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The Phenotype of Persons Having Mosaicism for Trisomy 21/Down Syndrome Reflects the Percentage of Trisomic Cells Present in Different Tissues

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Little is known about the pathogenesis of the phenotype in individuals with trisomy 21 mosaicism and Down syndrome. The primary goal of this study was to identify factors contributing to the observed phenotypic variation by evaluating 107 individuals having trisomy 21 mosaicism. To investigate a potential "threshold" effect due to trisomic imbalance, lymphocyte and buccal mucosa nuclei were scored using FISH. Overall, buccal cells showed a significantly higher frequency of trisomy than lymphocytes ($P < 0.0001$). Using latent class analysis, two phenotypic classes were identified based on the clinical findings of the propositi. Patients from class 1 had significantly fewer traits and a lower percentage of trisomic cells (mean of 37.3% lymphocytes; 34.5% buccal mucosa cells) when compared to those stratified into class 2 (54.0% lymphocytes; 53.4% buccal mucosa cells). Tissue-specific influences were also detected, with buccal mucosa trisomy levels being significantly correlated with IQ ($P = 0.0094$; both ectodermal derivatives), while congenital heart defects were significantly correlated with lymphocytes ($P = 0.0286$; both mesodermal embryonic derivatives). In conclusion, allowing for the distinction of two groups, we observed variation in phenotype, associated with the percentage of trisomic cells. We also observed tissue-specific effects on phenotype. The results of this study should enable geneticists and other health care professionals to provide information regarding optimal diagnostic approaches and anticipated clinical outcomes.

Key words: trisomy 21 mosaicism; mosaicism; Down syndrome; karyotype/phenotype correlation; fluorescence in situ hybridization; aneuploidy

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INTRODUCTION

Down syndrome is the most common chromosomal abnormality in live-born individuals, occurring at a frequency of about 1/800 live births [reviewed by Patterson and Costa, 2005]. Most (90–95%) individuals with Down syndrome have trisomy for chromosome 21 [Pangalos et al., 1994]. Two to four percent of individuals with Down syndrome have a trisomic dose of the long arm of chromosome 21 as a result of a structural chromosomal abnormality (translocation or isochromosome) [Pangalos et al., 1994]. Mosaicism is seen in another two to four percent of individuals diagnosed with Down syndrome [Hamerton et al., 1965; Richards, 1969; Mikkelsen, 1977; Hook, 1981]. Mosaicism is a condition in which an individual has two or more genetically distinct cell lines that develop from a single zygote [Nussbam et al., 2001]. In the case of trisomy 21 mosaicism and Down syndrome, affected individuals have both trisomic (47,XX,+21 or 47,XY,+21) and euploid (46,XX or 46,XY) cell lines. The primary goal of this study was to determine if there are correlations between the phenotypic

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traits and the proportion of trisomic cells present in individuals having mosaicism.

An investigation involving individuals with trisomy 21 mosaicism can further our understanding of the mechanisms underlying the phenotype of Down syndrome since individuals with trisomy 21 mosaicism show a broad spectrum of clinical findings, ranging from traits typically seen in "complete" trisomy 21 (people having trisomy 21 in every cell) to that of a near normal phenotype [Finley et al., 1966; Shipe et al., 1968; Johnson and Abelson, 1969; Fishler and Koch, 1991; Bhatt et al., 1995]. Our knowledge of factors influencing the clinical outcome in individuals with MDS has been limited due to the relatively small number of cases of this rare condition that are available for study, with most investigators presenting either single case reports or findings from a small number of patients [reviewed by Papavassiliou, 2007]. These previously reported investigations were also accomplished prior to the advent of fluorescent in situ hybridization (FISH) techniques, and were thus limited to the study of metaphase chromosomes following in vitro culturing, the latter of which could potentially skew the levels of trisomy detected.

Possible factors contributing to the broad spectrum of traits observed in persons with trisomy 21 mosaicism include (but are not limited to) variation in the proportion of trisomic cells present: (1) between individuals; and (2) from tissue to tissue within and/or between individuals. In this study of persons having trisomy 21 mosaicism, we quantified and compared the percentage of trisomic cells present in nuclei from both lymphocytes (cultured and uncultured) and buccal mucosa cells (uncultured). Thus, the level of trisomy was not only assessed in different tissues, but also from cells with and without the effect of in vitro culturing. The phenotypic profiles of the study subjects were also analyzed, using a latent class analysis (LCA), to determine if subgroups could be identified based on the patients' phenotype, and if these distinct groups could be distinguished from one another based on their ratio of euploid to trisomic cells. The specific aims of this study were to test the following hypotheses: (1) The proportion of trisomic cells present in different tissues (blood and buccal mucosa) influences the phenotypic outcome associated with mosaicism for trisomy 21 in Down syndrome; and (2) Trisomic levels in buccal mucosa cells are more closely correlated with phenotypic findings of ectodermal origin, while trisomic levels in lymphocytes are more strongly correlated with findings of mesodermal origin.

MATERIALS AND METHODS

Human Subject Involvement

A total of 107 individuals with mosaicism for trisomy 21 (propositi) was ascertained through parental support groups [National Down Syndrome Congress (NDSC), International Mosaic/Down Syndrome Association meetings, newsletters, and websites]. The only selection criterion was that the child had a chromosomally confirmed diagnosis of mosaicism for trisomy 21. After giving their informed consent (assent for children) (Virginia Commonwealth University IRB Committee, protocol # 0179) the study participants had the option to provide: (1) blood samples; (2) buccal smears (one from each cheek); (3) a completed questionnaire (which included queries regarding physical characteristics, health history, developmental progress, pregnancy history, and lifestyle); (4) pictures; (5) medical records; and/or (6) IQ reports/developmental information. Participating families could elect to provide all or only a portion of the items noted above. Questionnaire and medical record information was also collected from individuals with complete trisomy 21 (positive control group), who were also ascertained through parental support groups.

Evaluation of the Proportion of Cells With Trisomy 21

Lymphocyte preparations. Duplicate lymphocyte cultures were established and harvested according to standard protocols [Moorhead et al., 1960]. In order to quantify the proportion of trisomic cells in uncultured specimens, peripheral blood smear slides were made from each sample according to standard protocols.

Buccal mucosa cellular preparations. Buccal mucosa slides were made by the study subjects (or with parental assistance for younger subjects). The cells were collected by the subjects rubbing the inside of their cheek with a toothbrush and then spreading the collected cells directly onto positively charged slides (Fisher Scientific, Pittsburgh, PA). This procedure was repeated for the other cheek, with both slides being air-dried prior to shipment of the specimens. Upon receipt in the lab, these buccal smear slides were stored at −20°C until FISH was performed (approximately 1 week to 2 months later).

