Tobu Medical Center for Children with Developmental Disabilities Ethics Committee (29MoriTobuCe256-1).

CONFLICT OF INTEREST

We have no financial relationships to disclose.