

Apparent Influence of the X Chromosome on Timing of 73 Ossification Centers

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ABSTRACT Among 73 postnatal ossification centers, sister-sister (SS) correlations involving age at appearance tend to exceed brother-brother (BB) and sister-brother (SB) correlations by an average of 0.16. This excess of SS over BB and SB ossification timing similarity is not a function of type of center, limb or location, and is in accordance with the hypothesis of partial X-linkage. It is estimated that the larger proportion of genetically determined variance in postnatal ossification timing may be attributed to genes on the X chromosome.

There is now considerable evidence for partial X-chromosomal involvement in postnatal developmental timing. This we first found for the teeth (Garn, Lewis and Polachek, '60; Garn and Rohmann, '62a; Garn, Lewis and Kerewsky, '65a,b) and later for postnatal hand-wrist timing (Garn and Rohmann, '62b; Garn, Rohmann and Davis, '63). Consistently, sisters show a higher communality in postnatal ossification timing than is true for either brother-sister or brother-brother pairs, and father-daughter similarities in postnatal ossification timing exceed father-son, mother-son, and mother-daughter hand-wrist timing resemblances. *In toto*, this evidence is consistent with the hypothesis of X-chromosomal involvement, the excess of sister-sister over brother-brother similarity coefficients indicating the relative contribution of genes on the X chromosomes and those on the remaining autosomes.

In the present study we have newly extended data analysis to encompass all available postnatal ossification centers of the hand, foot, elbow, knee, shoulder, and hip (including the adductor sesamoid of the thumb) and covering the age range 1 month through 15 years in age at appearance. Sister-sister, sister-brother and brother-brother similarity coefficients have been calculated center by center and then pooled in order to provide the best possible estimates of sex-specific and cross-sex similarities in postnatal ossification timing.

METHODS AND MATERIALS

This study is based on the magnitude of sibling resemblances in the age at appearance of 73 postnatal ossification centers as ascertained from serial, longitudinal radiographs of long-term participants in studies of growth and aging. In every instance the age at appearance of a given postnatal ossification center was verified by reference to previous and succeeding radiographs and no value was reported where there was ambiguity because of subject positioning, radiographic quality, or missed visits. The approach, radiographic analysis and analytical techniques are those previously described by us, particularly in Garn, Rohmann and Blumenthal ('66) and Garn, Rohmann and Silverman ('67).

Raw scores for age-at-appearance were first converted into sex-specific normalized T-scores, following McCall's method, and employing the machine program described by Black ('66). This procedure effectively eliminated the effects of skewness inherent in dichotomous growth data. For further details see Garn and Shamir ('58), Lacey ('56) and Black ('66).

Separate correlations were made for (a) sisters, (b) brothers and (c) cross-sexed sibling pairs, so as to test for possible influences of the X and Y chromosomes. Mean values of *r* were also computed for

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73 ossification correlations for each type of comparison (SS, BB and BS) and for all 219 correlations, both after weighting for sample size and, separately using unweighted values. Throughout, mean values of r were calculated from the corresponding z transforms of r as described by Fisher ('58).

Further data analysis included grouping by (1) type of center (*i.e.*, round bones, metacarpal and metatarsal epiphyses etc.) and (2) by age at appearance, in order to explore differential genetic effects on (a) type of center and location or (b) sequence in the total pattern of postnatal ossification. Finally, an attempt was made to apportion sex-chromosomal and autosomal influences by comparison of mean values of r for sister pairs and brothers pairs respectively.

Findings

In the first step of the data analysis, sister-sister, sister-brother and brother-brother age-at-ossification correlations were tabulated, center by center. With a total of 73 postnatal ossification centers considered, this amounted to 219 correlations, and 8256 ossification center pairings in all. Whether pooled as the mean unweighted r for all 73 correlations for each type of sibling pairing, or when employing the mean r from the mean z transform of r , the results were then in the same direction. Sister-sister correlations were the highest (mean $r = 0.49$ and 0.52 respectively) and considerably exceeded sister-brother correlations (0.36 and 0.38 respectively) which were slightly higher than brother-brother correlations (0.32 and 0.35). Overall, sister-sister (SS) ossification correlations approximated 0.5 , sister-brother correlations approximated 0.37 and brother-brother correlations 0.33 . The rankings were then $r_{SS} > r_{SB/BB}$.

