

Spina Bifida Cystica Families X-Ray Examination and HLA Typing

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Summary

Thirty-three families of individuals with a history of spina bifida with myelomeningocele were studied. Each member was HLA typed and had a spinal X-ray. The incidence of spina bifida occulta and laminal asymmetry of the lumbo-sacral spine in sibs and parents is high. No association between these abnormalities and the HLA system was found.

Spina bifida cystica with myelomeningocele is one of the most frequent and severe congenital malformations of the spinal cord. The etiology of this condition is still unknown.

The recurrence risk of this malformation is high in sibs (3% from a review study) (10). The recurrence risk rises as the number of affected sibs and the population incidence increase, but in general is lower than 25%. In parents of affected children, there is a high incidence of spina bifida occulta (7). Amos *et al.* (1) have described a large family with 98 members in whom they found an inherited association of spina bifida occulta and vertebral joint facet asymmetry, which they ascribed to a single locus. This locus would be on chromosome 6 at about 30 centimorgans distance from the HLA group of loci.

A human equivalent of the T-locus of the mouse has been looked for, and there is suggestive, albeit circumstantial evidence for this in man (4). The T-locus complex in the mouse is located on chromosome 17, at about 12 to 14 centimorgans from the centromere. It is linked to the H-2 complex and is the site of a great number of mutations, some of which are responsible for tail and vertebral abnormalities. Sperm production, genetic recombination and other aspects of embryonic development are sometimes affected by some specific mutations of the T-locus.

In an earlier study, we performed HLA, Pi (alpha-1-antitrypsin), Gm, and Km (immunoglobulins) typing in 53 spina bifida cystica cases (11). The present study looks for an association between HLA haplotypes and spinal abnormalities, especially spina bifida occulta and laminal asymmetry.

MATERIALS AND METHODS

For 8 years, 146 children with spina bifida cystica have been treated by the pediatric surgical service of Rouen. A study of both parents was possible in 33 families, of only one parent in 14 families, of sibs in 40 families, and of relatives such as aunts, uncles, and grandparents in 19 families. The objective and limits of our study had been explained to each subject. Most of them permitted a venepuncture and an anteroposterior X-ray examination of the lumbosacral spine.

Complete red-blood-cell, Gm and Km, Pi and HLA haplotypes were ascertained. HLA determination was accomplished by the microlymphocytotoxicity technique with local and national re-

agents. HLA haplotypes were determined by family studies. Lumbo-sacral X-rays of relatives were read by two radiologists. Only spina bifida occulta and vertebral laminal asymmetry were considered to be abnormal for the purposes of this study. The first abnormality is defined as an absence of fusion of each half of the posterior vertebral arch; the second is the result of an asymmetric fusion of these arches (Fig. 1). It should be remembered that each lateral half of the vertebral arch originates from a separate centre of ossification. They fuse during the first year of life in the lumbar vertebrae (5).

The average incidence of these abnormalities was determined in two control studies. The first study was of 299 adult patients who had an X-ray examination for other pathologic reasons, and the second was of 178 children who had an X-ray examination for abdominal pain.

RESULTS

In the adult study, the incidence of lumbo-sacral vertebral lesions, as defined above, was higher in males (27.9%) than in females (16.9%). The difference is significant ($P < 0.05$) (Table 1).

In the children studied, there was neither a significant variation in the incidence between youngest and oldest nor between males and females (Table 2).

The frequency of spina bifida occulta, with or without laminal asymmetry, was higher in children (27.5%) than in adults (10.4%) but the frequency of laminal asymmetry was lower (5 and 14.4%, respectively).

The incidence of these abnormalities in sibs of spina bifida cystica individuals is 53% and in parents 47.3%. When this was compared to the frequencies found in the two control populations, the differences were significant ($P < 0.01$ and $P < 0.001$, respectively) (Table 4).

In second degree relatives, a moderate increase in frequency was noted in aunts and uncles, but no variation was seen in the small number of grandparents studied (Table 4).