FISH methodology. The number of chromosomes 21 in interphase nuclei from cultured and uncultured lymphocytes and uncultured buccal mucosa cells was determined using FISH. The test probe used was one that is localized to 21q22.13–21q22.2 (D21S259\D21S341\D21S342) (Abbott Laboratories, Des Plaines, Illinois). In addition, a control probe for chromosome 13 (RB1 locus at 13q14; Abbott Laboratories) was also evaluated. The FISH on cultured lymphocytes was performed according to the probe manufacturer's protocol (Abbott Laboratories). FISH on the uncultured specimens (buccal mucosa cells and uncultured lymphocytes) was done as described previously by Leach et al. [2004], with the adaptation that the uncultured lymphocytes were digested in a protease pretreatment solution (NaSCN; Abbott Laboratories) for 10 min rather than 30 min, as is done for the buccal cells.

Scoring frequency of trisomic cells. Probe signals were visualized using a Zeiss epifluorescent Axiskop equipped with single (SpectrumOrange™, SpectrumGreen™) and triple band pass filters. When scoring each cell, all focal planes were viewed to ensure the detection of all signals in the three-dimensional nuclei. Interphase nuclei that did not have clear borders or that were overlapping were excluded from the analysis. A total of 1000 cultured blood lymphocyte nuclei, 500 uncultured blood nuclei, and 500 buccal cells (250 per cheek) were scored for each study participant. Thus, using the probability cutoff value of 0.05, the evaluation of 1000 or 500 cells with our probes (which demonstrated 0.99% analytic sensitivity level) allowed for the detection of as low as 1.6% or 1.8% trisomic cells, respectively [Dewald et al., 1998]. In addition to interphase nuclei, 30 to 50 metaphase spreads were also analyzed from cultured lymphocyte samples.

Phenotype Assessment

The phenotypic findings present in each propositus having mosaicism were identified by certified (American Board of Medical Genetics) medical geneticists. Evaluations were done using one or more of the following assessment tools: (1) direct physical examination; (2) evaluations of photographs; and/or (3) a review of survey responses and medical records, the latter of which were obtained, with permission, as described above. Clinically relevant findings were summarized based on the criteria established by Epstein et al. [1991].

Statistical Analyses and Data Interpretation

A paired *t*-test was used to compare the frequencies of trisomic cells present amongst the collected samples (e.g., cultured blood vs. uncultured blood; cultured blood vs. buccal mucosa cells). Trait comparisons were done using a Pearson correlation analysis or a contingency Chi-square test. All statistical comparisons were performed using a significance level of $\alpha = 0.05$.

Latent class analysis (LCA) was used to model subtypes of mosaicism by representing them as latent ("unmeasured") variables [Muthén and Muthén, 1998]. Models were tested with 1, 2, and 3 classes. The model parameters were: (1) the prevalence of each latent class; and (2) the conditional response probabilities (the probability that a randomly selected member of particular latent class would have a positive response to a query regarding a particular phenotypic item). A subset of 25 phenotypic variables (Tables II and III) that were reported (present or absent) in 65% or more of the cases was used in the LCA analysis.

RESULTS

To date, a total of 107 individuals having mosaicism for trisomy 21 and Down syndrome have been ascertained from families living in the USA, Australia, Canada, Denmark, England, Ireland, Israel, and Portugal. The samples/data materials provided by these

TABLE I. Summary of Data Collected and Mean Trisomic Levels for Study Subjects

		Lymphocytes			
		Cultured		Research Study	
Sample/information collected	Number of samples	Diagnostic metaphases	Nuclei	Metaphases	Uncultured nuclei	Buccal nuclei
Blood[a]						
Diagnostic reports	95	51.53 ± 2.92 (4.0–95.0)	—	—	—	—
Cultured lymphocytes	70	—	35.12 ± 3.52 (3.3–97.0)	—	—	—
Uncultured lymphocytes	44	—	—	—	30.37 ± 3.80 (5.0–91.0)	—
Buccal smears[b]	59	—	—	—	—	48.89 ± 3.02 (4.0-89.4)
Paired specimens[c]						
Diagnostic cultured blood/ research cultured blood	58	46.31 ± 3.76 (4.0–94.0)	33.63 ± 3.57 (3.3–91.6)	—	—	—
Research						
Cultured blood/uncultured blood	44	—	33.15 ± 4.72 (3.3–93.3)	—	30.37 ± 3.80 (5.0–91.0)	—
Cultured blood/buccal	49	—	30.13 ± 3.99 (3.6–93.3)	—	—	48.06 ± 3.44 (4.0–89.4)
Cultured blood interphase/ cultured blood metaphase	57	—	34.06 ± 4.02 (4.8–94.4)	34.15 ± 4.12 (3.3–93.3)	—	—
Phenotypic information						
Questionnaire	90	—	—	—	—	—
Pictures	60	—	—	—	—	—
Medical records	104	—	—	—	—	—
Direct physical exam	23	—	—	—	—	—
IQ reports	55	—	—	—	—	—
Developmental information	81	—	—	—	—	—

[a]The mean ± standard error (range) is shown for all subjects for whom this lymphocyte data was available.
[b]The mean ± standard error (range) is shown for all subjects for whom this buccal mucosa cell data was available.
[c]The mean ± standard error (range) is shown for all subjects for whom paired comparisons were available (e.g., not all study subjects providing buccal samples also provided blood samples (and vice versa), so the number of paired specimens is less than the total for each specimen type).