Much the same picture emerged from a center-by-center comparison, and use of a sign test. Overall, SS ossification-timing correlations were higher than the corresponding SB correlations for 51.5 centers ($\chi^2 = 12.3$) and higher than the corresponding BB correlations for 49.5 centers ($\chi^2 = 9.3$).² Though exact tests of significance are not practicable, because postnatal ossification centers are positively if often slightly correlated, and because of re-

peated sampling from the same population sample, the trend is nevertheless clear. Sisters are appreciably more similar in postnatal ossification timing than either brothers or brother-sister pairs.

In the second step of the analysis the correlations were arrayed according to (1) type of center, (2) location on the body frame, and (3) age at appearance, in order to explore anatomical and timing variables. Arrangement as to type of center provided no particular illumination. As shown in table 2, round bones, epiphyses of the long bones, metacarpal epiphyses etc. generally followed the $SS > SB/BB$ rule. Similarly, comparison of the upper and lower limbs provided no surprises, except to indicate their overall similarity despite the assumption of the upright posture. Analysis in terms of timing (*i.e.* age at appearance) proved more valuable, however.

Breaking the data into three cycles (0.00 – 0.99 years, 1.00 – 9.99, and 10.00 – X years) did prove revealing. Using the 3-cycle approach, since percent sexual dimorphism in postnatal ossification timing does, in fact, fit a 3-cycle plot (*cf.* Garn, Rohmann and Silverman, '67), the present data also provided a useful fit. For centers 1 through 10 (0.0 to 0.8 years) the excess of SS over BB was only 0.04. For centers 11 through 65 (1.0 to 9.7 years) it was 0.11, and for centers 66 – 73 (11.2 through 15.3 years) the excess of SS over BB ossification correlations was then greater than 0.50. In a general way, then, the hypothesis of X-chromosomal involvement is most tenable for the centers of ossification that appear well after the first year of life.

Since $SS > SB/BB$ it is then possible to make some numerical estimate of the relative involvement of the X chromosome. This can be done under the assumption that r_{BB} represents both the autosomal contribution and that of one X chromosome, while r_{SS} represents the further contribution of the paternal X chromosome. Since r_{SS} approximates 0.51, while r_{BB} approximates 0.33, as mentioned above, the relative contribution of the paternal X chromosome to total interpersonal variance may then be estimated as 0.15, *i.e.* $0.51^2 - 0.33^2$. Since all genes in common account for approximately 25% of timing variance in these

² Including tied values.

TABLE 1

Sister-sister, sister-brother and brother-brother similarities in ossification timing