The situation is quite similar for the two types of vertebral abnormalities, *i.e.*, spina bifida occulta and laminal asymmetry. In 33 families, we have observed the following different distributions of anomalies:

- in 17 families, one parent had a vertebral abnormality;
- in 8 families, there was no vertebral lesion in the parents;
- in 8 families, both parents had at least one lesion and:
 - in two, a sib had a normal neural tube;
 - in three, all sibs had a vertebral abnormality;
 - in three, the propositus had no sib.

HLA antigen frequencies in propositi (spina bifida cystica) are reported in Table 4. HLA-A9 and HLA-B5 antigen frequencies are increased. It is noteworthy that HLA-B27 is decreased.

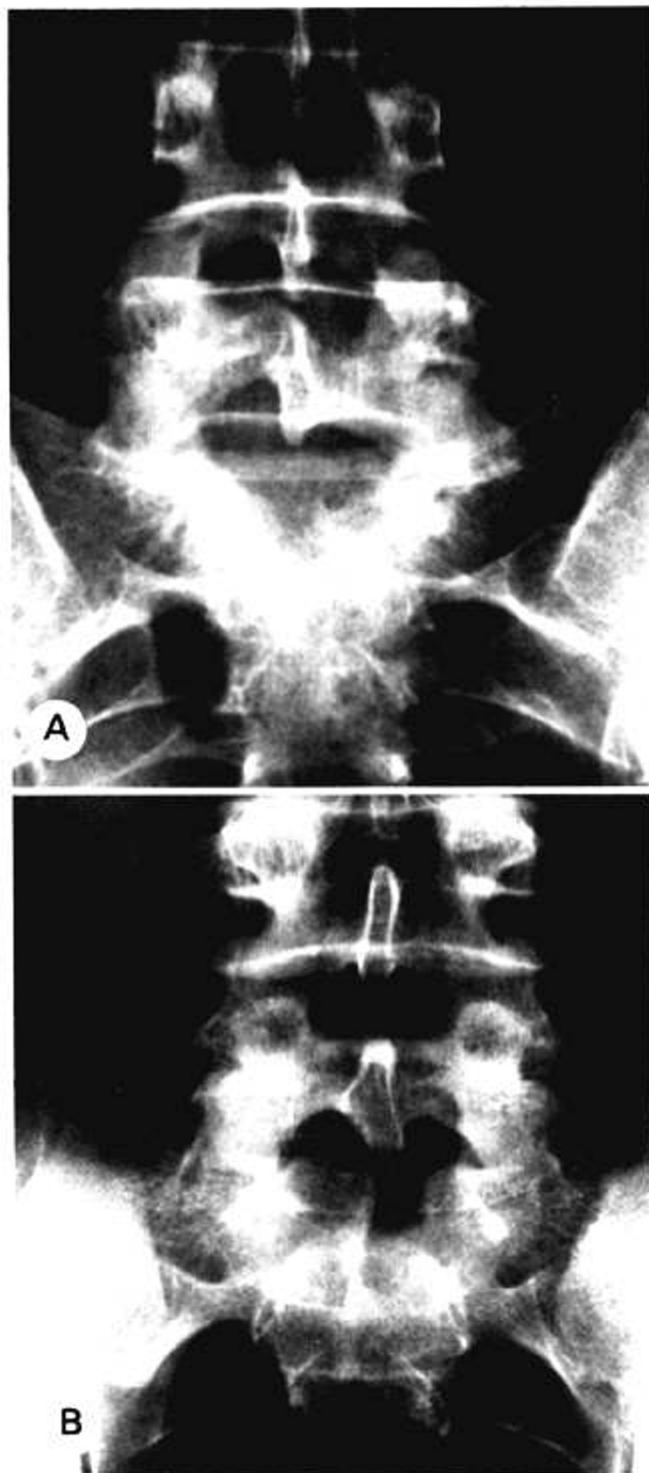


Fig. 1. A, laminal asymmetry (L5); B, spina bifida occulta (S1).