TABLE II. Summary of Physical Stigmata Reported in 104 Mosaic Probands

Clinical finding	Proportion[a] having trait	% reporting data (response rate)
Cyanotic CHD[b]	1/99 = 0.010	95%
Celiac disease	1/100 = 0.010	96%
Hirschsprung's disease	1/100 = 0.010	96%
Imperforate anus	1/100 = 0.010	96%
Esophageal stricture	2/100 = 0.020	96%
Congenital/acquired cataracts	2/93 = 0.022	89%
Leukemia	3/98 = 0.031	94%
Duodenal stenosis/atresia	4/100 = 0.040	96%
Cleft palate/uvula	4/43 = 0.093	41%
Umbilical hernia	10/100 = 0.100	96%
GI reflux	11/100 = 0.110	96%
Constipation	12/100 = 0.120	96%
Hypothyroidism	10/80 = 0.125	77%
Obesity	14/91 = 0.154	88%
Hearing loss	17/89 = 0.191	86%
Nystagmus	6/23 = 0.261	22%
ADHD	14/44 = 0.318	42%
Atlanto-axial instability	14/42 = 0.333	40%
Acyanotic CHD[b]	43/103 = 0. 417	99%
Presence of any CHD[b]	44/103 = 0.427	99%
Obstructed lacrimal ducts	12/28 = 0.429	27%
Dry skin	39/91 = 0.429	88%
Malformed teeth	20/46 = 0.435	44%
Narrow ear canals	14/31 = 0.452	30%
High arched palate	21/45 = 0.467	43%
Strabismus	27/58 = 0.466	56%
Single transverse palmar crease	44/90 = 0.489	87%
Protruding tongue	51/101 = 0.505	97%
PE tube placement(s)	40/78 = 0.513	75%
Frequent ear infections	45/97 = 0.464	93%
Myopia/hyperopia	47/90 = 0.522	87%
Brushfield spots	28/48 = 0.583	46%
Webbing, short, and/or broad neck	56/96 = 0.583	92%
Incurved 5th finger	30/50 = 0.600	48%
Stubborn behavior	29/48 = 0.604	46%
Epicanthal folds	61/98 = 0.622	94%
Small ears	46/74 = 0.622	71%
Short hands and fingers	29/45 = 0.644	43%
Low set ears	48/74 = 0.649	71%
Hyper-extensibility of joints	61/93 = 0.656	89%
Hypotonia	65/98 = 0.663	94%
Thin, and/or sparse hair	66/98 = 0.673	94%
Up-slanted eyes	76/100 = 0.760	96%
Brachycephaly	54/71 = 0.761	68%
Flat face	78/100 = 0.780	96%
Flat nasal bridge	68/80 = 0.850	77%
Increased gap between 1st and 2nd toes	44/51 = 0.863	49%

[a]Number of findings present/total number reported (present or absent).
[b]Specific CHD findings are noted in Table IV.

TABLE III. Summary of Physical Stigmata Reported in 54 Individuals With Non-Mosaic Down Syndrome (Positive Controls)

Clinical finding	Proportion having trait[a]	% reporting data (response rate)
Celiac disease	0/54 = 0	100%
Esophageal stricture	0/54 = 0	100%
Umbilical hernia	0/54 = 0	100%
Cyanotic CHD[b]	1/54 = 0.019	100%
Hirschsprung's disease	1/54 = 0.019	100%
Imperforate anus	1/53 = 0.019	98%
Leukemia	1/53 = 0.019	98%
Duodenal stenosis/atresia	3/54 = 0.056	100%
GI reflux	3/54 = 0.056	100%
Congenital/acquired cataracts	4/52 = 0.077	96%
Hearing loss	4/49 = 0.082	91%
Atlanto-axial instability	3/20 = 0.150	37%
Constipation	10/54 = 0.185	100%
ADHD	4/16 = 0.250	30%
Hypothyroidism	13/43 = 0.302	80%
Nystagmus	5/16 = 0.313	30%
Obesity	19/53 = 0.358	98%
Obstructed lacrimal ducts	9/25 = 0.360	46%
Strabismus	9/23 = 0.391	43%
Frequent ear infections	22/53 = 0.415	98%
Brushfield spots	8/19 = 0.421	35%
PE tube placement(s)	24/51 = 0.471	94%
Protruding tongue	25/53 = 0.472	98%
Acyanotic CHD[b]	27/54 = 0.500	100%
Presence of any CHD[b]	28/54 = 0.519	100%
Flat face	27/53 = 0.509	98%
Webbing, short, and/or broad neck	27/52 = 0.519	96%
Myopia/hyperopia	26/50 = 0.520	93%
Incurved 5th finger	13/23 = 0.565	43%
Thin, and/or sparse hair	31/52 = 0.596	96%
Dry skin	36/54 = 0.667	10%
Single transverse palmar crease	36/52 = 0.692	96%
Low set ears	15/21 = 0.714	39%
Flat nasal bridge	19/26 = 0.731	48%
Stubborn behavior	19/26 = 0.731	48%
Small ears	18/24 = 0.750	44%
Increased gap between 1st and 2nd toes	15/20 = 0.750	37%
Short hands and fingers	19/25 = 0.760	46%
Malformed teeth	18/23 = 0.783	43%
Epicanthal folds	43/51 = 0.843	44%
Up-slanted eyes	45/53 = 0.849	48%
Hypotonia	46/54 = 0.852	100%
Hyper-extensibility of joints	46/53 = 0.868	98%
Narrow ear canals	1/1 = 1	2%
Cleft uvula and palate	1/1 = 1	2%
High arched palate	3/3 = 1	6%
Brachycephaly	4/4 = 1	7%

[a]Number of findings present/total number reported (present or absent).
[b]Specific CHD findings are noted in Table IV.

propositi are summarized in Table I. Medical records and completed questionnaires were provided for nearly all (97% and 84%, respectively) individuals having mosaicism. The majority of propositi also elected to provide blood specimens (65%) and/or buccal mucosa specimens (55%). Direct physical examinations were completed for 23 of the individuals having mosaicism (the exams were completed in conjunction with the first and third International Mosaic Down Syndrome Association Conventions). Informative photographs (Fig. 1) were provided for 56% of the propositi, with developmental information being provided for 76% of the study subjects having mosaicism.

Tissue-Specific Assessments of the Percentage of Trisomic Cells

The percentages of trisomic cells in the specimens evaluated for the propositi having mosaicism are summarized in Table I and Figure 2. The range was not significantly different in the cultured lymphocytes (3.3–97%) compared to buccal mucosa cells (4.0–89.4%). However, the mean percentage of trisomic cells noted in the buccal mucosa specimens (48.06% ± 3.44%; mean ± SE) was significantly different from that detected in the cultured lymphocytes (30.13 ± 3.99; $P < 0.0001$), with higher values being seen in 35 of the 49 (71.4%) cases providing both specimens ($P < 0.0001$). Interestingly, the distribution of trisomic cells observed in the cultured lymphocyte specimens showed a non-random pattern (more cases having lower trisomic values). In contrast, the buccal specimen showed a random pattern ($P > 0.05$; Fig. 2).

To determine the effect, if any, of in vitro culturing on lymphocytes, the proportion of trisomic cells present in cultured lymphocytes (33.15 ± 4.72%.) was compared to that observed in the uncultured blood smears (30.37 ± 3.80%). These values were not significantly different ($P > 0.05$; paired t-test) and were positively correlated ($r = 0.97$, $P < 0.0001$).

