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ORIGINAL ARTICLE

Application of Interphase Fluorescent in Situ Hybridization: a Screening Tool for the Diagnosis of Microdeletion Syndrome

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SUMMARY

Background: **Most laboratories adopt the results of metaphase fluorescent in situ hybridization (FISH) for the diagnosis of microdeletion syndromes. To investigate the discrepancy between the results of interphase and metaphase, we compared the quantitative results of FISH for 5 kinds of microdeletion syndrome and gender determination disorders (SDD).**

Methods: **A total of 282 (135 for DiGeorge syndrome, 20 for Kalmann syndrome, 7 for Miller-Dieker syndrome, 38 for Prader Willi/Angelman syndrome, 62 for Williams syndrome, and 20 for SDD (*SRY* FISH)) were enrolled. For *SRY* FISH, we artificially mixed fresh blood of male and female with various ratios and then compared the results of metaphase and interphase *SRY* FISH. Using a bio-cell chip, we performed interphase FISH in 168 patients with microdeletion syndromes and compared the results with manual interphase.**

Results: **The concordance rate between the results of metaphase and interphase was 100% in microdeletion syndrome. In the disorders of gender development, *SRY* FISH showed 100% concordance between interphase and metaphase when we counted 50 metaphase cells and 100 interphase cells. Comparison with mixtures of male and female blood at various ratios also showed 100% concordance. The results of bio-cell chip showed 100% concordance between previous interphase FISH results.**

Conclusions: **Considering the complete concordance between interphase and metaphase in microdeletion syndrome, the application of interphase FISH without performing metaphase FISH can be a screening test for microdeletion syndrome. Confirmation by metaphase FISH can be performed only in cases with abnormal results by interphase FISH.**

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Supplementary Tables and Figures

Table S1. Results of *SRY* probe with artificially mixed peripheral blood samples - Analyst 1 vs. Analyst 2.

