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Genetic aspects of nutritional rickets

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Doxiadis, S., Angelis, C., Karatzas, P., Vrettos, C., and Lapatsanis, P. (1976). *Archives of Disease in Childhood*, **51**, 83. **Genetic aspects of nutritional rickets.** Amino acid excretion was investigated in 21 rachitic infants and in 22 of their parents. There was (a) increased α-amino acid excretion in one-third of the infants a long time after the rickets had healed, (b) an abnormally high excretion of α-amino nitrogen and of phosphorus in many of the parents, (c) an abnormal pattern of amino acid excretion in all 9 infants tested, and (d) a good correlation between the excretion of individual amino acids by an infant and by its parents. Our findings suggest that in at least some cases of nutritional rickets there is a genetic element which may manifest itself only under adverse environmental conditions.

Before the discovery of vitamin D and its relation to rickets there was some speculation about whether the disease was hereditary, but this was forgotten when the role of such definite environmental factors as lack of sunshine or of vitamin D in the diet became established. Jonxis and his associates (Jonxis, Smith, and Huisman, 1952), studying the aminoaciduria of vitamin-D sensitive rickets, found that some members of the families of rachitic infants were also excreting abnormally large amounts of amino acids. They therefore suggested that there might be a hereditary factor in the pathogenesis of the disease. The possibility received additional support when sex differences in infants with nutritional rickets were found by Childs, Cantolino, and Dyke (1962) in hospital patients, and by us (Lapatsanis, Deliyanni, and Doxiadis, 1968) in a random sample of infants in urban and rural areas. We suggested that vitamin D deficiency rickets might be a hereditary disease manifesting itself only under adverse environmental conditions.

In this paper we report on the amino acid excretion of a group of rachitic infants and their parents. We found in some of the infants that a biochemical defect persisted after the rickets had healed and that the same defect was present in some of the parents of these infants. This finding gives added support for the belief that there is a genetic factor in nutritional rickets.

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Patients and methods

Twenty-one rachitic infants aged 3 to 18 months were studied. They were considered to be rachitic because their serum alkaline phosphatase was above 20 King-Armstrong (K-A) units in the absence of clinical signs of liver disease. Furthermore, they had received either no vitamin D or only minimal amounts and they had not been exposed to sunlight. After the initial investigation the infants were treated with 4000 IU of vitamin D daily and they were examined every 4–6 weeks until their serum alkaline phosphatase fell to 20 K-A units or lower. This was taken as the time of biochemical healing of the disease (Fig. 1). Thereafter they continued to receive a daily prophylactic dose of 800–1000 IU vitamin D.

Twenty-two of the parents of these infants who had neither clinical nor biochemical evidence (serum calcium, phosphorus, and alkaline phosphatase levels) of active osteomalacia were also studied.

Ten healthy infants from a babies' centre and 2 healthy adults were used as controls. The 10 infants were under medical care and received vitamin D supplements of 800 IU daily. Their serum alkaline phosphatase, calcium, and phosphorus levels are shown in Table I.

Infants. The 21 rachitic infants were examined clinically and *x*-rays of their wrists were taken before treatment and at 4-weekly intervals until biochemical and radiological healing. Serum calcium, phosphorus, alkaline phosphatase, and total aminoaciduria were measured at the same time. In those infants in whom amino acid excretion was still raised at the time of biochemical healing measurements of total amino-

TABLE I

Serum alkaline phosphatase, calcium, and phosphorus levels in 10 normal infants

Case no.	Sex	Age (m)	Phosphatase (K-A units)	Ca (mg/100 ml)	P (mg/100 ml)
1	M	8	12	9·8	5·1
2	M	9	14	8·8	4·6
3	M	13	20	9·0	5·9
4	M	8	18·5	9·6	4·7
5	M	13	15·5	9·0	5·9
6	M	6	16·5	8·6	5·9
7	M	3	14	9·0	5·4
8	M	8	16	9·2	5·8
9	M	7	17	9·5	5·0
10	M	6	18	9·3	4·8