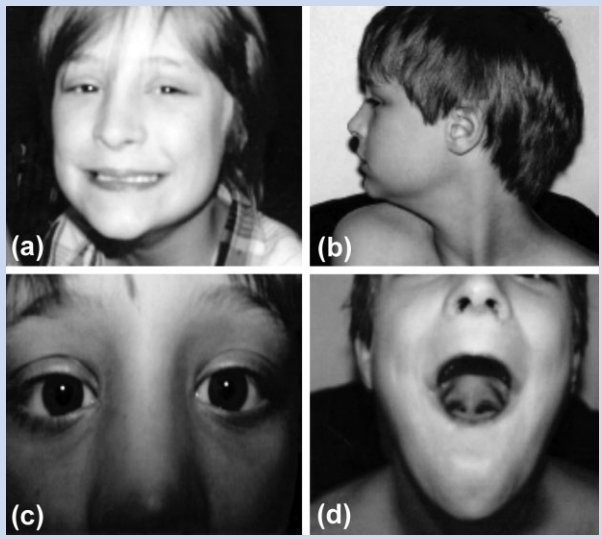

FIG. 1. Evaluation of propositi with mosaicism for trisomy 21/Down syndrome using photographs. This figure shows photographs of an 11-year-old male who had trisomy for chromosome 21 noted in 20% of his lymphocytes in his diagnostic chromosomal report. The trisomy 21 levels observed at the time of his participation in this research study (at age 11) were 50% for his buccal mucosa cells and 7% for his lymphocytes. The photographs shown here are representative of those used to confirm phenotypic findings reported in medical records and by parents. The types of images requested from the families included (but were not limited to) those showing a full facial view (a); a side profile (b); a close-up view of the mid-face (c); and (d) a view of the mouth. This individual's ears are slightly small, but normal in shape and position. He also has a mildly broad, but not flat nasal bridge; subtle epicanthal folds; and a high arched palate. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

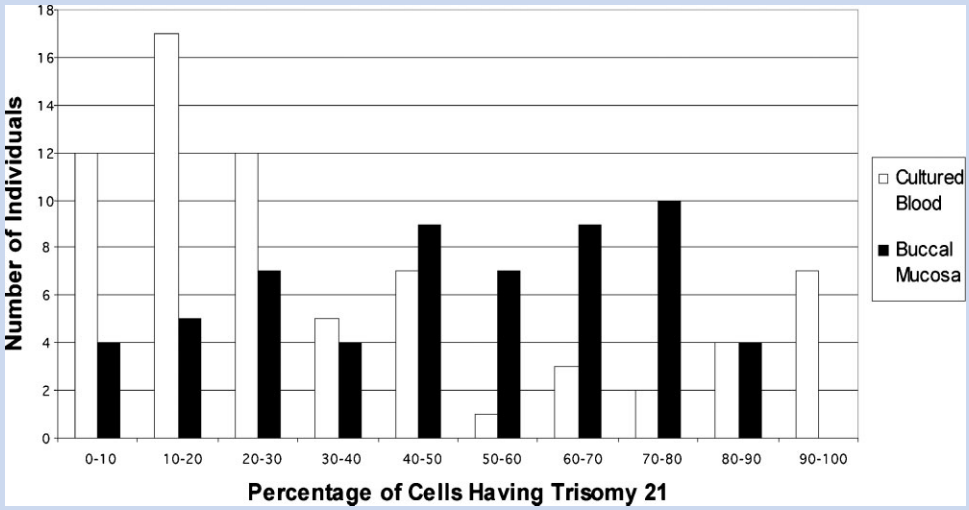

FIG. 2. Distribution of the percentage of the cells of the proposita having trisomy for chromosome 21 in cultured lymphocytes and buccal mucosa cells. For the buccal mucosa specimens, the percentage values observed were not significantly different from random expectations. However, for the cultured lymphocytes, a significantly increased number of cases having trisomy 21 in 30% or fewer cells was observed.

Assessment of Percentage of Trisomic Cells in Diagnostic Compared to Research Samples

The average age of the propositi at the time of diagnosis was 1.8 years and ranged from the prenatal period to 4 years after birth. The average age at the time of our research study was 6.6 years, with participation ages ranging from 1 month after birth to 43 years of age. In the subset of 58 cases providing both diagnostic reports and research blood specimens, the values reported for the diagnostic samples ($46.31 \pm 3.76\%$) were significantly different from those noted in the cultured lymphocytes at the time of this research study ($33.63 \pm 3.57\%$) [paired *t*-test ($P < 0.0001$)]. To determine if the observed difference might be attributable to methodology used for these evaluations (metaphase chromosomes were assessed with GTG-banding in the diagnostic report compared to interphase nuclei using FISH in the research studies), the percentage of trisomic cells observed in both metaphase spreads and interphase nuclei from the same research sample was quantified (Table I). No significant differences were observed between these measurements, the latter of which were significantly correlated ($r = 0.99$, $P < 0.0001$).

Evaluations of Phenotypic Findings

Phenotypic information was obtained from medical records, on-site physical examinations, and/or photographs for 104 propositi. The number of clinical traits observed/reported for the propositi with trisomy 21 mosaicism (13.26 ± 0.54) was not significantly different from that reported for the non-mosaic positive control group (13.00 ± 0.80)(Fig. 3). The specific physical stigmata evaluated (38 traits) in the individuals with mosaicism and complete trisomy 21/Down syndrome (non-mosaic) are summarized (in increasing frequencies) in Tables II and III, respectively.

The overall frequency of congenital heart defects (CHD) was not significantly different between the propositi having mosaicism (41.7%) compared to the non-mosaic positive control group (50%) (Table IV). In particular, the most commonly seen finding in the individuals having mosaicism was an atrial septal defect, which was noted in 29.2% of cases. An atrioventricular canal defect was reported in only 11.1% of the mosaic cases (compared to 22.6% in the positive control group).

In addition to physical traits, IQ testing results were obtained for individuals with mosaicism for trisomy 21 and with complete trisomy 21/Down syndrome (Table IV). The average IQ in individuals with mosaicism for trisomy 21 was significantly higher (mean of 67.35 ± 2.31 with a range of 41 to 117) than that of individuals with complete trisomy 21/Down syndrome (mean of 57.67 ± 3.17 and range of 36–75; $P = 0.03$).

The ages at which developmental milestones where achieved in individuals with mosaicism ($n = 81$), their siblings ($n = 106$), and individuals with complete trisomy 21/Down syndrome ($n = 50$) were also compared (Fig. 4). Individuals with mosaic trisomy 21/Down syndrome attained all milestones at a significantly later age than their chromosomally normal siblings ($P < 0.05$), but tended to attain milestones earlier than the age-matched, non-mosaic individuals. Significant decreases were observed for the age that the mosaic propositi first crawled ($P = 0.004$), walked ($P = 0.013$), and independently dressed themselves ($P = 0.030$) when compared to the individuals having complete trisomy 21.