			Vysis Probe XY/total counted cells			Cytocell Probe XY/total counted cells		
	Peripheral blood mixed ratio		Analyst 1	Analyst 2	p-value	Analyst 1	Analyst 2	p-value
1	M:F = 1:99	Interphase	2/200 (1%)	4/200 (2%)	0.6851	4/200 (2%)	2/200 (1%)	0.6851
		Metaphase	1/50 (2%)	2/50 (4%)	1.0000	0/50 (0%)	1/50 (2%)	1.0000
2	M:F = 5:95	Interphase	9/200 (4.5%)	14/200 (7%)	0.3908	16/200 (8%)	13/200 (6.5%)	0.7004
		Metaphase	4/50 (8%)	4/50 (8%)	1.0000	3/50 (10%)	3/50 (6%)	1.0000
3	M:F = 10:90	Interphase	21/200 (10.5%)	22/200 (11%)	0.2883	15/200 (7.5%)	23/177 (11.5%)	0.2323
		Metaphase	4/50 (8%)	5/50 (10%)	1.0000	3/50 (6%)	6/50 (12%)	0.4870
4	M:F = 15:85	Interphase	32/200 (16%)	26/200 (13%)	0.4779	29/200 (14.5%)	31/200 (15.5%)	0.8888
		Metaphase	6/50 (12%)	9/50 (18%)	0.5766	5/50 (10%)	9/50 (18%)	0.3881
5	M:F = 20:80	Interphase	38/200 (19%)	40/200 (20%)	0.8996	39/200 (19.5%)	37/200 (18.5%)	0.8987
		Metaphase	12/50 (24%)	11/50 (22%)	1.0000	9/50 (18%)	9/50 (18%)	1.0000
6	M:F = 30:70	Interphase	71/200 (35.5%)	75/200 (37.5%)	0.7554	59/200 (29.5%)	53/200 (26.5%)	0.5778
		Metaphase	20/50 (40%)	16/50 (32%)	0.5323	20/50 (40%)	15/50 (30%)	0.4019
7	M:F = 40:60	Interphase	94/200 (47%)	93/200 (46.5%)	1.0000	98/200 (49%)	89/200 (44.5%)	0.4228
		Metaphase	23/50 (46%)	22/50 (44%)	1.0000	23/50 (46%)	23/50 (46%)	1.0000
8	M:F = 50:50	Interphase	103/200 (51.5%)	98/200 (49%)	0.6892	101/200 (50.5%)	98/200 (49%)	0.8415
		Metaphase	29/50 (58%)	26/50 (52%)	0.6879	27/50 (54%)	26/50 (52%)	1.0000
9	M:F = 60:40	Interphase	128/200 (64%)	126/200 (63%)	0.9173	124/200 (62%)	129/200 (64.5%)	0.6783
		Metaphase	33/50 (66%)	30/50 (60%)	0.6790	32/50 (64%)	28/50 (56%)	0.5406
10	M:F = 70:30	Interphase	145/200 (72.5%)	155/200 (77.5%)	0.2987	135/200 (67.5%)	137/200 (68.5%)	0.9147
		Metaphase	35/50 (70%)	39/50 (78%)	0.4945	36/50 (72%)	33/50 (66%)	0.6658
11	M:F = 80:20	Interphase	168/200 (84%)	167/200 (83.5%)	0.1277	165/200 (82.5%)	166/200 (83%)	1.0000
		Metaphase	41/50 (82%)	43/50 (86%)	0.7858	42/50 (84%)	41/50 (82%)	1.0000
12	M:F = 85:15	Interphase	178/200 (89%)	175/200 (87.5%)	0.7565	181/200 (90.5%)	175/200 (87.5%)	0.4246
		Metaphase	43/50 (86%)	46/50 (92%)	0.5246	45/50 (90%)	41/50 (82%)	0.3881
13	M:F = 90:10	Interphase	190/200 (95%)	188/200 (94%)	0.8270	184/200 (92%)	183/200 (91.5%)	1.0000
		Metaphase	45/50 (90%)	46/50 (92%)	1.0000	46/50 (92%)	46/50 (92%)	1.0000
14	M:F = 95:5	Interphase	194/200 (97%)	195/200 (97.5%)	1.0000	185/200 (92.5%)	192/200 (96%)	0.1966
		Metaphase	46/50 (92%)	48/50 (86%)	0.6777	46/50 (92%)	47/50 (94%)	1.0000
15	M:F = 99:1	Interphase	197/200 (98.5%)	196/200 (98%)	1.0000	198/200 (99%)	196/200 (98%)	0.6851
		Metaphase	49/50 (98%)	49/50 (98%)	1.0000	50/50 (100%)	50/50 (100%)	1.0000

Table S2. Results of *SRY* probe with artificially mixed peripheral blood samples - Metaphase vs. Interphase.

