Genet. Res., Camb. (1991), **57**, *pp.* 29–35 *With 2 text-figures Printed in Great Britain*

Tich: a mutant causing disproportional growth in the mouse

JAMES R. ARCHER[1*], VICTORIA A. ARCHER[2], MICHAEL F. W. FESTING[3] AND MIRIAN A. GHIRALDINI[4]

[1] *Bone, Tooth and Biomaterials Group, Bone and Joint Research Unit, London Hospital Medical College, London E1 2AD*
[2] *Britton's School, Rainham, Essex*
[3] *MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey*
[4] *Escola Paulista de Medicina, São Paulo, Brazil*

(*Received 1 June 1989 and in revised form 19 April 1990*)

Summary

A spontaneous mutation 'tich' (gene symbol *tch*) appeared as a recessive mutation in inbred mice of strain A.TL. Homozygotes are rather dumpy mice of approximately normal weight but with short limbs and tail. Skeletal measurements on backcross siblings show that the mandible bones are almost normal but long bones and some parts of the pelvic and pectoral girdles are short. Although tich resembles brachypodism phenotypically it is not linked to agouti, and does not match the description of any other skeletal mutation. There was some evidence for weak linkage with albinism on chromosome 7. The mutation has reappeared amongst the A.TL mice of a UK commercial breeder and may have been accepted as the norm for A.TL amongst some European users of this mouse.

1. Introduction

We report the occurrence of a spontaneous mutation affecting bone growth in a subline of A.TL mice (H-2 congenic with A). It should provide a new co-isogenic inbred line for the study of bone development.

2. Materials and methods

(i) *History*

A.TL mice were originally imported into the United Kingdom in 1974 by one of the authors (J.R.A.) from Dr Chella David, then at Ann Arbor, Michigan, to the Animal Laboratories of GD Searle & Co., High Wycombe, where they were bred according to a Traffic Light system. Shortly after their arrival it was noticed that some litters contained animals with abnormally short limbs and tails. These were removed for genetic studies and proved to carry a Mendelian recessive gene (see below), which was given the provisional name of tich with the gene symbol *tch*. In 1978 they were transferred to the authors' (J.R.A., V.A.A.) home, and bred there for 2 years before being moved to Britton's School, where they were maintained until 1987. Attempts to establish a colony at the London Hospital Medical College failed until 1987, when there was an unexplained improvement in breeding performance. Animals described in this paper were bred either in the Searle colony or at the London Hospital Medical College.

Meanwhile, in 1979, the original Searle colony was transferred to OLAC (1976) Ltd. Inspection of mice from this colony (now Harlan Olac Ltd) in 1988 showed that it consisted entirely of the tich phenotype, which we are informed has been the case since 1979. These mice have been distributed amongst several European laboratories, and we suspect that any lines derived from this source are also tich.

(ii) *Description*

The mutant phenotype has short legs, a short tail and generally dumpy appearance, so much so that the older males can appear, at first glance, to be pregnant (Fig. 1). With experience, tich and normal phenotypes from the same litter can be distinguished by the time they are 7 days old. Normal and tich siblings do not differ significantly in weight.

(iii) *Mice*

Mice used in genetic studies were bred at the laboratories of GD Searle & Co. between 1974 and 1978 and at the London Hospital Medical College (1989). Strains used were A.TL, A.TL-*tch*/*tch*, and