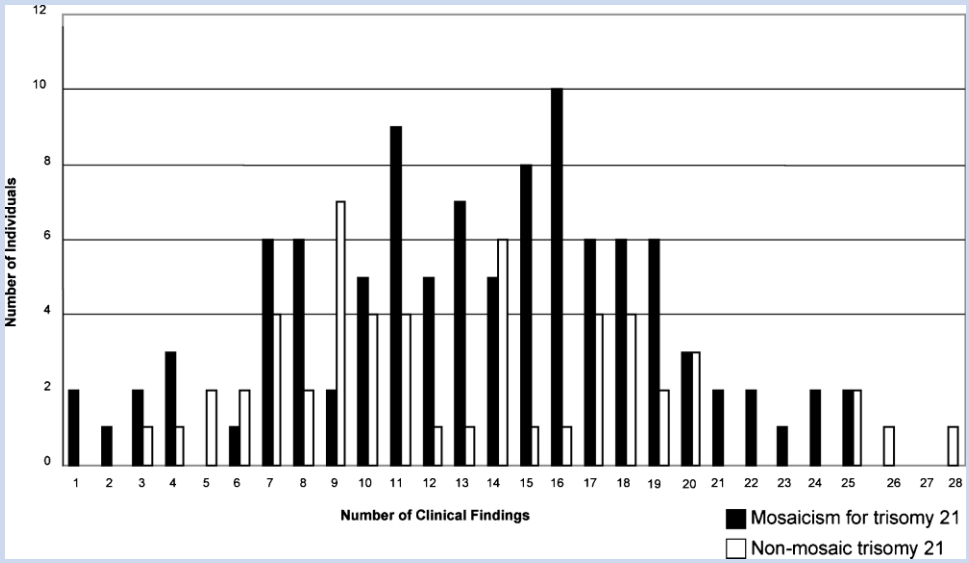

FIG. 3. Distribution of clinical findings in study subjects having mosaicism compared to non-mosaic (complete) trisomy 21. Individuals with mosaic Down syndrome (n = 104) presented with a range of 1–25 findings, compared to cases having complete trisomy 21 (n = 54) in whom a range of 3–28 findings was noted.

TABLE IV. Congenital Heart Defects (CHD) and IQ Values Reported in Individuals With Mosaicism for Trisomy 21 and Non-Mosaic Trisomy 21

Trait	Mosaic trisomy 21 (n = 103)	Complete trisomy 21 (n = 54)
(1) Cases having congential heart defect(s)	43	27
Types of congenital heart defects		
Atrial septal defects	21 (2)[a]	6 (5)
Patent ductus arteriosus	17 (2)	9 (4)
Ventricular septal defect	12 (4)	7 (3)
Atrioventricular canal defect	8 (8)	7 (7)
Pulmonic stenosis	6 (0)	0 (0)
Bicuspid aortic valve	2 (0)	0 (0)
Mitral valve prolapse	2 (0)	2 (0)
Congenital missing mitral valve	1 (0)	0 (0)
Hypoplastic left ventricle	1 (1)	0 (0)
Pre-excitation syndrome	1 (0)	0 (0)
Tetralogy of fallot	1 (1)	0 (0)
Total	72 (18)[b]	31 (19)[b]
(2) IQ values		
Reported ranges		
30–40	0	1
41–50	7	7
51–60	12	1
61–70	18	6
71–80	8	3
81–90	3	0
91–100	3	0
>100	4	0

[a]Number of propositi having heart defect (number surgically corrected).
[b]Several subjects had more than one heart defect. Thus, the total number of specific defects is greater than the total number of individuals having a congenital heart defect.

Latent Class Analysis (LCA) of Phenotype

To determine if the subjects with mosaic trisomy 21 had sufficient differences in their phenotype (presence or absence of traits) to allow them to be partitioned into distinct groups, 104 subjects were evaluated using a LCA. The best fit for the data was obtained for a model that partitioned the propositi into two groups having fewer (class 1) or more (class 2) phenotypic traits (1 vs. 2 class model $P = 0.0022$; 2 vs. 3 class model $P = 0.1203$). The proportion of trisomic cells (from diagnostic reports) of the 60 individuals assigned to class 1 ($43.84 \pm \%$) was significantly lower than that of the 44 individuals assigned to class 2 ($59.76 \pm \%$; $P = 0.0072$). In addition, the mean percentage of trisomic cells in the buccal samples from individuals in class 1 (42.09%) was significantly lower than the values from the propositi in class 2 (57.11%) ($P = 0.0156$). The greatest discrepancies in phenotype that allowed for the distinction between the members of class 1 and class 2 were seen for the presence/absence of: (1) acyanotic congenital heart defects (CHD); (2) total (acyanotic and cyanotic) CHD; and (3) umbilical hernias (Fig. 5a).

A second LCA analysis was also done to determine if improvements in phenotypic distinctions for the mosaic propositi (same 104 subjects evaluated above) could be made by comparing their phenotypic data with that of the 54 positive control individuals having complete trisomy 21 (total n = 158; Fig. 5b). In this model, the subjects with complete non-mosaic trisomy 21 were restricted to a single class, but the individuals with mosaicism for trisomy 21 could be partitioned into any class, based on their phenotype. Using this approach, a two-class model still provided the best fit to the data. The 20 individuals categorized into class 1 presented with fewer phenotypic findings and had a significantly lower proportion of trisomic cells (mean of 37.34%) than the 84 mosaic propositi categorized as belonging to class 2 (phenotype similar to complete trisomy 21/Down syndrome; mean of 53.97%; $P = 0.0189$). This second LCA model, using data from positive control subjects as "training variables," resulted in non-overlapping distributions for 19 of the 25 traits observed in the propositi with mosaicism (Fig. 5b) and allowed for improvements (visualized as higher values for the differences between class probabilities) in the distinction of classes (compared to model 1) for all traits but hearing loss, acyanotic CHD, total CHD, hernias, and constipation. Under model 2, the greatest distinctions between the 2 classes were observed for the presence/absence of the following phenotypic traits: (1) epicanthal folds; (2) brachycephaly; (3) hyperextensibility of joints; and (4) webbing, short, or broad neck.