Ossification center	Sister-sister		Sister-brother		Brother-brother	
	N	r	N	r	N	r
1. Head of humerus	7	0.09	23	0.69	12	0.69
2. Proximal epiphysis, tibia	13	.00	33	.28	20	.55
3. Coracoid proc., scapula	2	.00	5	-.25	1	.00
4. Cuboid	31	-.05	55	-.09	39	.04
5. Capitate	22	.05	64	-.05	36	-.06
6. Hamate	23	.18	65	.04	35	-.02
7. Capitellum of humerus	14	.58	55	.38	35	.46
8. Head of femur	16	.61	50	.47	37	.18
9. Lateral cuneiform	10	.79	39	.05	29	-.07
10. Greater tuberosity — humerus	10	.72	28	.15	9	.84
11. Primary center, middle phalanx, 5th toe	15	.42	54	.56	39	.51
12. Distal epiphysis, radius	29	.71	76	.44	42	.34
13. Epiphysis, distal phalanx, 1st toe	14	.66	52	.46	33	.47
14. Epiphysis, middle phalanx, 4th toe	6	.17	18	.39	13	.47
15. Epiphysis, prox. phalanx, 3rd finger	28	.52	74	.15	44	.20
16. Epiphysis, middle phalanx, 3rd toe	13	.28	43	.03	26	.37
17. Epiphysis, prox. phalanx, 2nd finger	30	.66	77	.28	44	.32
18. Epiphysis, prox. phalanx, 4th finger	31	.65	73	.18	42	.33
19. Epiphysis, distal phalanx, 1st finger	30	.39	77	.39	43	.28
20. Epiphysis, prox. phalanx, 3rd toe	15	.69	60	.40	36	.21
21. Epiphysis, 2nd metacarpal	32	.53	77	.37	41	.19
22. Epiphysis, prox. phalanx, 4th toe	16	.92	58	.34	33	.19
23. Epiphysis, prox. phalanx, 2nd toe	17	.65	58	.43	35	.46
24. Epiphysis, 3rd metacarpal	32	.52	79	.42	41	.41
25. Epiphysis, prox. phalanx, 5th finger	35	.36	87	.43	44	.46
26. Epiphysis, middle phalanx, 3rd finger	30	.41	78	.47	43	.45
27. Epiphysis, 4th metacarpal	34	.44	77	.54	43	.43
28. Epiphysis, middle phalanx, 2nd toe	16	.60	55	.49	34	.58
29. Epiphysis, middle phalanx, 4th finger	29	.46	79	.38	43	.34
30. Epiphysis, 5th metacarpal	35	.36	80	.49	46	.40
31. Medial cuneiform	18	.58	53	.45	31	.18
32. Epiphysis, 1st metatarsal	20	.65	60	.02	34	.39
33. Epiphysis, middle phalanx, 2nd finger	30	.57	79	.54	48	.59
34. Epiphysis, prox. phalanx, 1st toe	20	.72	54	.39	33	.34
35. Epiphysis, distal phalanx, 3rd finger	29	.42	77	.43	45	.29
36. Triquetral	35	.47	79	.34	49	.30
37. Epiphysis, distal phalanx, 4th finger	31	.31	80	.51	47	.42
38. Epiphysis, prox. phalanx, 5th toe	20	.45	58	.27	35	.22
39. Epiphysis, 1st metacarpal	35	.36	99	.55	57	.44
40. Intermediate (middle) cuneiform	22	.68	53	.49	30	.28
41. Epiphysis, 2nd metatarsal	20	.55	53	.39	28	.09
42. Greater trochanter	28	.47	66	.21	35	.50
43. Epiphysis, prox. phalanx, 1st finger	36	.36	95	.37	52	.38
44. Navicular of foot	21	.42	56	.50	29	.61
45. Epiphysis, distal phalanx, 2nd finger	40	.57	100	.47	53	.39
46. Epiphysis, distal phalanx, 5th finger	39	.37	95	.47	56	.30
47. Epiphysis, middle phalanx, 5th finger	36	.60	90	.44	51	.26
48. Proximal epiphysis of fibula	18	.21	52	.28	25	.16
49. Epiphysis, 3rd metatarsal	23	.53	55	.42	26	.42
50. Epiphysis, distal phalanx, 5th toe	16	.18	30	-.03	21	.54
51. Patella	12	.90	39	.75	25	.88
52. Epiphysis, 4th metatarsal	23	.59	55	.37	26	.28
53. Lunate	37	.66	89	.60	47	.39
54. Epiphysis, distal phalanx, 3rd toe	21	.07	51	.45	26	.56
55. Epiphysis, 5th metatarsal	24	.68	50	.46	26	.38
56. Epiphysis, distal phalanx, 4th toe	21	-.00	53	.32	26	.54
57. Epiphysis, distal phalanx, 2nd toe	22	.21	51	.32	26	.52
58. Capitulum of radius	17	.66	54	.54	33	.31
59. Navicular of hand (scaphoid)	37	.61	70	.51	41	.35
60. Trapezium	38	.77	77	.44	41	.41
61. Trapezoid	38	.62	76	.58	44	.40
62. Medial epicondyle of humerus	17	.33	53	.44	33	.47

TABLE 1 (continued)
Sister-sister, sister-brother and brother-brother similarities in ossification timing