Table 5 shows HLA phenotypes in sibs. Parental haplotype segregation and X-ray examination are reported in Table 3.

There is no significant difference between paternal and maternal haplotype distribution in sibs with or without vertebral abnormalities (Table 6).

DISCUSSION

In our study, the frequency of spina bifida occulta (with or without laminal asymmetry) in adults (10.4%) is lower than that reported by R. H. Post (9), which was near 25% (L5 and S1), varying from 3.6 to 48%.

Such variations are probably related to ethnic differences, but also to the methods of detection; we did not take into account cervical and dorsal lesions, and these are frequent (9).

The incidence of lumbosacral spina bifida occulta is higher in children than in adults. Some cases of spina bifida occulta probably disappear by fusion after the age of sixteen.

Vertebral laminal asymmetry seems to be correlated with spina bifida occulta. Its frequency increases in our adult controls, whereas spina bifida occulta frequency decreases, and they have the same localization; these facts could suggest that the laminal asymmetry could be the result of an asymmetrical fusion of the posterior arches.

The frequency of lumbosacral vertebral lesions is lower in adult females than in males. The sex ratio is near unity before the age of sixteen. A symmetrical fusion may be more frequent in females. Wynne-Davies found a female preponderance of nearly 3:1 for patients with multiple vertebral abnormalities, with or without spina bifida (occulta or cystica), and a 3:2 female preponderance for solitary vertebral abnormalities such as hemivertebrae, anterior body defects, narrowing of disc spaces or fused vertebrae (12).

In a study in Glasgow, Richards *et al.* (10) found a male:female sex ratio near 4:5 for spina bifida cystica, but Lorber and Levick (7) in a survey of parents of children with spina bifida cystica, showed an excess of fathers with spina bifida occulta. The male:female sex ratio for parents with spina bifida occulta is approximately 2:1 and was of the same order for his control population.

It seems, therefore, that the male:female sex ratio is above unity for minor vertebral abnormalities such as spina bifida occulta or laminal asymmetry and under unity for the more severe abnormalities (*e.g.*, spina bifida cystica or multiple vertebral lesions).

In our study, we have found a high frequency of low spinal lesions in families with cases of spina bifida cystica. These findings agree with the results of Lorber *et al.* (7) and suggest a partial common heredity for these lesions.

The high recurrence risk of spina bifida cystica in sibs is consistent with the existence of genetic factors in the aetiology of this condition. Some of them are probably common with spina bifida occulta, as seen above. However, the different sex ratios suggest genetic differences.

Table 1. Lumbo-sacral spina bifida occulta, laminal asymmetry, and their lumbar or sacral localization in 299 adult controls undergoing X-rays for other pathological conditions

	Localization	Females (n = 130)	Males (n = 169)	Total (n = 299)
S.O. ¹	L5-S1	1	1	2
	S1	8	14	22
	S1-S2	0	1	1
	S.O. total	9 (6.9) ²	16 (9.4)	25 (8.4)
L.A.	L4-L5	1	1	2
	L5	2	6	8
	L5-S1	0	2	2
	S1	8	17	25
	L.A. total	11 (8.5)	26 (15.4)	37 (12.4)
S.O. +	L4-L5 (L.A.) + S1 (S.O.)	1	1	2
	L5 (L.A.) + S1 (S.O.)	1	1	2
	L5-S1 (L.A. + S.O.)	0	1	1
	S1 (L.A. + S.O.)	0	1	1
Total (S.O. + L.A.)	2 (1.5)	4 (2.4)	6 (2)	
Total		22 (16.9)	46 (27.2)	68 (22.8)

¹ S.O., spina bifida occulta; L; lumbar; S, sacral; L.A., laminal asymmetry.

² Numbers in parentheses, percentages.