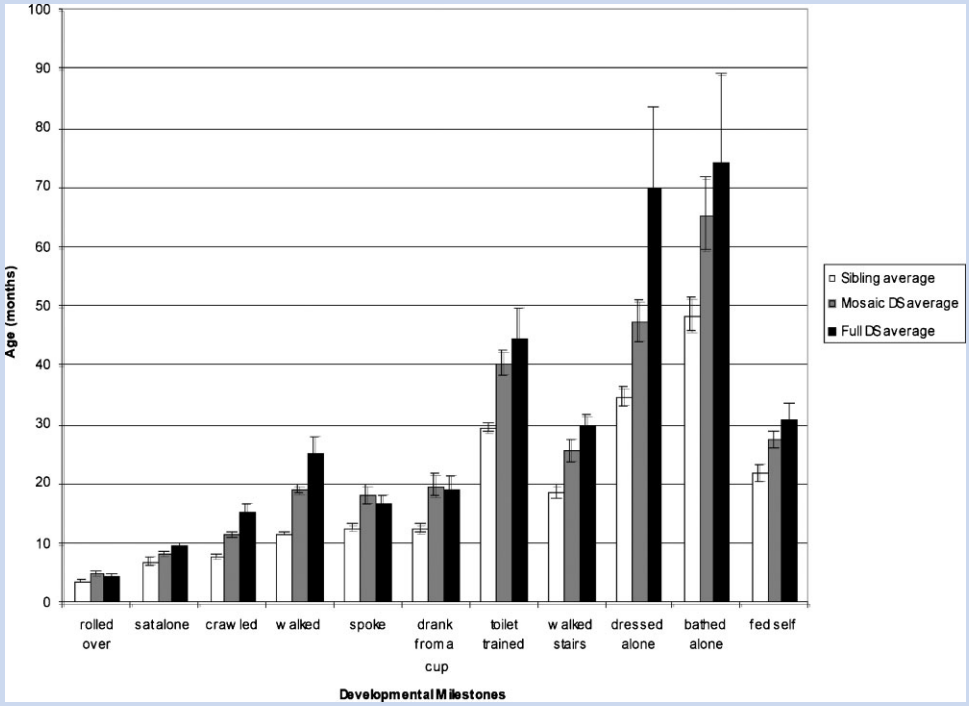

FIG. 4. Comparison of the average ages of developmental milestone attainment. Individuals with mosaic Down syndrome (gray histograms) attained all milestones at a significantly later age than their chromosomally normal siblings (white histograms). However, with the exception of rolling over, speaking and drinking from a cup, there was a general trend for individuals with mosaic trisomy 21/Down syndrome to achieve milestones at an earlier age than individuals with full (complete) trisomy 21 Down syndrome (black histogram). (Bars denote standard error)

Correlations Between Percentage of Cells With Trisomy 21 and Tissue-Specific Findings

Interestingly, a significant inverse correlation ($r = -0.53$; $P = 0.0094$) was observed between the percentage of trisomic cells present in the buccal samples and the IQ scores of the mosaic propositi. Although a similar trend was observed between the IQ values and percentage of trisomic cells in the blood samples, this correlation was not significant ($P = 0.1998$). In addition, a contingency chi-square test was used to determine if there was any relationship between the proportion of trisomic cells in the study subjects and the presence of congenital heart defects. Intriguingly, there was a significant relationship between the presence of CHD and the proportion of trisomic cells in blood specimens ($P = 0.0286$), with higher blood trisomy levels ($59.26 \pm 4.38\%$) being associated with CHD, while lower trisomic percentages ($43.84 \pm 3.81\%$) were associated with fewer cases of CHD. Although a similar trend was seen between buccal mucosa trisomy levels and CHD, this relationship was not significant.

DISCUSSION

The differing percentages of trisomic and euploid cells in individuals with mosaicism for trisomy 21 provide an opportunity to assess the extent to which phenotypic variability is related to the varying proportion and/or tissue distribution of the trisomic cells. In this study, buccal cells showed a significantly higher level of trisomy than lymphocytes. This difference could not be attributed solely to in vitro culturing since the percentage of trisomic cells in cultured and uncultured lymphocytes was not significantly different.

One possible factor contributing to the observed difference in trisomy levels between specimens is that these tissues have varying in vivo selection rates due to inherently different cell turnover and/or growth rates of blood and buccal samples. In one of the few reports detailing the kinetics of normal lymphocytes and buccal epithelia, Hellerstein et al. [1999] noted that, in healthy individuals, CD4 and CD8 T cells have a half-life of approximately 87 and 77 days, respectively. In contrast, the turnover times seen in buccal epithelial cells have been reported to vary from 5 to 25 days [Gillespie, 1969; Alvares et al., 1972]. Collectively, the findings of these two studies suggest that lymphocytes have a lower turnover rate than buccal cells. Therefore, if in vivo selection (acting against replicating trisomic cells) is largely responsible for the difference in trisomy levels observed between the tissues of the subjects in this study, one might expect to detect an overall higher trisomic percentage for the lymphocytes compared to buccal cells since they have a lower cell turnover rate. However, the opposite trend was observed. Thus, the observed variation in trisomy level between tissues may, instead, be a manifestation of differences in the distribution of mosaic cells during development or possibly be attributable to selection differences that are not simply a reflection of the number of cell cycles completed after birth.