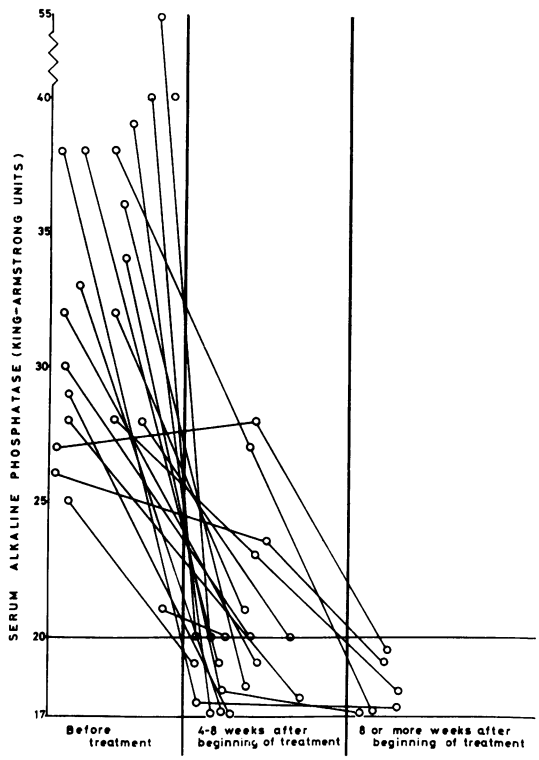

FIG. 1.—*Serum alkaline phosphatase levels in rachitic infants before and after beginning treatment.*

aciduria were repeated 8 to 12 weeks later. In 9 infants we estimated the pattern of excretion of amino acids in 24-hour specimens. In the 10 controls serum calcium phosphorus and alkaline phosphatase, urine total amino acid excretion, and, in 2 of them, the pattern of excretion of amino acids were estimated.

Adults. In the parents serum calcium, phosphorus, and alkaline phosphatase and 24-hour total amino acid, phosphorus, and creatinine excretion were measured. In 10 of the parents the pattern of excretion of amino acids in 24-hour specimens of urine was recorded. In the 2 healthy adult controls total amino acid excretion and the pattern of excretion of amino acids were recorded.

In both infants and adults two 24-hour specimens of urine were collected; if the creatinine excretion differed between the two by more than 20% we considered the collection unreliable and repeated the procedure.

Chemical methods. Blood samples were collected at about 9 a.m. and the serum separated immediately. Serum alkaline phosphatase was measured by the method of King and Armstrong (1934), serum and urinary phosphorus by the method of Fiske and Subbarow (1925), serum calcium by flame photometry. The method of Sobel *et al.* (1957) was used for estimating the 24-hour urinary total α-amino-nitrogen, and the Beckman 120C amino acid analyser was used for measuring the pattern of excretion of amino acids in the urine.

Results

Table II shows the serum alkaline phosphatase, calcium, and phosphorus levels in the 21 infants before treatment. It also shows the incidence of rachitic changes in the *x*-rays of the wrists. The serum calcium in 10 infants was below 8 mg/100 ml and serum phosphorus was below 4 mg/100 ml in 7. Only 3 of the 21 infants had no or doubtful *x*-ray changes of the wrists.

Fig. 2 shows the total amino acid excretion in mg/kg per 24 h of the 21 rachitic infants and of the 10 controls. In only one of the rachitic infants was the amino acid excretion normal before treatment. When as a result of treatment the alkaline phosphatase values returned to normal (below 20 K-A units) 4 to 10 weeks later, the amount of amino acids excreted fell considerably in the group as a whole. However, despite the biochemical and radiological healing amino acid excretion was still raised in 11 of the 19 infants examined. In 10 of

TABLE II

Serum alkaline phosphatase, calcium, and phosphorus levels and x-ray signs in the wrist in 21 rachitic infants