			Vysis Probe XY/total counted cells			Cytocell Probe XY/total counted cells		
	Peripheral blood mixed ratio		Interphase	Metaphase	p-value	Interphase	Metaphase	p-value
1	M:F = 1:99	Analyst 1	2/200 (1%)	1/50 (2%)	0.4895	4/200 (2%)	0/50 (0%)	0.5867
		Analyst 2	4/200 (2%)	2/50 (4%)	0.3446	2/200 (1%)	1/50 (2%)	0.4895
2	M:F = 5:95	Analyst 1	9/200 (4.5%)	4/50 (8%)	0.2995	16/200 (8%)	3/50 (10%)	0.7728
		Analyst 2	4/200 (7%)	4/50 (8%)	0.0533	13/200 (6.5%)	3/50 (6%)	1.000
3	M:F = 10:90	Analyst 1	21/200 (10.5%)	4/50 (8%)	0.7934	15/200 (7.5%)	3/50 (6%)	1.000
		Analyst 2	22/200 (11%)	5/50 (10%)	1.000	23/177 (11.5%)	6/50 (12%)	1.000
4	M:F = 15:85	Analyst 1	32/200 (16%)	6/50 (12%)	0.6597	29/200 (14.5%)	5/50 (10%)	0.4950
		Analyst 2	26/200 (13%)	9/50 (18%)	0.3663	31/200 (15.5%)	9/50 (18%)	0.6687
5	M:F = 20:80	Analyst 1	38/200 (19%)	12/50 (24%)	0.4332	39/200 (19.5%)	9/50 (18%)	1.000
		Analyst 2	40/200 (20%)	11/50 (22%)	0.8445	37/200 (18.5%)	9/50 (18%)	1.000
6	M:F = 30:70	Analyst 1	71/200 (35.5%)	20/50 (40%)	0.6226	59/200 (29.5%)	20/50 (40%)	0.1746
		Analyst 2	75/200 (37.5%)	16/50 (32%)	0.5143	53/200 (26.5%)	15/50 (30%)	0.5993
7	M:F = 40:60	Analyst 1	94/200 (47%)	23/50 (46%)	1.000	98/200 (49%)	23/50 (46%)	0.7531
		Analyst 2	93/200 (46.5%)	22/50 (44%)	0.8741	89/200 (44.5%)	23/50 (46%)	0.8747
8	M:F = 50:50	Analyst 1	103/200 (51.5%)	29/50 (58%)	0.4326	101/200 (50.5%)	27/50 (54%)	0.7521
		Analyst 2	98/200 (49%)	26/50 (52%)	0.7531	98/200 (49%)	26/50 (52%)	0.7531
9	M:F = 60:40	Analyst 1	128/200 (64%)	33/50 (66%)	0.8696	124/200 (62%)	32/50 (64%)	0.8710
		Analyst 2	126/200 (63%)	30/50 (60%)	0.7450	129/200 (64.5%)	28/50 (56%)	0.3264
10	M:F = 70:30	Analyst 1	145/200 (72.5%)	35/50 (70%)	0.0880	135/200 (67.5%)	36/50 (72%)	0.6120
		Analyst 2	155/200 (77.5%)	39/50 (78%)	1.000	137/200 (68.5%)	33/50 (66%)	0.7371
11	M:F = 80:20	Analyst 1	168/200 (84%)	41/50 (82%)	1.000	165/200 (82.5%)	42/50 (84%)	1.000
		Analyst 2	167/200 (83.5%)	43/50 (86%)	0.8299	166/200 (83%)	41/50 (82%)	0.8363
12	M:F = 85:15	Analyst 1	178/200 (89%)	43/50 (86%)	0.6211	181/200 (90.5%)	45/50 (90%)	1.000
		Analyst 2	175/200 (87.5%)	46/50 (92%)	0.4659	175/200 (87.5%)	41/50 (82%)	0.3556
13	M:F = 90:10	Analyst 1	190/200 (95%)	45/50 (90%)	0.1893	184/200 (92%)	46/50 (92%)	1.000
		Analyst 2	188/200 (94%)	46/50 (92%)	0.5340	183/200(91.5%)	46/50 (92%)	1.000
14	M:F = 95:5	Analyst 1	194/200 (97%)	46/50 (92%)	0.1165	185/200 (92.5%)	46/50 (92%)	1.000
		Analyst 2	195/200 (97.5%)	48/50 (86%)	0.6297	192/200 (96%)	47/50 (94%)	0.4637
15	M:F = 99:1	Analyst 1	197/200 (98.5%)	49/50 (98%)	1.000	198/200 (99%)	50/50 (100%)	1.000
		Analyst 2	196/200 (98%)	49/50 (98%)	1.000	196/200 (98%)	50/50 (100%)	0.5867

Abbreviations: M - male, F - female.

Table S3. Approximate numbers of Metaphases or Interphases required to identify Mosaicisms or Chimerisms at 95% or 99% power by using probes with analytical sensitivities of 90%, 95%, or 99% [1].

Frequency (%) of second cell line [b]	95% Power [a]			99% Power [a]		
	Analytical sensitivity			Analytical sensitivity		
	90%	95%	99%	90%	95%	99%
≥ 50	11	7	5	18	12	8
≥ 40	19	12	7	30	19	12
≥ 30	39	20	11	61	33	18
≥ 20	133	46	19	203	74	34
≥ 10	ID	291	54	ID	447	92
≥ 5	ID	ID	171	ID	ID	282

[a] Power in this context is the probability that the test rejects the null hypothesis, H_0, when, in fact, H_0 is false. [b] Frequency = 1 - p_a.
Abbreviations: ID - indistinguishable form background.