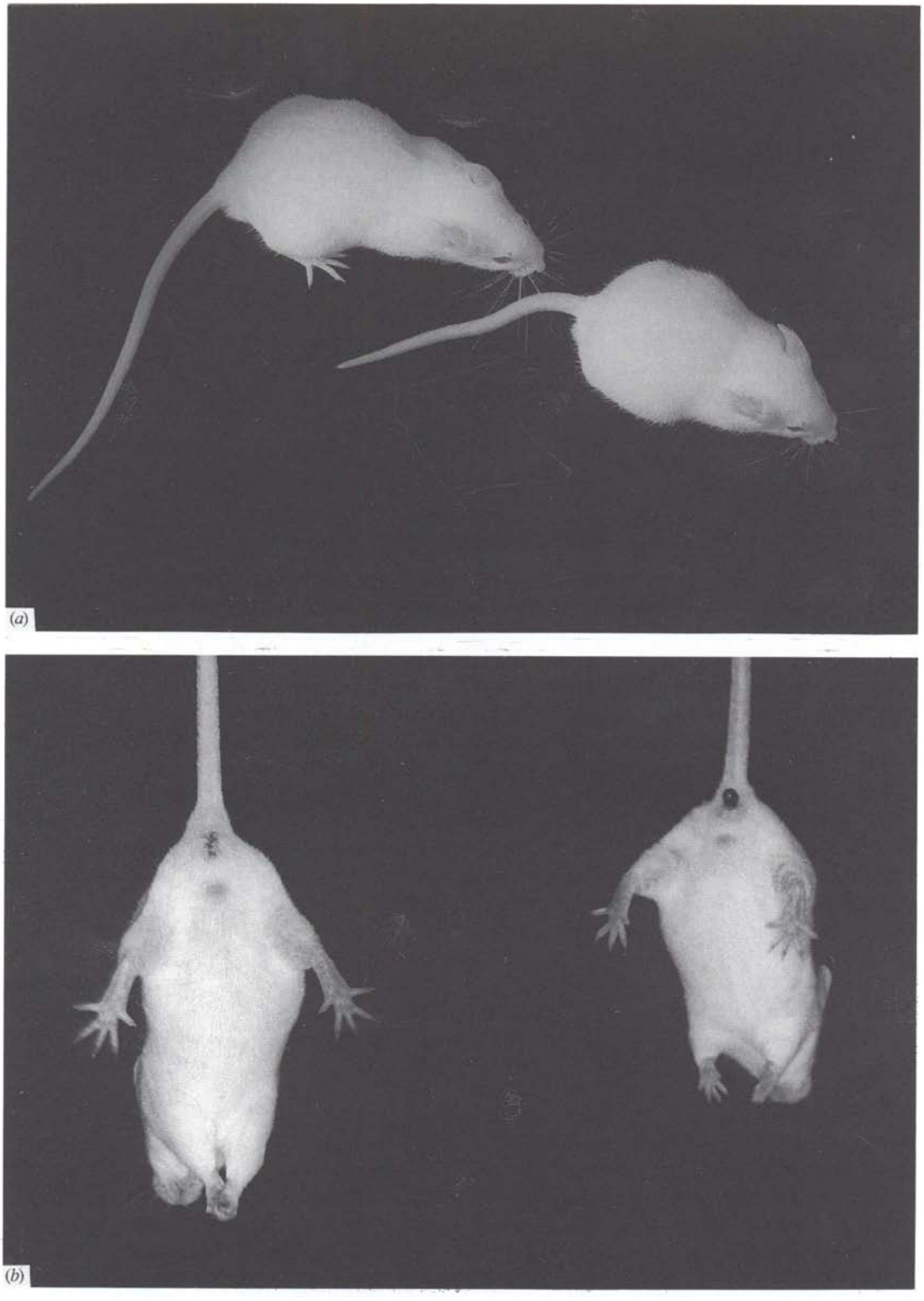

Fig. 1. Tich phenotype. 53-day-old siblings from a (C3H × ATL.*tch*/*tch* × ATL.*tch*/*tch* backcross. Left +, right tich.

C3H/HeDa. C3H differs from A.TL in the coat-colour genes *A* (chromosome 2), *B* (chromosome 4) and *C* (chromosome 7). C3H/HeDa is *H-2*^{k} and A.TL is *H-2*^{t1} (chromosome 17).

Mice used for skeletal studies [(BALB/c × A.TL-*tch*/*tch*) × A.TL-*tch*/*tch*] were bred in the animal laboratories of the London Hospital Medical College. They were 7–8 weeks old when killed.

https://doi.org/10.1017/S0016672300029001 Published online by Cambridge University Press