Table 2. Lumbo-sacral spina bifida occulta and laminal asymmetry in 178 children with an abdominal pain

	Age (yr)					Total (n = 178)
	1-3 (n = 33)	3-5 (n = 36)	5-7 (n = 35)	7-12 (n = 36)	12-16 (n = 38)	
S.O. ¹	7	6	10	13	9	45 (25.3)
L.A.	0	2	2	0	2	6 (3.4)
S.O. + L.A.	0	0	2	1	0	3 (1.7)
Total	7 (21.2)	8 (22.2)	14 (40)	14 (38.9)	11 (28.9)	54 (30.3)
$X^2 = 3.66$ for 4 d.f.					N.S.	

	Sex			Total (n = 178)
	Females (n = 91)	Males (n = 87)		
S.O.	22 (24.2)	23 (26.4)		45 (25.3)
L.A.	4 (4.4)	2 (2.3)		6 (3.4)
S.O. + L.A.	1 (1.1)	2 (2.3)		3 (1.7)
Total	27 (29.7)	27 (31.0)		54 (30.3)
$X^2 = 0.03$			N.S.	

¹ S.O., spina bifida occulta; L.A., laminal asymmetry.

Table 3. Parental HLA haplotypes segregation and X-ray examination in sibs of spina bifida cystica-affected subjects

Family	Father—A/B	Mother—C/D	S.B.— affected children	S.O.—affected sibship	L.A.—af- fected sibship	Normal children
1	2, 12/30, 40	30, 5/3, 7	A/C			B/D
2	10, Y/29, 12	2, 12/2, 40	A/C	B/D		A/C ² ; B/C
3	19, 17/19, 12	1, 17/19, 40	A/C			B/C
4	1, 8/X, Y	1, 17/3, 7	A/C	1, 8/D ³		
5	29, 14/29, 12	28, 40/29, 12	A/C			B/C; B/D
6	10, 21/3, 7	X, 5/11, 12	A/C	A/C ² ; A/D		
7	11, Y/9, 17	3, 18/29, Y	A/C			B/C
8	9, 12/3, 7	9, 5 or 27/2, 5 or 27	A/9, 5 ⁴	A/9, 27 ⁴		
9	28, 5/1, 13	3, Y/1, 17	A/C	B/D		
10	11, 22/9, 12	1, 5/11, 27	A/C	B/D		
11	11, 8/29, 12	9, 12/19, 5	A/9, 5 ⁵	E/C ⁶		
12	9, 7/19, Y	19, Y/2, Y	A/C			E/D ⁶
13	2, 5/9, 12	2, 40/9, 40	2 A/C	2 × B/D; E/D ⁶		
14	1, 35/26, 35	2, Y/X, 27	A/C			B/C
15	2, 18/9, Y	X, 18/19, 14	A/C	2 × B/D; A/C ² ; A/D	B/D	
16	9, 40/X, Y	X, 7/3, Y	A/C			E/C ⁶
17	9, 12/9, 21	1, 7/9, Y	A/C	A/D		B/C
18	2, 13/9, 18	X, 14/29, Y	A/C			B/D
19	9, 18/19, 40	9, 8/3, 35	A/C			A/D
20	19, 35/11, 12	11, 40/19, 8	A/C			2 × A/C ² ; 2 × B/C
21	2, 15/X, 21	2, 12/9, 21	A/C	B/C	B/C	
22	X, 40/X, 27	2, Y/1, 13	A/C		A/D	
23	2, 39/X, 35	1, 5/2, 12	A/C	A/C ²		A/D
24	10, 18/1, 21	2, 12/28, 12	A/C	E/C ⁶	A/C ²	A/C ²
25	3, Y/1, 12	19, 12/3, 7	A/C			B/D
26	10, 18/3, 7	2, 12/10, 38	A/C	B/D		
27	28, 12/X, 35	3, 7/1, 40	A/C			A/D
28	1, 7/19, 22	2, 12/2, 15	A/C	A/D		A/D
29	X, 21/9, 12	2, 17/2, 8	A/C			B/D

¹ S.B., spina bifida cystica; S.O., spina bifida occulta; L.A. laminal asymmetry.

² Sib HLA identical to the affected child.

³ Father's genotype undetermined for one haplotype.

⁴ Crossing over for one child.

⁵ Crossing over proved by family study.