Case no.	Sex	Age (m)	Serum			*X*-ray signs*
			Phosphatase (K-A units)	Ca (mg/100 ml)	P (mg/100 ml)	
1	M	11	26	9·4	4·4	+
2	M	3	27	6·4	6·2	−
3	F	18	38	8·8	3·8	+ + +
4	M	5	33	7·0	4·3	+ + +
5	M	5	28	7·0	4·5	+ +
6	M	6	30	6·8	3·2	+ +
7	M	6	29	7·0	3·9	+ +
8	M	4	28	8·0	4·1	−
9	M	13	34	6·6	4·4	+
10	M	7	33	7·2	3·4	+
11	F	12	36	8·2	3·6	+
12	M	12	28	7·4	5·1	±
13	M	3	40	7·0	5·9	+
14	M	3	40	5·8	5·7	+
15	F	5	32	8·2	4·6	+
16	M	12	54	8·6	3·8	+
17	F	9	39	10·5	3·8	+
18	F	8	32	10·0	6·1	+
19	M	6	27	9·2	6·0	+
20	M	8	25	9·8	5·6	+
21	M	3	21	8·0	4·4	+

**X*-ray signs: +, Rarefaction of the irregular fraying of provisional zone of calcification of ulna and/or radius. + +, Rarefaction plus cupping and widening of distal ends of ulna and/or radius. + + +, The first two plus diffuse osteoporosis of ulna and/or radius.

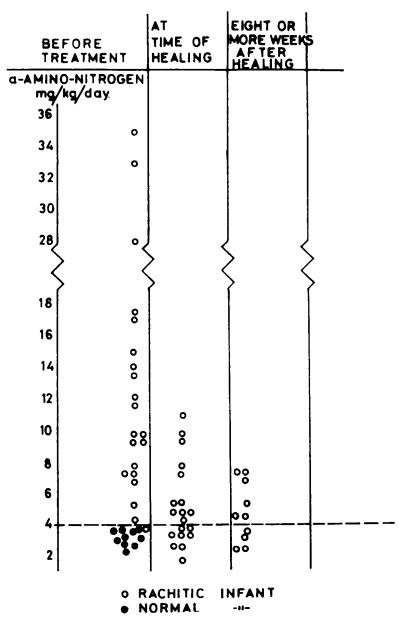

FIG. 2.—*Urinary total amino acid excretion in rachitic infants before, at time of healing, and 8 or more weeks after healing compared with total amino acid excretion in normal infants.*

these 11 infants further estimations were made 8 to 12 weeks later, and in 6 excretion was still high.

This continuing aminoaciduria after biochemical and radiological healing of the disease suggested the presence, at least in some cases of rickets, of a more persistent biochemical defect that was only partly influenced by vitamin D treatment. Its persistence, though in a milder degree, raised the suspicion that it may have preceded the development of rickets and might be of genetic origin. If this were so a similar biochemical defect might be detectable in the parents. We therefore measured their amino acid excretion, phosphorus clearance, and urine phosphorus: urine creatinine concentration ratio. We made a distinction between the parents of infants whose aminoaciduria disappeared and those of the infants in whom it persisted, without implying that there was a qualitative difference between the two.

Table III shows the results of these tests in 13 parents of 9 infants whose abnormal aminoaciduria *disappeared* after treatment with vitamin D. Of the 13 parents examined, 7 had an abnormally high clearance of phosphorus, 6 an abnormally high urine phosphorus: urine creatinine concentration ratio, and 2 an abnormally high α-amino-nitrogen excretion.

Table IV shows the same measurements in 9 parents of 5 rachitic infants whose pathological

TABLE III

Phosphorus clearance, urine phosphorus : urine creatinine concentration ratio, and urinary α-amino-nitrogen excretion in 13 parents of 9 rachitic infants, whose aminoaciduria disappeared after treatment

Case no.	Parents	Phosphorus clearance (ml/min)	UP : UCr ratio	α-amino-N excretion (mg/kg per 24 h)
2	M	14	0·60	2·7
	F	20*	0·73*	3·2
9	M	14·1	0·54	3·6
	F	—	—	—
15	M	—	—	—
	F	9·2	0·6	2·4
16	M	18·3*	0·6	4·05*
	F	10	0·5	3·2
17	M	23·5*	0·6	2·1
	F	15·4	1·2*	2·3
18	M	—	—	—
	F	24*	0·7*	4·1*
19	M	29·4*	0·98*	1·6
	F	29*	0·8*	3·1
20	M	—	—	—
	F	11	0·48	2·2
21	M	—	—	—
	F	25*	1*	3·4

*Abnormal values.
UP, phosphorus in urine (mg/100 ml). UCr, creatinine in urine (mg/100 ml).