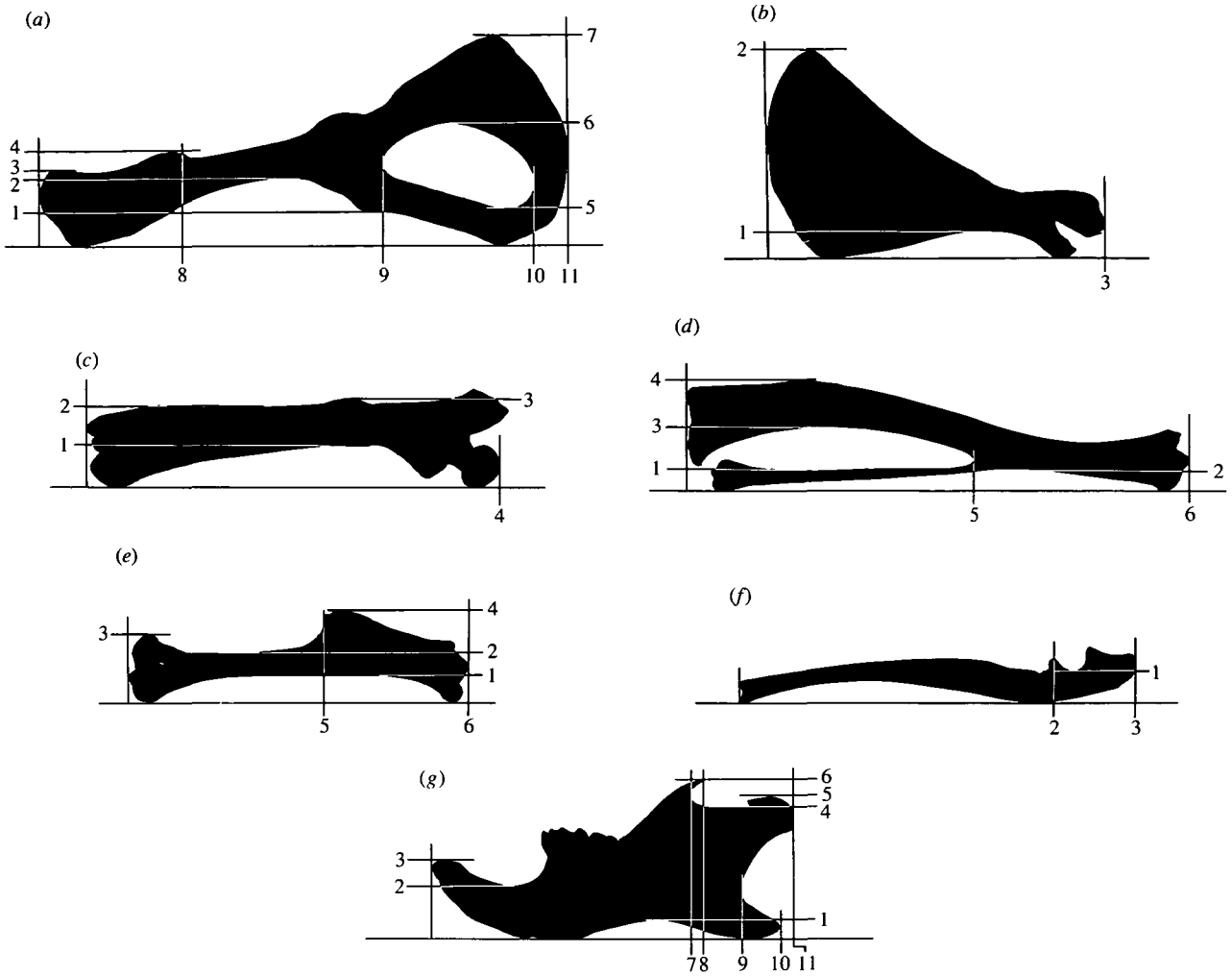

Fig. 2. Bone meausurements on (*a*) right pelvis, (P1–P11), (*b*) left scapula (S1–S3), (*c*) right femur without ephyses (F1–F4), (*d*) left tibio-fibula (T1–T6) (*e*) right humerus without head (H1–H6), (*f*) ulna (U1–U3) and (*g*) right mandible (M1–M11) in a +/+ mouse. Note that each measurement represents the length along the *x*-axis, or the height along the *y*-axis of the tangent to the curve of the bone, indicated by the line.

