Detection of Chromosomal Mosaicism by Computer Methods

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Modern techniques of cell culture have led to widespread use of chromosome analysis as a diagnostic tool in human disease. In normal subjects, variations of chromosome counts about the modal number are generally attributed to artefact. This assumption is supported by the fact that the distribution is negatively skewed around the modal number, logically attributable to loss of chromosomes from broken cells (Court Brown, Jacobs, and Doll, 1960). It is reasonable to suppose, however, that mitotic errors sometimes give rise to cells in somatic tissues with chromosome numbers other than the modal number. Non-disjunction during gametogenesis or in early cleavage stages of the zygote is probably the cause of most chromosomal abnormalities in man. Thus an occasional individual may be a mosaic, having some tissues composed of two or more stem-lines of cells with different chromosome numbers. The accurate detection of mosaicism is highly desirable in the ultimate evaluation of chromosomal abnormalities, especially in regard to potential variation in disease syndromes due to mosaicism. An even more pressing problem is the evaluation of parents of children with trisomy 21 (Down's syndrome or mongolism), since mosaicism in an apparently normal parent may account for an increased risk for bearing mongoloid offspring (Weinstein and Warkany, 1963).

The problem facing the cytogeneticist is when to suspect mosaicism and how to confirm or deny his suspicion. First, the number of cells with aneuploid counts must be more than can reasonably be expected by chance alone. Ideally, the excess of aneuploidy should be observed in two or more examinations, and in two or more tissues. Karyotype analysis should support the conclusion by providing evidence that a high proportion of the aneuploid cells have the same karyotype. Calcula-

tions of the probability of finding aneuploidy should give a clue to the presence of mosaicism in any given individual chromosome analysis. Since culture techniques and criteria for accepting or rejecting individual cells for counting vary from one laboratory to another, probabilities should be established by each laboratory group to test the significance of its own data. Utilizing the IBM 1401 computer, we have calculated probabilities printed in the form of tables.

For this purpose, chromosome analyses from peripheral blood leucocyte cultures were divided into three groups: 1. *Normal*, which included both truly normal individuals who had no known disease, and individuals with disease states in which no chromosomal abnormality was found. 2. *Abnormal other than cancer*, which included all individuals with a recognizable chromosomal abnormality. 3. *Cancer cells*.

In each group, cell counts were arranged in 3 classifications: hypomodal, modal, and hypermodal, omitting polyploids. There were 3,088 cells scored in 69 chromosome analyses from the normal group with a distribution of 0.0398 hypomodal, 0.9378 modal, and 0.0223 hypermodal. There were 1,005 cells scored in 20 analyses from individuals with chromosome abnormalities other than cancer, with a distribution of 0.0497 hypomodal, 0.9363 modal, and 0.0139 hypermodal. These additional cases were omitted, since they had been recognized as probably being mosaics. Probabilities of selecting individual cell counts were calculated by the following formula: Let A, B, C, equal probabilities of modal, hypomodal, and hypermodal counts respectively with respective frequencies a, b, c, from a sample of size n, where $a + b + c = n$ and $A + B + C = 1$. A, B, and C are derived from compiled cell count data. Then $\frac{n!}{a! b! c!}$

$A^a B^b C^c = P$ = probability (a, b, c) occurring by chance alone. For practical application the formula

was programmed for the computer so that probability tables could be printed out at multiples of 10 cells counted. The computer programme applicable to most computers and set to stop when P becomes consistently < 0.00001 is listed as programme 1. This programme requires that the dominant probability be entered as the variable PA, corresponding to the modal population.

The counting of 30 cells as a minimum number for the determination of chromosome count distribution has been generally accepted (Baikie, Jacobs, McBride, and Tough, 1961). In the accompanying illustration, the computer print-out is shown for probabilities of finding individual cell counts within the framework of our laboratory technique. Column A is modal, B hypomodal, C hypermodal; the next column is the possible number of combinations to arrive at such a count, and the final decimal figure is the probability for that individual count. Additional tables are printed at cell count levels of 40, 50, etc. Since the probabilities fall off so rapidly with decreasing numbers of modal cell counts, no more than 100 lines of print-out are necessary, even at the cell