TABLE IV

Phosphorus clearance, urine phosphorus : urine creatinine concentration ratio, and urinary α-amino-nitrogen excretion in 9 parents of 5 rachitic infants with persistent amino aciduria

Case no.	Parents	Phosphorus clearance (ml/min)	UP : UCr ratio	α-amino-N excretion (mg/kg per 24 h)
3	M	32·8*	0·73*	7*
	F	17·0	0·46	5·4*
4	M	19·6*	0·54	6·2*
	F	15·6	0·5	4·02*
10	M	16·4	0·64	5*
	F	—	0·89*	3·2
12	M	—	—	—
	F	32*	0·65*	7*
13	M	23*	1·9*	5·4*
	F	22*	0·3	4·7

*Abnormal values.
Abbreviations as in Table III.

aminoaciduria *persisted* for 8 or more weeks after the complete biochemical and radiological healing of rickets. 5 parents had an abnormally high phosphorus clearance, 4 an abnormally high urine phosphorus : urine creatinine ratio, and 8 an abnormally high α-amino nitrogen excretion.

Table V shows the number of the renal tests in which the results were abnormal in the two groups of parents. All 9 parents of infants with persisting aminoaciduria had at least one abnormal test and 5 had two or three. 8 of the 13 other parents had at least one abnormal test.

We measured the pattern of amino acid excretion in 9 rachitic and 2 healthy infants before treatment (Table VI). Of the 9 rachitic infants, 4 (Cases 4, 10, 12, and 13) were later found to have persistent aminoaciduria and the remainder nonpersistent aminoaciduria. Amino acid excretion in the 2 healthy control infants was within normal limits (O'Brien, Ibbott, and Rodgerson, 1968). Excretion of from 3 to 11 amino acids was abnormally high in all 9 rachitic infants. In 7 the excretion of lysine, serine, glycine, alanine, and tyrosine was abnormally high.

TABLE V

Number of abnormal results of renal function tests in parents of rachitic infants

	No. of parents	No. of abnormal renal tests			
		3	2	1	Nil
Parents of infants whose aminoaciduria disappeared	13	1	5	2	5
Parents of infants with persistent aminoaciduria	9	3	2	4	—
Total	22	4	7	6	5

TABLE VI

Pattern of amino acid excretion (μmol/min per 1·73 m^2) in rachitic infants and in controls (only abnormally high values are recorded in the case of rachitic infants)

Amino acid	Normal values*	Healthy controls		Rachitic infants								
		Case 1	Case 2	Case 4	Case 10	Case 12	Case 13	Case 16	Case 18	Case 19	Case 20	Case 21
Lysine	0·04–0·21	0·11	0·08	0·94	0·57	0·99	0·42	0·98	0·47	0·22	0·26	
Histidine	0·11–1·00	0·28	0·32	1·21	1·17		1·08	1·41	1·12			
Arginine	0·01–0·04											
Aspartic acid	tr–0·07	tr	0·004				0·13					
Threonine	0·04–0·17	0·05	0·05	1·15	1·39		0·69		0·45	0·29		
Serine	0·09–0·34	0·13	0·23	1·85	1·95	0·83	1·82	1·50	1·10	0·94	0·93	1·21
Glutamic acid	0·01–0·13	0·05	0·04	0·24	0·37	0·26	0·26	0·19	0·29			
Proline	tr–0·04	tr	tr	0·22			0·26					
Glycine	0·33–1·50	0·16	0·19	3·47	3·11	1·72	3·08	2·38	2·45	1·98		2·62
Alanine	0·04–0·35	0·14	0·12	0·99	1·06	0·45	1·15	0·78	0·67	0·57	0·39	0·82
Cysteic acid	—	0·56	0·22									
Valine	tr–0·08	tr	0·015									
Methionine	0·01–0·04	0·022	0·013				0·08		0·11			
Isoleucine	0·01–0·07	0·014	0·014									
Leucine	0·02–0·11	0·021	0·023									
Tyrosine	0·03–0·12	0·06	0·04	0·45		0·25	0·30	0·29	0·17	0·14	0·16	
Phenylalanine	0·01–0·11	0·019	0·027	0·17				0·13				

*O'Brien, Ibbott, and Rodgerson (1968). tr = trace

Table VII shows the pattern of amino acid excretion in 10 parents of the 9 rachitic children whose pattern is shown in Table VI and in 2 controls. Excretion in the 2 healthy adult controls was within the normal ranges (O'Brien *et al.*, 1968) with the exception of histidine, excretion of which was a little high in both controls. In all of the 10 parents of rachitic infants excretion of at least one amino acid was high, and in all 5 parents of infants with persistent aminoaciduria the excretion of from 3–7 amino acids was abnormally high.