Ossification center	Sister-sister		Sister-brother		Brother-brother	
	N	r	N	r	N	r
63. Distal epiphysis of ulna	27	.68	68	.48	37	.37
64. Epiphysis of calcaneus	20	.55	43	.44	22	.36
65. Olecranon process of ulna	18	.84	42	.54	19	.52
66. Lateral epicondyle of humerus	24	.60	48	.32	20	.34
67. Tibia tubercle	15	.74	24	.31	11	-.24
68. Adductor sesamoid of thumb	23	.59	58	-.05	35	.36
69. Os acetabulum	9	.71	14	.43	4	.09
70. Acromial process	9	.03	28	.13	15	.10
71. Epiphysis of iliac crest	7	.80	17	.31	6	-.43
72. Accessory epiphysis, coracoid proc.	9	.53	23	.45	12	-.19
73. Ischial tuberosity	5	.76	13	.52	4	-.58
Mean unweighted r	1645	0.490	4229	0.362	2382	0.319
Mean unweighted r from z transform of r		0.527		0.376		0.350

TABLE 2
Sibling similarities in ossification timing by type of center

Type of center	Sister-sister mean r	Sister-brother mean r	Brother-brother mean r	All pairings mean r
Round bones	0.48	0.32	0.23	0.36
Epiphyses of long bones	0.50	0.35	0.23	0.35
Epiphyses of metacarpals	0.44	0.48	0.37	0.43
Epiphyses of metatarsals	0.60	0.33	0.31	0.41
Epiphyses of proximals	0.60	0.32	0.31	0.41
Epiphyses of middles	0.44	0.40	0.43	0.42
Epiphyses of distals	0.32	0.38	0.43	0.38

sister-sister comparisons, there is then reason to believe that the greater part of genetically determined variance in postnatal ossification timing may have an X chromosomal basis, certainly in the female and probably in the male as well.

Postnatal ossification timing of 73 centers of the hand-wrist, foot-ankle, elbow, knee, shoulder and hip thus shows sibling resemblances of 0.30 to 0.50 with a notable excess of sister (SS) similarity over brother-sister (BS) and brother-brother (BB) similarity. From the numerical data there is the not unreasonable suggestion that genes on the X chromosomes have somewhat more influence on postnatal ossification timing than genes on the remaining autosomes.

DISCUSSION

The findings in this particular study are simple to summarize. Sister-sister (SS) correlations in postnatal ossification timing generally exceed sister-brother (SB) and

brother-brother (BB) correlations, and these systematic differences are suggestive of X-chromosomal involvement. With some differences associated with the three cycles of age at ossification, with few consistent differences attributable to type of center or anatomical location, and with individual values of r subject to sampling fluctuations, one generalization is indeed clear. Overall, for 73 postnatal bony nuclei, appearing over a 15 year span, SS correlations approximate 0.51, SB correlations average close to 0.37, and BB correlations nearer 0.33.

These data, supported by relevant parent-child similarities in postnatal ossification timing, are consistent with the hypothesis of autosomal plus X-linked inheritance. As a first approximation, the relative involvement of autosomal genes and genes on the X chromosome can be ascertained by the comparison of father-daughter and father-son similarities, since the latter pairing shares no X chromosomes

in common. (This is also true of the sons of brothers.) As a second approximation, sister-sister and brother-brother comparisons provide some indication, for sisters share the paternal X and have a 50:50 chance of sharing the same maternal X chromosome in common.

In the order $SS > SB/BB$, postnatal ossification resembles postnatal tooth formation (Garn, Lewis and Polacheck, '60; Garn and Rohmann, '62a; Garn, Lewis and Kerewsky, '65a,b) for the same population sample. Moreover, again for the same population sample, crown-size dimensions (mesiodistal and buccolingual) also show an excess of SS over SB and BB correlations (cf. Garn et al., '67), a finding that has been substantiated by Lewis and Grainger ('67) for Burlington, Ontario, children and by Goose ('67) for a Liverpool, England, sample. Further, in our still unpublished data on tibial length from birth to maturity, and for statural data, SS again exceeds SB and BB . It would appear, therefore, that the phenomenon of partial X-linkage, or better the partial influence of the X chromosome, is common to many developmental features.