PA	0.93780	PB	0.03980	PC	0.02230	N	30	Normal
						Frequency		Probability
A 30	B 0	C 0				1.		0.145649
A 29	B 0	C 1				30.		0.103902
A 29	B 1	C 0				30.		0.185439
A 28	B 0	C 2				435.		0.035825
A 28	B 1	C 1				870.		0.127877
A 28	B 2	C 0				435.		0.114115
A 27	B 0	C 3				4060.		0.007951
A 27	B 1	C 2				12180.		0.042571
A 27	B 2	C 1				12180.		0.075979
A 27	B 3	C 0				4060.		0.045201
A 26	B 0	C 4				27405.		0.001276
A 26	B 1	C 3				109620.		0.009111
A 26	B 2	C 2				164430.		0.024391
A 26	B 3	C 1				109620.		0.029021
A 26	B 4	C 0				27405.		0.012949
A 25	B 0	C 5				142506.		0.000158
A 25	B 1	C 4				712530.		0.001408
A 25	B 2	C 3				1425060.		0.005027
A 25	B 3	C 2				1425060.		0.008971
A 25	B 4	C 1				712530.		0.008006
A 25	B 5	C 0				142506.		0.002858
A 24	B 0	C 6				593775.		0.000016
A 24	B 1	C 5				3562650.		0.000167
A 24	B 2	C 4				8906625.		0.000747
A 24	B 3	C 3				11875500.		0.001778
A 24	B 4	C 2				8906625.		0.002380
A 24	B 5	C 1				3562650.		0.001699
A 24	B 6	C 0				593775.		0.000505

FIG. A portion of computer printed table listing probabilities for individual 30 cell counts to occur by chance alone, based on compiled laboratory data.

PROGRAMME 1

Seq	STMNT	Fortran Statement
1		Dimension title (5)
2	1	Read 101, PA, PB, PC, N, title
3		Print 201, PA, PB, PC, N, title
4	201	Format (3H1PA, F10.5, 3X, 2HPB, F10.5, 3X, 2HPC, F10.5, 3X, 1HN, I5, 5X, 5A8)
5	101	Format (3F10.0, I5, 4X, 5A8)
6		Print 202
7	202	Format (1HO, 34X, 9HFrequency, 5X, 10HProbability)
8		IF (PA+PB+PC-1.0) 2, 2, 16
9	2	PAL=LogF (PA)
10		PBL=LogF (PB)
11		PCL=LogF (PC)
12		FN=N
13		I=0
14		J=0
15		K=0
16		IF (I) 4, 3, 4
17	3	FI=1.0
18		Go to 5
19	4	FI=FI*FN
20		FN=FN-1.0
21		I=I+1
22	5	K=I
23		APR=0.0
24	6	J=I-K
25		IF (K) 8, 7, 8
26	7	FK=1.0
27		Go to 10
28	8	FKK=1.0
29		DO 9 KF=1, K
30		FKF=KF
31	9	FKK=FKK*FKF
32		FK=FKK
33	10	IF (J) 12, 11, 12
34	11	FJ=1.0
35		Go to 14
36	12	FJJ=1.0
37		DO 13 JF=1, J
38		FJJ=FJJ*FJF
39	13	FJ=FJJ
40		FJ=FJJ
41	14	EI=N-I
42		EJ=J
43		EK=K
44		PF=FJ*FK
45		PF=FI/PF
46		PR=PF*EXPF (EI*PAL+EJ*PBL+EK*PCL)
47		II=N-I
48		IF (II) 1, 17, 17
49	17	Print 102, II, J, K, PF, PR
50	102	Format (1X, 1HA, 13, 3X, 1HB, 13, 3X, 1HC, 13, 5X, F20.0, 5X, F10.6)
51		APR=APR+PR
52		IF (J-I) 15, 18, 15
53	15	K=K-1
54		Go to 6
55	16	Pause
56		Go to 1
57	18	IF (APR-.00001) 1, 1, 4
58		End

count level of 90. Computer time is negligible, since the IBM 1401 computer prints each line at approximately 1-second intervals. To use the tables, one simply looks up the probability of finding any individual count, having counted 20 cells. If the probability of finding that count is within acceptable limits, then no further counts need to be made. Although the limits of probability must satisfy the individual investigator, P values greater than 0.01 have been regarded as being within the limits of normal (Baikie *et al.*, 1961). If a count produces a borderline probability, then successive ten cell counts can be added, and the corresponding tables consulted. If the proportion of aneuploidy previously found continues, then the probability that the analysis falls within the normal range becomes progressively less. Confirmation of any particular stem-line must of course be carried out by karyotyping cells of that line.