We found that the abnormally high urinary excretion of amino acids in 9 rachitic infants correlated well with a high excretion in their parents in respect of amino acids (Table VIII). In the case of 5 amino acids (threonine, glutamic acid, proline, alanine, and tyrosine) excretion was abnormally high in many more infants than parents. Since in the case of only one infant were we able to examine both parents this finding was not unexpected. The opposite was also observed in a smaller number of cases.

Discussion

This study has confirmed the findings of Jonxis *et al.* (1952) that most infants with nutritional rickets excrete abnormally high amounts of amino acids in the urine. They stated that the aminoaciduria disappears with treatment and healing (Jonxis and Huisman, 1953; Jonxis, 1955) but their published data do not seem to support this. Furthermore, their finding of persisting aminoaciduria in older children and adults with healed rickets implies that the abnormal amino acid excretion in nutritional rickets may not always disappear. Amino acid excretion was still abnormally high in

TABLE

Pattern of amino acid excretion (μmol/min per 1·73 m²) in 10 parents of 9 in the case

Amino acid	Normal adult values*	Healthy controls			
		Case 1	Case 2	Case 4 (M)	Case 10 (M)
Lysine	0·02–0·20	0·14	0·20	0·30	
Histidine	0·15–0·53	0·64	0·85	2·14	1·69
Arginine	0·02–0·20	—	—		
Aspartic acid	0·03–0·09	0·02	0·01		
Threonine	0·09–0·14	0·12	0·11	0·55	
Serine	0·09–0·31	0·51	0·56	1·71	1·48
Glutamic acid	0·008–0·16	0·06	0·05		
Proline	nil	nil	nil		
Glycine	0·40–0·90	0·87	0·89	3·41	5·80
Alanine	0·09–0·27	0·26	0·22	0·43	0·56
Cysteic acid	0·02–0·08	0·08	0·06		
Valine	tr–0·05	0·017	0·035		
Methionine	0·02–0·04	0·022	0·022		
Isoleucine	0·01–0·04	0·025	0·027		
Leucine	0·02–0·05	0·024	0·031		
Tyrosine	0·06–0·10	0·075	0·081	0·16	
Phenylalanine	0·04-0·07	0·034	0·046		

*O'Brien, Ibbott, and Rodgerson (1968).

about half of our infants after all other signs of rachitic activity had cleared, indicating that raised amino acid excretion is the most persistent of the various biochemical disorders associated with rickets. We also observed that in 6 of the 10 infants the raised excretion of amino acids persisted 8 to 12 weeks after the rickets had healed. Since all infants continued to receive vitamin D in prophylactic doses and the alkaline phosphatase level remained normal, the persisting aminoaciduria could not have been due to a relapse of the rickets. It could mean that in some cases of rickets the raised excretion of amino acids is a permanent biochemical feature and as such may be genetically determined.

TABLE VIII

Numbers of rachitic infants, parents of rachitic infants, and infant-parent pairs whose urinary excretion of 11 named amino acids was abnormally high

	Infants (n=9)	Parents (n=10)	Pairs
Lysine	8	6	6
Histidine	5	7	4
Aspartic acid	1	1	1
Threonine	5	2	2
Serine	9	8	7
Glutamic acid	6	0	0
Proline	2	0	0
Glycine	8	9	7
Alanine	9	4	4
Methionine	2	3	2
Tyrosine	7	2	2