(iv) *Genetics*

Colour phenotypes were assessed by inspection and comparison with a known set of freeze-dried skins. The presence of *H-2*^{k} was sought using serum A175 (A.SW anti-B10.BR spleen-enlarging lymphoma; *H-2*^{s} anti-*H-2*^{k}) by a standard complement-mediated ^{51}Cr-release cytotoxicity test (Archer *et al.* 1974). This combination assumes that antibodies to the *H-2*^{k} class II region, derived from both A.TL and C3H, are distinguishable in the backcross from *H-2*^{k} class I (C3H only).

(v) *Skeletal measurements and observations*

Skeletons were prepared by standard methods, and a total of 44 measurements (Fig. 2) were made on seven different bones as described by Festing & Roderick (1989). Briefly, the bones were placed over a piece of mm graph paper which had been photographically reduced to $\frac{1}{4}$ size, touching in a standardized way a perspex '*X*' and '*Y*' axis which had been glued to the graph paper. They were then viewed with a low power microscope, and the dimensions were read off and recorded. Each measurement represents the tangent to a suitably chosen curve, read off either along the *x* (bone length) or *y* (bone height) axis. The values were read to the nearest $\frac{1}{8}$ mm. A total of 16 male tich, 13 male wild type, 15 female tich and 7 female wild type were available from a backcross of A.TL-*tch*/*tch* with (A.TL-*tch*/*tch* × BALB/c). Bones were also studied under a dissecting microscope for obvious abnormalities, though a detailed study of non-metric characters was not attempted.

(vi) *Statistical analysis*

The data from skeletal measurements were analysed using multivariate statistical methods as used by Festing & Roderick (1989). Univariate *F* tests for the hypothesis that measurements of bones from each sex by phenotype subclass were identical were also used to give a quick indication of which measurements differed most. Following Green (1981), χ^2 tests were calculated

https://doi.org/10.1017/S0016672300029001 Published online by Cambridge University Press

without Yates's correction for continuity. Dixon & Massey (1983) point out that χ^2 corrected for continuity has a lower power than the uncorrected statistic, though there appears to be no real consensus on exactly when Yates's correction should be used.

3. Results

(i) *Genetics*

Early results of crossing A.TL-*tch*/*tch* with normal mice, and of setting up backcrosses to A.TL and F2s are given in Table 1. They show that *tch* behaves as an autosomal Mendelian recessive gene. Linkage data available from backcrosses to A.TL-*tch*/*tch* from F1 hybrids of C3H and A.TL-*tch*/*tch* [(C3H × A.TL-*tch*/*tch*) × A.TL-*tch*/*tch*] are shown in Table 2 (which includes the data from the mice in Table 1 as well as mice born later). In this larger sample there was some evidence for a deficiency of tich animals in the backcrosses used to test linkage to the agouti and brown loci ($\chi^2 = 4{\cdot}96$, $P < 0{\cdot}05$), suggesting that under some circumstances the viability of *tch*/*tch* mice may be reduced. This is consistent with the difficulties we experienced in breeding tich at the London Hospital Medical College. However, in the pooled backcross data the slight deficiency of *tch*/*tch* homozygotes (86 wild type and 69 *tch*/*tch*) was not significantly different from the expected 50%. Absence of linkage to *a* indicates that *tch* is not identical to brachypodism (*bp*) (chromosome 2) and non-linkage to *b* shows that it is not achondroplasia (*cn*) (chromosome 4). There was

Table 1. *Evidence for autosomal recessive inheritance*

Mating	Pairs	Wild type M	Wild type F	tich M	tich F
Cross with wild type (+) (F1)					
A.TL$^+$ × A.TL-*tch*/*tch*	4	25	29	0	0
C3H$^+$ × A.TL-*tch*/*tch*	2	6	8	0	0
Totals	6	68		0	
Backcross					
(A.TL$^+$ × A.TL-*tch*/*tch*) × A.TL-*tch*/*tch*	2	7	4	7	4
Intercross (F2)					
(A.TL$^+$ × A.TL-*tch*/*tch*) × (A.TL$^+$ × A.TL-*tch*/*tch*)	3	17	18	7	6
Totals	3	35		13	