A similar computer programme was devised to determine probabilities for specific samples where the total number of cells counted was larger than would be feasible for print-out in tabular form. This formula includes an approximation of the combinatorial weight which increases in accuracy when each value for a, b, or c is greater than 5.

Let $X = (n + 1/2) \log_e n + a [\log_e A - \log_e a] + b [\log_e B - \log_e b] + c [\log_e C - \log_e c] - \log_e (2\pi) - 1/2 \log_e (abc)$ where $X \leq 0$.

Then probability $[a, b, c] = e^X$ where $\log_e$ and e have Napierian or natural base.

Note: $1/2 \log_e (abc) = 1/2 [\log_e a + \log_e b + \log_e c]$. Other notations A, B, C, etc. are the same as in the previous formula. To accommodate extremely low probabilities, the E format is used, so that answers appear as decimal figures followed by exponent values representing powers of 10. This computer programme is listed as programme 2.

The three cases from this laboratory suspected to be mosaics indicate the extremely low probabilities found in actual practice.

1. Mongol

Chromosomes per cell	45	46	47	Total
Number of cells	2	35	16	53
$P = 0.49668839E - 17 = 0.5 \times 10^{-17}$.				

2. Turner's syndrome

Chromosomes per cell	44	45	46	47	Total
Number of cells	3	71	28	1	103
$P = 0.36458727E - 29 = 0.4 \times 10^{-29}$.					

3. Mongol

Chromosomes per cell	45	46	47	Total
Number of cells	2	10	19	31
$P = 0.36933054E - 08 = 0.4 \times 10^{-8}$.				

PROGRAMME 2

```

Seq  STMT  Fortran statement
1      Const=1.837877
2      1      Read 101, PA, PB, PC, A, B, C,
          T1, T2, T3
3      101    Format (3F10.0, 3F5.0, 4X, 3A8)
4      Print 201, T1, T2, T3
5      201    Format (1H0, 3A8)
6      IF (PA+PB+PC-1.0) 2, 2, 15
7      2      IF (A+B+C-200.0) 3, 3, 15
8      3      FN=A+B+C
9      FNL=LogF (FN)
10     IF (PA) 15, 15, 4
11     IF (PB) 15, 15, 5
12     IF (PC) 15, 15, 6
13     6      PAL=LogF (PA)
14     PBL=LogF (PB)
15     PCL=LogF (PC)
16     AL=0.0
17     BL=0.0
18     CL=0.0
19     IF (A) 15, 8, 7
20     7      AL=LogF (A)
21     8      IF (B) 15, 10, 9
22     9      BL=LogF (B)
23     10     IF (C) 15, 12, 11
24     11     CL=LogF (C)
25     12     X=(FN+0.5)*FNL+A*(PAL-AL)
          +B*(PBL-BL)+C*(PCL-CL)
          -CONST-0.5*(AL+BL+C)
          1L)
26     IF(X) 13, 13, 15
27     13     Prob=ExpF(X)
28     14     Print 102, PA, PB, PC, A, B, C, Prob
29     Go to 1
30     102    Format (6H PA=, F10.6, 5X5HPB=,
          F10.6, 5X5HPC=, F10.6, 6X4HA=,
          F5.0, 6X4HB=, F5.0, 6X4HC=,
          F5.0, 6X7HProb=, E15.8)
31     15     Prob=9999.9
32     Go to 14
33     End

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In the study of cancer cells, chromosome analysis frequently indicates a modal number readily recognizable as being outside the range of normal without resort to statistical analysis. However, in malignant disease involving the haemopoietic system, such as multiple myeloma, leukaemia, and Waldenstrom's macroglobulinaemia, probability estimates on specific results of chromosome counting may be helpful.

Summary

A computer programme was devised to print tables of probabilities for obtaining specific cell counts in chromosome analysis. Utilizing the distribution of hypomodal, modal, and hypermodal counts obtained by any given laboratory, similar

tables applicable to that laboratory can be conveniently printed by computer. Such tables should be helpful in the detection of mosaicism, and in the determination of an adequate sample size for any given analysis in which mosaicism is suspected.

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