Seventeen of the parents of our rachitic infants had abnormalities of phosphorus and/or amino acid excretion, and the incidence of such abnormalities was higher in the group of parents whose infants had a raised aminoaciduria persisting 8 to 12 weeks after complete radiological and biochemical healing of their disease. In all the rachitic infants excretion of at least 3, and in some infants up to 11, amino acids was abnormally high. The excretion of serine and alanine was abnormally high in all 9 infants tested, and of lysine and glycine in 8 of them. The amino acid excretion in 10 parents of those 9 infants (independently of their sex or of the sex of the affected infant) was abnormally high of at least one amino acid and of at least 3 in the parents of those infants with persisting aminoaciduria. Furthermore, there was a good correlation between infants and parents in respect of the amino acids which they secreted in large amounts. The exceptions to this could be partly explained by the fact that in the case of 8 of the 9 infants only one parent was examined. A high excretion of one amino acid by a parent and not the infant may obviously be explained by a recessive mode of transmission.

All these findings strongly suggest that in vitamin D sensitive rickets there is a genetically determined biochemical disorder, which is evident also in the parents of affected infants. Adverse environmental factors will lead to the development of clinical

VII

rachitic infants and in 2 controls (only abnormally high values are recorded of parents)

Parents (M, F) of rachitic infants							
Case 12 (F)	Case 13 (M)	Case 13 (F)	Case 16 (M)	Case 18 (F)	Case 19 (F)	Case 20 (F)	Case 21 (F)
		0·27	0·53	0·66	0·39	0·58	
1·11		1·56		1·44	1·41		1·12
	0·22						
				0·38			
0·69	0·80	1·10		0·90	1·20		0·98
1·89	1·32	1·98		1·65	2·62	1·96	3·00
					0·30	0·32	
		0·06		0·06	0·05		
				0·08	0·06		
				0·06			
					0·12		

vitamin-D sensitive rickets. In our field survey (Lapatsanis *et al.*, 1968) only 25% of the infants who were not exposed to sunlight and who did not receive vitamin D in their diet developed rickets. These infants, we surmise, had the genetic defect preceding the development of the disease.

The nature of the primary defect cannot be determined from our findings. The abnormally high amino acid excretion, present in all but one of the 21 infants, seems to be partly corrected by treatment with vitamin D, and therefore secondary, and partly persistent both in infants and their parents. At present the way in which the genetic and environmental factors interact in clinical rickets is also not known. Clearly it is not an 'all or none' disorder, and there are quantitative variations covering a wide spectrum of manifestations. At one end are the infants with no, or mild, aminoaciduria, disappearing after treatment with vitamin D and with a smaller number of parents with abnormal tests of proximal tubular dysfunction. At the other end are infants with aminoaciduria persisting for many weeks after the standard biochemical criteria of vitamin-D sensitive rickets have returned to normal and with a higher proportion of abnormal tests in their parents. It may well be that the various forms of vitamin-D resistant rickets are extreme variants of the same spectrum.

Our inability to provide a complete explanation of all the observed facts extends also to the question of sex linkage. Childs *et al.* (1962) and Childs (1965) first suggested that there may be a locus on the X chromosome which has something to do with rickets and vitamin D, and our later findings (Lapatsanis *et al.*, 1968) have added weight to this hypothesis. However, the present work does not support a pure sex-linked inheritance of a genetic defect. Although we have been unable to examine both parents of all children we have found cases where abnormal tests were present in the father of an affected male or the mother of an affected female infant. On the other hand, in our present material males again predominate both in number and also perhaps in the severity of the disorder. Furthermore, in 7 male infants there were abnormal tests in their mothers. Since, however, the fathers were not examined in all of them, we cannot consider a sex-linked effect certain.

Vitamin-D sensitive rickets is not therefore, as is usually described, a single aetiological entity but the result of an interaction between hereditary elements—one of them sex-linked—and environmental influences. Abnormally high amino acid excretion seems to be part of the hereditary mechanism, but a secondary pathological aminoaciduria may also develop as a result of vitamin D deficiency. The primary genetic disorder varies in its severity from infant to infant or from family to family, and probably cases of vitamin-D resistant rickets differ only quantitatively from vitamin-D sensitive rickets and they belong to one end of a wide spectrum

of disorders of proximal renal dysfunction, the milder part of this spectrum being covered by cases of what we call vitamin-D sensitive rickets.

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