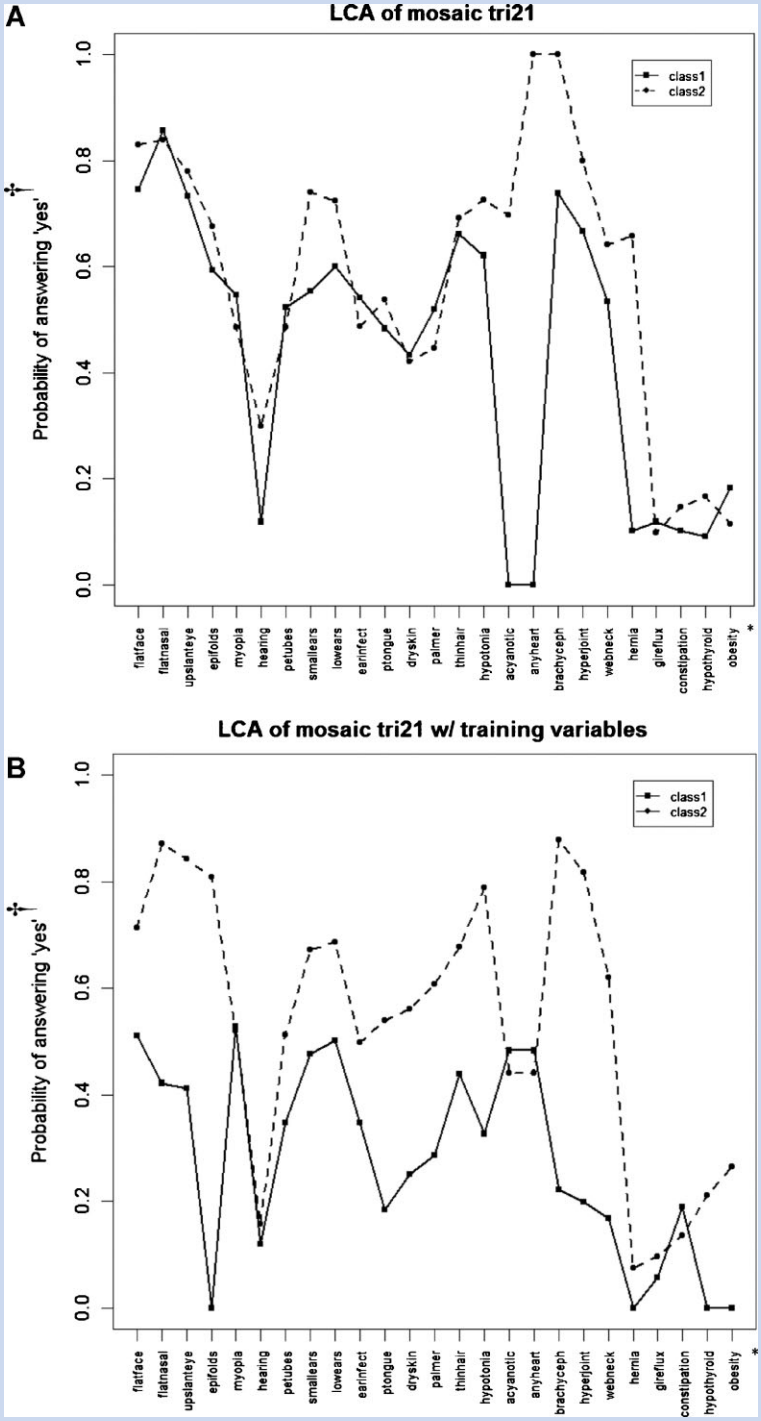

FIG. 5. Latent class analysis of individuals with mosaicism for trisomy 21 (a) without training variables (model 1) and (b) with training variables (individuals with full trisomy 21) [model 2]. a: A graphical representation of the conditional response probabilities for class 1 (squares [solid line]) and class 2 (circles [hatched line]) are shown. The traits that most clearly distinguished the two classes included hearing loss; small ears; low-set ears; cardiac anomalies; brachycephaly; and hernias. b: A graphical representation of the conditional response probabilities for both class 1 (squares [solid line]) and class 2 (circles [hatched line]) are shown. Under this model (which incorporated training variables) the data point curves are separated for the majority (76%) of traits studied. X-axis abbreviations: * Flat Face (flatface); Flat Nasal Bridge (flatnasal); Upslanted Eyes (upslanteye); Epicanthal Folds (epifolds); Myopia (myopia); Hearing Loss (hearing); PE Tubes (petubes); Small Ears (smallears); Low-set Ears (lowears); Frequent Ear Infections (earinfect); Protruding Tongue (ptongue); Dry Skin (dryskin); Single Transverse Palmar Crease (Palmar); Thin, or Sparse Hair (Thinhair); Hypotonia (hypotonia); Acyanotic Congenital Heart Defects (acyanotic); Presence of Cyanotic and Acyanotic Heart Defects (anyheart); Brachycephaly (brachyceph); Hyperextensibility of Joints (hyperjoint); Webbing, Short, or Broad Neck (webneck); Umbilical Hernia (hernia);GI Reflux (gireflux); Constipation (constipation); Hypothyroid (hypothyroid); Obesity (obesity). †Y-axis shows the probability of answering "yes" to a query regarding the presence of a particular phenotypic feature.

In addition to differences in the level of trisomy between tissues, investigators have also reported changes in the percentage of cells having trisomy 21 over time in individuals with both mosaicism for trisomy 21 and non-mosaic (complete) trisomy 21 [Taylor, 1968, 1970; Taysi et al., 1970; Wilson et al., 1980; Jenkins et al., 1997]. In this current study, diagnostic analyses (which tended to be completed in the first 2 years of life) showed significantly higher levels of trisomy than assessments at later ages (as part of this research protocol). Possible explanations for the observed differences include (but are not limited to): (1) in vivo selection against trisomic lymphocytes that may occur over time; (2) a bias in the initial diagnostic study to identify metaphases having a trisomic compared to euploid complement; or (3) a difference in the proportion of trisomic cells present in interphase nuclei compared to metaphase spreads. However, this latter hypothesis was not supported by our data, which showed no significant difference in the proportion of trisomic cells noted in the metaphase compared to interphase cells evaluated from 57 individuals. Thus, the causes for the observed changes in the frequency of trisomic cells over time, if present, remain unclear (requiring additional assessment in a long-term, longitudinal study design [ideally assessing buccal cells as well as lymphocytes] to better characterize).

When evaluating phenotype, the mean IQ level of the mosaic propositi in this study was significantly higher than the mean IQ reported for individuals with complete trisomy 21 (10 points higher). This result is in agreement with the findings of previous investigations, where IQ values were noted to be 10–30 points higher for cases with mosaicism [Wunsch, 1957; Fishler et al., 1976]. This result, coupled with the observation that the average age at which mosaic propsoti attained developmental milestones was between the average ages reported for their siblings and for individuals with complete trisomy 21/Down syndrome, suggests that as a group, individuals with mosaicism for trisomy 21 tend to have less intellectual and developmental impairments than individuals with complete trisomy 21. Nevertheless, it is important to point out that, just as in the case of chromosomally normal individuals, one cannot accurately predict the specific intellectual capacity of individuals with trisomy 21 mosaicsim or complete trisomy 21 since this trait is determined by many factors.

In order to better characterize the phenotype among individuals with mosaicism for trisomy 21 in Down syndrome, a latent class analysis was performed. Models run with and without training variables resulted in the distinction of two phenotypic subtypes. The mosaic propositi in the class having more clinical traits had higher frequencies of trisomic cells. This finding suggests that the level of trisomy is positively correlated to the severity of the phenotype.

As a final assessment, correlations between the phenotypic findings and the tissue specific level of trisomic cells were examined. A significant inverse correlation was observed between the percentage of trisomic cells present in the propositi's buccal samples and their IQ scores (but not lymphocytes). Thus, the level of trisomy 21 in buccal samples may be a better predictor of developmental progress than lymphocytes. One can speculate that this correlation results from the fact that brain cells, like buccal cells, are ectodermally derived [Larsen, 2001]. Interestingly, the presence or absence of CHD was more closely associated with trisomy 21 levels in lymphocytes than buccal cells. This may reflect the fact that both blood and heart tissues are derived from the mesoderm layer [Larsen, 2001]. An unexpected finding of this study was the observation that the individuals with mosaicism for trisomy 21 presented with more types of CHD than the positive control group having complete trisomy 21/Down syndrome. Interestingly, they also had a significantly lower number of defects that required surgery. Given that atrioventricular canal defects are treated surgically, the reduced frequency of this type of CHD in the mosaic population was of clinical relevance since it lessened their risk for cardiac morbidity.