Table 2. *Backcross: linkage tests with four markers*

	(C3H × A.TL-*tch*/*tch*) × A.TL-*tch*/*tch*				
	wild type	tich	Total	Assortment χ^2	P
Chromosome 2					
Non-agouti	31	20	51		
Wild type (agouti)	24	14	38		
Totals	55	34	89	0·01	> 0·05
Chromosome 4					
Brown	28	16	44		
Wild type	27	18	45		
Totals	55	34	89	0·10	> 0·05
Chromosome 7					
Albino	31	35	66		
Wild type	55	34	89		
Totals	86	69	155	4·03	< 0·05
Chromosome 17					
H-2^{t1}/*H-2^k*	1	3	4		
H-2^k/*H-2^k*	4	5	9		
Totals	5	8	13	0·05[a]	> 0·05

[a] Includes Yates correction.

https://doi.org/10.1017/S0016672300029001 Published online by Cambridge University Press

Table 3. *Means of bone measurements (mm) for mice of each phenotype by sex group, pooled within-group standard deviations, measurements for tich expressed as a percentage of the wild type group, and univariate F values*

		Males			Females				
Bone code	Number...	tich 16	Wild type 13	%	tich 15	Wild type 7	%	S.D.[a]	F^b
P1		0·5	0·9	58·2	0·5	1·2	39·2	0·104	115·5
P2		1·7	2·0	88·5	1·5	2·2	68·9	0·120	63·0
P3		2·0	2·3	88·8	2·1	2·2	93·7	0·183	5·6
P4		2·5	2·7	93·3	2·3	2·6	89·2	0·166	10·3
P5		1·1	1·5	74·9	0·7	1·7	41·7	0·181	66·8
P6		3·7	4·0	92·5	3·3	4·2	78·4	0·201	43·6
P7		6·1	6·6	92·3	5·6	6·8	82·2	0·283	43·7
P8		3·9	5·0	78·3	3·8	5·3	72·2	0·382	43·5
P9		10·0	11·5	86·9	9·9	12·0	82·6	0·458	59·8
P10		13·5	16·1	83·8	13·7	17·2	79·8	0·431	190·3
P11		14·1	17·3	81·2	14·3	18·5	77·2	0·495	219·5
S1		0·7	0·8	86·9	0·7	0·9	81·0	0·080	11·2
S2		6·7	7·2	93·5	6·4	6·9	93·9	0·327	11·5
S3		9·8	10·6	92·1	9·5	10·6	90·3	0·282	47·6
F1		1·3	1·5	89·7	1·4	1·6	88·5	0·121	7·7
F2		2·7	2·8	99·0	2·7	2·8	99·4	0·129	0·2
F3		3·6	3·4	105·1	3·5	3·2	110·5	0·241	4·8
F4		10·5	13·3	78·7	10·8	13·2	81·6	0·436	152·0
T1		0·6	0·6	87·4	0·5	0·8	66·7	0·035	4·2
T2		0·6	0·8	78·3	0·6	0·9	65·3	0·016	28·1
T3		2·1	2·0	100·8	2·0	2·1	92·2	0·009	2·4
T4		3·8	3·6	106·1	3·6	3·6	99·3	0·014	0·7
T5		6·5	9·0	71·4	6·5	8·9	72·4	0·010	72·4
T6		12·4	16·1	76·7	12·5	16·0	77·9	0·006	118·8
H1		0·8	0·9	88·1	0·8	1·0	82·1	0·016	6·4
H2		1·7	1·8	92·9	1·7	1·8	91·5	0·007	7·7
H3		2·3	2·5	95·6	2·3	2·6	91·1	0·006	9·8
H4		3·2	3·3	94·6	3·0	3·3	90·6	0·015	2·3
H5		5·1	5·8	88·8	5·1	5·8	87·6	0·003	114·1
H6		9·7	11·0	88·9	9·5	10·8	88·5	0·005	39·6
U1		1·0	0·9	111·8	1·0	0·9	104·8	0·116	2·5
U2		7·4	9·6	76·8	7·5	9·8	76·4	0·456	98·2
U3		9·6	12·2	78·0	9·7	12·4	78·2	0·544	98·2
M1		0·8	0·8	99·2	0·7	0·8	96·7	0·076	6·1
M2		1·8	1·8	98·7	1·8	1·9	95·1	0·075	2·7
M3		2·5	2·5	98·6	2·5	2·5	99·0	0·049	1·7
M4		4·8	4·8	100·7	4·7	4·8	98·7	0·134	1·2
M5		5·3	5·4	98·9	5·2	5·4	95·2	0·188	3·9
M6		5·9	5·9	99·4	5·8	5·9	98·3	0·167	2·9
M7		8·1	8·2	98·6	8·1	8·3	98·8	0·125	3·7
M8		8·5	8·6	99·2	8·5	8·7	97·5	0·187	2·3
M9		10·2	10·4	97·8	10·1	10·4	97·5	0·146	11·1
M10		11·5	11·9	97·1	11·4	11·8	96·4	0·216	14·5
M11		11·5	11·8	97·8	11·5	11·8	96·9	0·183	11·2