Estimating the relative influence of genes on the X chromosome and of autosomal genes is admittedly hazardous. Analytical errors tend to attenuate product moment correlations, and both maternal effects and postnatal nutritional effects tend to raise them. However, if sisters sharing the paternal X exceed brothers by 0.14 to 0.16 (see above) then the relative combination of at least one X chromosome can be estimated as $(0.51)^2 - (0.33)^2$ or 0.15. By this method of computation it would appear that X chromosomal involvement is at least as large as that of the autosomes in determining brother-brother resemblance and that the X chromosome (or rather the X chromosomes) together account for the major portion of genetically-determined timing variance or postnatal ossification.

Further, however, the raw-order correlations given in the first table tend to suggest a degree of uniformity that would not, in fact, occur under the assumption of partial X-linkage. With the choice of one of a pair of maternal X chromosomes one set of brothers could be much alike in

postnatal ossification and another pair (of the same parentage) quite unlike both in tempo and pattern of ossification.³ Finally, the far greater similarity of sisters than brothers by virtue of the additional paternal X chromosome shared in common should extend itself to monozygotic twins, with single egg girl twins then being quantitatively more alike than boy twins. As with further attention to parent-child ossification timing similarities and those of male fraternal cousins, comparison of male and female single-egg twins should further illuminate the role of the X chromosome in ossification.

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LITERATURE CITED

- Black, C. R. 1966 A computer approach to the parametric and non-parametric description of distributions and their subsequent noramlization using a polynomial to obtain normalized T-scores. *Ann. N. Y. Acad. Sci.*, 134: 538-540.
- Garn, S. M., A. B. Lewis and R. S. Kerewsky 1965a Genetic, nutritional, and maturational correlates of dental development. *J. Dent. Res.*, 44: 228-242.
- 1965b X-linked inheritance of tooth size. *J. Dent. Res.*, 44: 439-441.
- Garn, S. M., A. B. Lewis and D. L. Polacheck 1960 Sibling similarities in dental development. *J. Dent. Res.*, 39: 170-175.
- Garn, S. M., A. B. Lewis, D. Swindler and R. S. Kerewsky 1967 Genetic control of sexual dimorphism in tooth size. *J. Dent. Res. Supplement* (A. A. Dahlberg, ed.), 46: 963-972.
- Garn, S. M., A. B. Lewis and A. J. Walenga 1968 Evidence for a secular trend in tooth size over two generations. *J. Dent. Res.*, 47: 503.
- Garn, S. M., and C. G. Rohmann 1962a X-linked inheritance of developmental timing in man. *Nature*, 196: 695-696.
- 1962b Parent-child similarities in hand-wrist ossification. *Am. J. Dis. Child.*, 103: 603-607.

³ Since fathers and sons share no X chromosomes in common, the assumption of X-linkage affords the possibility of relatively large intergenerational differences. Such an effect we have found for crown size (cf. Garn, Lewis and Walenga, '68).

- Garn, S. M., C. G. Rohmann and T. Blumenthal 1966 Ossification sequence polymorphism and sexual dimorphism in skeletal development. *Am. J. Phys. Anthrop.*, 24: 101-115.
- Garn, S. M., C. G. Rohmann and A. A. Davis 1963 Genetics of hand-wrist ossification. *Am. J. Phys. Anthrop.*, 21: 33-40.
- Garn, S. M., C. G. Rohmann and F. N. Silverman 1967 Radiographic standards for postnatal ossification and tooth calcification. *Med. Radiogr. Photogr.*, 43: 45-66.
- Garn, S. M., and Z. Shamir 1958 *Methods for Research in Human Growth*. Charles C Thomas, Springfield, Illinois.
- Goose, D. H. 1967 Preliminary study of tooth size in families. *J. Dent. Res.*, 46: 959-962.
- Lacey, J. I. 1956 The evaluation of autonomic responses: toward a general solution. *Ann. N. Y. Acad. Sci.*, 67: 144-150.
- Lewis, D. W., and R. M. Grainger 1967 Sex-linked inheritance of tooth size: a family study. *Arch. Oral Biol.*, 12: 539-544.