In conclusion, the data derived from our studies suggest that the proportion of trisomic cells present in tissues of different embryologic origin (e.g., blood and buccal mucosa) influences the phenotypic outcome associated with mosaicism for trisomy 21 in Down syndrome. Thus, an analysis of different tissue samples should be considered when evaluating individuals with trisomy 21 mosaicism and Down syndrome. Collectively, the findings of our studies have helped to further clarify our understanding of the phenotypic variation seen in persons with mosaicism for trisomy 21.

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REFERENCES

Alvares O, Skougaard MR, Pindborg JJ, Roed-Petersen B. 1972. In vitro incorporation of tritiated thymidine in oral homogeneous leukoplakias. Scand J Dent Res 80:510–514.

Bhatt S, Schreck R, Graham JM, Korenberg JR, Hurvitz CG, Fischel-Ghodsian N. 1995. Transient leukemia with trisomy 21: Description of a case and review of the literature. Am J Med Genet 58:310–314.

Dewald G, Stallard R, Al Saadi A, Arnold S, Bader PI, Blough R, Chen K, Elejalde BR, Harris CJ, Higgins RR, Hoeltge GA, Hsu W-T, Kubic V, McCorquodale DJ, Micale MA, Moore JW, Phillips RM, Scheib-Wixted S, Schwartz S, Siembieda S, Strole K, VanTuinen P, Vance GH, Wiktor A, Wise L, Yung J-F, Zenger-Hain J, Zinsmeister A. 1998. A multi-center investigation with interphase in situ hybridization using X- and Y-chromosome probes. Am J Med Genet 76:318–326.

Epstein CJ, Korenberg JR, Anneren G, Antonarakis SE, Ayme S, Courchesne E, Epstein LB, Fowler A, Groner Y, Huret JL, Kempler TL, Lott IT, Lubin BH, Magenis E, Opitz J, Patterson D, Priets JH, Pueschel SM, Rapoport SI, Sinet PM, Tanzi RE, de la Cruz F. 1991. Protocols to establish genotype-phenotype correlations in Down syndrome. Am J Med Genet 49:207–235.

Finley WH, Finley SC, Rosecrans CJ, Tucker CC. 1966. Normal/trisomy 21 mosaicism. Report of four cases and review of the subject. Am J Dis Child 112:444–447.

Fishler K, Koch R. 1991. Mental development in Down syndrome mosaicism. Am J Ment Retard 96:345–351.

Fishler K, Koch R, Donnell GN. 1976. Comparison of mental development in individuals with mosaic and trisomy 21 Down's syndrome. Pediatrics 58:744–748.

Gillespie GM. 1969. Renewal of buccal epithelium. Oral Surg Oral Med Oral Pathol 27:83–89.

Hamerton JL, Giannelli F, Polani PE. 1965. Cytogenetics of Down's syndrome (Mongolism) I. Data on a consecutive series of patients referred for genetic counseling and diagnosis. Cytogenetics 104:171–185.

Hellerstein M, Hanley MB, Cesar D, Siler S, Papageorgopoulos C, Wieder E, Schmidt D, Hoh R, Neese R, Macallan D, Deeks S, McCune JM. 1999. Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. Nat Med 5:83–89.

Hook EB. 1981. Down syndrome: Frequency in human populations and factors pertinent to variation in rates. In: de la Cruz FF, Gerald PS, editors. Trisomy 21 (Down syndrome): Research perspectives. Baltimore: University Park Press. pp 3–67.

Jenkins EC, Schupf N, Genovese M, Ye LL, Kapell D, Canto B, Harris M, Devenny D, Lee JH, Brown WT. 1997. Increased low-level chromosome 21 mosaicism in older individuals with Down syndrome. Am J Med Genet 68:147–151.

Johnson RC, Abelson RB. 1969. Intellectual, behavioral, and physical characteristics associated with trisomy, translocation, and mosaic types of Down's syndrome. Am J Ment Defic 73:852–855.

Larsen WJ. 2001. Development of the limbs. In: Sherman LS, Potter SS, Scott WJ, editors. Human embryology. New York: Churchill Livingstone. p 327.

Leach NT, Rehder C, Jensen K, Holt S, Jackson-Cook C. 2004. Human chromosomes with shorter telomeres and large heterochromatin regions have a higher frequency of acquired somatic cell aneuploidy. Mech Ageing Dev 125:563–573.

Mikkelsen M. 1977. Down syndrome: Cytogenetical epidemiology. Hereditas 86:45–50.

Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res 20:613–616.

Muthén LK, Muthén BO. 1998. Mplus: User's Guide. Los Angeles, CA:Muthén and Muthén.

Nussbam RL, McInnes RR, Willard HF. 2001. Thompson & Thompson: Genetics in Medicine, 6th edition. Philadelphia: W.B. Saunders Company. pp 157–162.

Pangalos C, Avramopoulos D, Blouin JL, Raoul O, deBlois MC, Prieur M, Schinzel AA, Gika M, Abazis D, Antonarakis SE. 1994. Understanding the mechanism(s) of mosaic trisomy 21 by using DNA polymorphism analysis. Am J Hum Genet 54:473–481.

Papavassiliou P. 2007. Karyotype-phenotype correlations for mosaic Down syndrome. Diss. Virginia Commonwealth University, 2007. Ann Arbor: ProQuest UMI Dissertations Publishing, 2008.AAT 3283127.

Patterson D, Costa ACS. 2005. Down syndrome and genetics—A case of linked histories. Nat Rev Genet 6:137–147.

Richards BW. 1969. Mosaic mongolism. J Ment Defic Res 13:66–83.

Shipe D, Reisman LE, Chung CY, Darnell A, Kelly S. 1968. The relationship between cytogenetic constitution, physical stigmata, and intelligence in Down's syndrome. Am J Ment Defic 72:789–797.

Taylor AI. 1968. Cell selection in vivo in normal/G trisomic mosaics. Nature 219:1028–1030.

Taylor AI. 1970. Further observation of cells election in vivo in normal/G trisomic mosaics. Nature 227:163–164.

Taysi K, Kohn G, Mellman WJ. 1970. Mosaic mongolism II. Cytogenetic studies. J Pediatr 76:880–885.

Wilson MG, Towner JW, Forsman I. 1980. Decreasing mosaicism in Down's syndrome. Clin Genet 17:335–340.

Wunsch WL. 1957. Some characteristics of Mongoloids evaluated in a clinic for children with retarded mental development. Am J Ment Defic 62:122–130.