[a] Pooled within-group standard deviation.
[b] *F* value (3 and 47 degrees of freedom) for the hypothesis that there are no differences between phenotype × sex subclasses. Note critical values for 5, 1 and 0·1% significance levels are approximately 2·8, 4·2 and 6·5, respectively.

statistically significant ($\chi^2 = 4{\cdot}03$, $P < 0{\cdot}05$) evidence for weak linkage with the albino locus (chromosome 7), with an estimated recombination frequency of $c = 0{\cdot}42 \pm 0{\cdot}04$ units. However, further work will be needed to establish whether this is a real effect or is due to chance. It just fails to reach statistical significance at $P = 0{\cdot}05$ if Yates's correction is used.

(ii) *Skeletal measurements and observations*

The results of the skeletal measurements are summarized in Tables 3 and 4. Multivariate statistical analysis indicated that differences between the sexes were small for most bones except the pelvis (see Table 4), so the univariate *F* values shown in Table 3 can largely be attributed to differences between the

https://doi.org/10.1017/S0016672300029001 Published online by Cambridge University Press

Table 4. *Summary of multivariate significance levels for four comparisons of interest for each bone*

Bone	Males tich *vs.* wild type	Females tich *vs.* wild type	Male wild type *vs.* female wild type	Male tich *vs.* female tich
Pelvis	***	***	***	***
Scapula	***	***	N.S.	*
Femur	***	***	N.S.	*
Tibio-fibula	***	***	N.S.	N.S.
Humerus	***	***	N.S.	N.S.
Ulna	***	***	N.S.	N.S.
Mandible	**	**	N.S.	*

N.S., not statistically significant.
* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

phenotypes. There were statistically highly significant differences for all bones, though differences in the lengths of the long-bones and the pelvis were most pronounced. Measurements P10 and P11, F4, T6, H5 and U2 and U3 in *tch/tch* mice ranged from 76 to 88% of normal. Most bone widths (e.g. U1, F2 and H2) were more normal, though the width of the pelvic bones as measured by P1 and P2 was markedly reduced to only 39 and 58% of normal in females and males, respectively. This was associated with a partial or complete failure of fusion of the ischium with the pubic bones, the only obvious qualitative skeletal abnormality observed.

The measurements of the mandible were much less affected. In no case was the measurement in *tch/tch* mice less than 95% of normal in females or 97% of normal in males, and a reduction of this magnitude in both length and height can be attributed to a proportional over-all reduction in the size of the mice.

As no homozygous normal skeletons were available, it is not possible to be absolutely sure that tich is completely recessive for all skeletal measurements. However, the ratios of lengths of long bones to the length of the mandible appear to be within the normal range in tich heterozygotes. For example, the ratio of ulna length to mandible length (U3/M11) ranged from 1·02 to 1·10 among the 12 strains of mice tested by Festing & Roderick (1989) compared with 1·03 and 1·05 for heterozygotes (i.e. clearly within this range) and 0·83 and 0·84 (well outside the range) for tich males and females, respectively.

4. Discussion

The results given in Tables 3 and 4 show that tich is a mutation affecting the skeleton, which causes disproportionate dwarfing. In *tch/tch* mice the mandibles were slightly reduced in size, but of very similar shape to those of the heterozygotes. In contrast, the long bones including the femur, tibio-fibula, and ulna were only about 80% of the normal length, though the humerus was about 88% of normal. Bone thicknesses were mostly near normal, except in the pelvis where measurements P1 and P2 were markedly less than normal, reflecting the extreme thinness of the bones at this point associated with the failure in the fusion of the ischium and pubis.