⁶ Half brother or sister.

There is an independent segregation between vertebral lesions and HLA haplotypes in families of spina bifida cystica cases. This is true for paternal haplotypes ($X^2 = 0.62$) as well as maternal haplotypes ($X^2 = 2.1$). Our study is concordant with the data of Bobrow *et al.* (2) and Kulakowski *et al.* (6).

An increase in the incidence of HLA-B5 in spina bifida has been described by de Bruyere *et al.* (3). By combining their data with ours, this increase is significant (11). Pietrzyk *et al.* (8) have shown an association between HLA-B27 and spina bifida, but in our study, the B27 antigen frequency is lower in patients than in

Table 4. Lumbo-sacral spina bifida occulta, spina bifida cystica, and laminal asymmetry in spina bifida cystica families

	S.O. ¹	S.B.	L.A.	SO + L.A.	Total	X ²	P
Sibs (n = 49)	20	1	4	1	26 (53.1) ²	8.69	<0.01
Parents (n = 74)	13	0	14	8	35 (47.3)	17.9	<0.001
Aunts & Uncles (n = 36)	6	0	6	3	15 (41.7)	6.17	<0.02
Grandparents (n = 33)	3	0	5	0	8 (24.2)	0.04	NS

¹ S.O., spina bifida occulta; S.B., spina bifida cystica; L.A., laminal asymmetry.

² Numbers in parentheses, percentages.

Table 5. HLA antigen frequencies in a population of spina bifida cases as compared to a control population (11)

	S.B. ¹ (n = 53)	C. (n = 412)	X ²
1st locus			
A1	26.4	25.7	
A2	47.2	51.5	
A3	13.2	19.0	
A9	30.2	16.7	5.11 ²
A10	17.0	9.5	2.7
A11	11.3	10.4	
A19	18.9	27.7	
A28	1.9	2.7	
X	34.0	36.7	
2nd locus			
B5	28.3	15.0	5.43 ³
B7	13.2	23.3	2.43
B8	11.3	14.1	
B12	37.7	31.8	
B13	1.9	5.1	
B14	3.8	11.2	2.62
B15	3.8	10.2	2.14
B17	11.3	6.8	
B18	11.3	8.7	
B21	5.7	3.9	
B22	3.8	3.1	
B27	3.8	11.7	2.86
B35	15.1	10.4	
B40	17.0	9.2	2.94
B38			
B39	1.9	1.7	
B41			
Y	30.2	33.7	

¹ S.B., spina bifida cystica; C., control.

² P < 0.05.

³ 0.01 < P < 0.02.

the control population. In their study, affected individuals also exhibit a small increase of HLA-B5.

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Table 6. Haplotype distribution in sibships of spina bifida cystica in comparison with lumbo-sacral vertebral abnormalities, spina bifida occulta, and laminal asymmetry

	Sibs with S.O. ¹ and/or L.A.	S.B., Without abnormality	X ²
Paternal			
a ²	11	8	X ² = 0.62
b	11	13	
e	4	2	
Maternal			
c	9	13	X ² = 2.1
d	16	10	
f	1	0	

¹ S.O., spina bifida occulta; S.B., spina bifida cystica, L.A., laminal asymmetry.

² Other distributions: a and b: paternal haplotypes c and d: maternal haplotypes a and c: propositus haplotypes e and f: others (exclusion, crossing-over, undetermined).

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14. D G R S T fellow during this work, 1978.
15. The authors are indebted to Professor Borde, paediatric surgeon, who enabled them to carry out this study. We also wish to thank Doctor Gaussin (Le Havre), Doctor Agratina (Vernon), Doctor Belin (Pont-Audemer), and Doctor Petit (Louviers), radiologists. We wish to thank Mrs. Ducimetièrre for her technical assistance in writing this manuscript.
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17. This research was supported by a Grant from INSERM (ATP n°36-76-68) (Institut National de la Santé et de la Recherche Médicale).
18. Received for publication December 11, 1979.
19. Accepted for publication October 22, 1980.