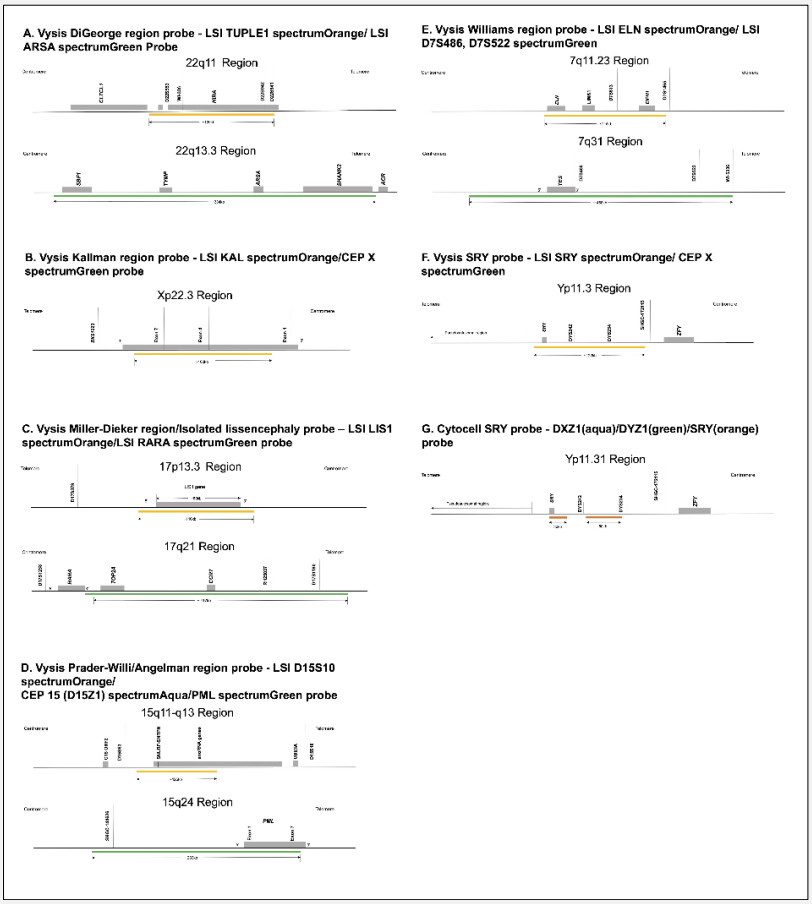

Figure S1. Probe size and map of each probe; (A) Vysis DiGeorge region probe - LSI TUPLE1 spectrumOrange/ LSI ARSA spectrumGreen Probe; (B) Vysis Kallman region probe - LSI KAL spectrumOrange/CEP X spectrumGreen probe; (C) Vysis Miller-Dieker region/Isolated lissencephaly probe - LSI LIS1 spectrumOrange/LSI RARA spectrumGreen probe; (D) Vysis Prader-Willi/Angelman region probe - LSI D15S10 spectrumOrange/CEP 15 (D15Z1) spectrumAqua/PML spectrumGreen probe; (E) Vysis Williams region probe - LSI ELN spectrumOrange/ LSI D7S486, D7S522 spectrumGreen; (F) Vysis SRY probe - LSI SRY spectrumOrange/ CEP X spectrumGreen; and (G) Cytocell SRY probe - DXZ1(aqua)/DYZ1(green)/ SRY(orange) probe.

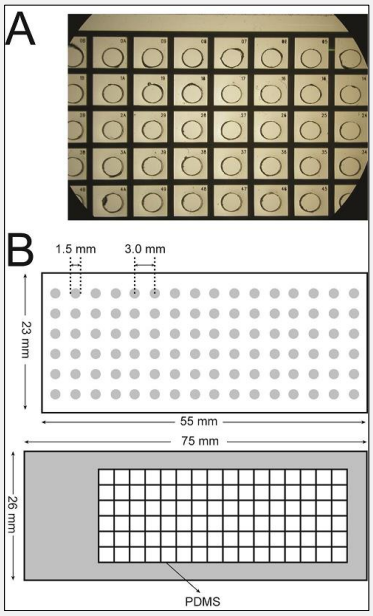

Figure S2. Fabricated bio-cell chip. (A) A perforated polydimethylsiloxane (PDMS) layer on the slide glass forms a total of 96 wells which are located within each lattice marked by an indexing number. (B) A blueprint of bio-cell chip [2].

References:

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