The multivariate analysis suggested that all bones were abnormal in *tch/tch* mice of both sexes, though this abnormality was less marked in the mandibles than the other bones. In this experiment there was no evidence for sexual dimorphism among the heterozygous mice for any bone except the pelvis, but among the *tch/tch* mice the scapula, femur and mandible also showed sexual dimorphism. However, this may simply be a reflection of the larger number of *tch/tch* animals which were available.

Tich does not appear to match the description of any of the 119 skeletal mutants described by Green (1989), with the possible exception of chubby (Wikström *et al.* 1987), which has an even more extreme shortening of the tibia and femur to 64 and 73% of normal, respectively. Breeding tests will be needed to determine whether chubby and tich, which are both fully penetrant and viable, are allelic. Phenotypically, it also resembles brachypodism (*bp*), showing similar disproportionate dwarfism, with both sexes fully viable and fertile. It is also somewhat similar to achondroplasia (*cn*) except that it does not have a domed skull, and is somewhat more viable than homozygous *cn/cn* mice. However, tich is not linked to the agouti or brown loci, thereby ruling out the possibility that it is another mutation at either of these loci.

There is some suggestion that tich is weakly linked to albino on chromosome 7, with a recombination frequency of about $0{\cdot}42 \pm 0{\cdot}04$, but more work will be needed to confirm or refute this observation. The skeletal mutation pudgy is approximately 36 cM proximal to the C locus. However the phenotype of

https://doi.org/10.1017/S0016672300029001 Published online by Cambridge University Press

pudgy is different from tich, with a shortening of the vertebral column, but without shortening of the long bones, so it seems unlikely that tich is another mutation at the pudgy locus.

Tich does not have the domed skull characteristic of brachymorphism (*bm*), a similar mutation causing disproportionate dwarfism which maps to chromosome 19, though no genetic data are available to rule out the possibility that it may be a variant allele at this or another locus associated with skeletal growth.

A number of other mutations causing disproportionate dwarfism also differ from tich either in phenotype or mode of inheritance. For example disproportionate micromelia (*Dmm*) has disproportionate shortening of the limbs, but differs in having a shortened head and a dominant mode of inheritance. Chubby (*cby*), chondrodysplasia (*cho*), phocomelia (*pc*) and droopy ear (*de*) all cause disproportionate dwarfism with recessive inheritance, but they also have various head and other abnormalities not seen in tich. Thus, it appears that tich differs from any of the mouse mutations causing disproportionate dwarfing described so far.

A.TL is a widely used strain which is congenic at the H-2 locus with strain A. Thus A.TL-*tch*/*tch* is a strain which is co-isogenic with A.TL. Presumably tich, like brachypodism, causes abnormalities of cartilage development leading to abnormal ephiphyseal end plates and hence abnormal growth of the long bones. It may provide a useful model for fundamental studies of bone development.

This work was supported by a grant from the Arthritis and Rheumatism Council.

References

Archer, J. R., Smith, D. A., Davies, D. A. L. & Staines, N. A. (1974). A skingraft enhancing antiserum which recognises two new B cell alloantigens determined by the major histocompatibility locus of the mouse. *Journal of Immunogenetics* **1**, 337–344

Dixon, W. J. & Morgan, F. J. (1983). *Introduction to Statistical Analysis*, 4th edn. London, New York: McGraw-Hill International Book Co.

Festing, M. F. W. & Roderick, T. H. (1989). Correlation between genetic distances based on single loci and on skeletal morphology in inbred mice. *Genetical Research* **53**, 45–55

Green, E. L. (1981). *Genetics and Probability in Animal Breeding Experiments*. London, Basingstoke: Macmillan Publishers Ltd.

Green, M. C. (1989). Catalog of mutant genes and polymorphic loci. In *Genetic Variants and Strains of the Laboratory Mouse* (ed. M. F. Lyon and A. G. Searle), pp. 12–403. Oxford, New York: Oxford University Press.

Wikström, B., Wallace, M. E., Hjerpe, A. & Engfeld, B. (1987). Chubby: a new autosomal recessive skeletal mutation producing dwarfism in the mouse. *Journal of Heredity* **78**, 8–14.