Spina bifida and maternal Rh phenotype

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Summary. Based on the sample in this study (members of the Spina Bifida Association of America), there are approximately 2.15 times as many mothers with Rh-blood type than would be expected in a similar sized sample of the general population.

The precise role of environmental agents (teratogens, mutagens) and/or the genetic background of the parents, as well as other factors, in the etiology of spina bifida and most other birth defects is either not known or controversial. Spina bifida is a relatively common (0.1–4.5 per 1000 live births) major birth defect[1]. It is associated with partial to total loss of sensory and/or motor function in the lower limbs, partial to total loss of sphincter (urinary and anal) control[1]. About 75–90% of the children born with spina bifida develop hydrocephalus[1]. While animal models of spina bifida exist (e.g. Manx cats[2], Curly tail mouse[3], Trypan blue injections[4]), the precise relationship between these models and the human conditions is not known. It is not clear how useful these models would be in the study of the etiology of this birth defect.

It is important to study the etiology of spina bifida and other major birth defects, so as to determine their cause and how to prevent their occurrence. By studying the etiology of each birth defect, it should be possible to discover if an environmental agent(s) is involved, and if so, to eliminate that agent. Similarly, if the birth defect is a genetic anomaly, it should be possible to estimate the risk of occurrence or recurrence. One of the obvious and logical places to start etiological studies is by doing retrospective studies in the population of persons with the birth defect, their sibs, parents, and other family members. Since the existing diagnostic aids (Alpha Feto protein levels in serum or amniotic fluid) for the detection of spina bifida are useful only after conception and require therapeutic abortions to prevent the birth of a child with spina bifida, it seems clear that it is important to determine if there are any diagnostic cues that are available to identify parents who are at risk for having a child with spina bifida before pregnancy.

For example, an early study of erythroblastosis fetalis suggested a possible relationship between erythroblastosis and spina bifida[5]. This means that the incidence of spina bifida might be correlated with Rh− blood type incompatibility, i.e. that mothers with Rh− blood type might be at greater risk for having a baby with spina bifida. However, others did not find any relationship between Rh or AB0 blood type and spina bifida[6,7]. We[8], in a small pilot study, found that there were approximately twice as many mothers with Rh-blood type in a population of mothers with a spina bifida child as would be expected when compared to a random sample drawn from the general population. We decided to pursue this problem by making a mailing to the members of the Spina Bifida Association of America, a nation wide organization of adults and teenagers with spina bifida and parents of children with spina bifida. The majority of the members of this organization are distributed in approximately 93 local and state chapters.

Methods. A Rh-AB0 blood type questionnaire was mailed to the 3512 members of the Spina Bifida Association of America. A sample questionnaire is shown in the figure. The data about the frequency of occurrence of the various Rh-AB0 blood types of the mothers and fathers of children with spina bifida as obtained from the questionnaires were compared to the expected frequency of occurrence of each blood type[9] using χ^2 methodology[10]. We also compared the incidence of spina bifida in AB0 incompatible pregnancies[11] using χ^2 methodology[10].

Results. Of the 3512 questionnaires that were mailed out, 738 or approximately 21% were returned. 63% of the replies came from 11 states (Cal., Ill., Maryland, Mass., Mich., N.J., N.Y., Ohio, Penn., Tex., and Wisc.), while the other 37% were distributed throughout the other states, with the

Spina bifida questionnaire

	Birthdate	Blood type	Jaundiced at birth?	Transfusions?	Birth defect? (Circle # from list below)	Current status
Mother	../../..	A B AB 0 Unknown Rh+ Rh− Unknown	Yes No	Yes No	1 2 3 4 5 6 7 8 9	Alive deceased
Father	../../..	A B AB 0 Unknown Rh+ Rh− Unknown	Yes No	Yes No	1 2 3 4 5 6 7 8 9	Alive deceased
Children (List in order of birth and include miscarriages and/or stillbirths)						
1.	../../..	A B AB 0 Unknown Rh+ Rh− Unknown	Yes No	Yes No	1 2 3 4 5 6 7 8 9	Alive deceased
2.	../../..	A B AB 0 Unknown Rh+ Rh− Unknown	Yes No	Yes No	1 2 3 4 5 6 7 8 9	Alive deceased
3.	../../..	A B AB 0 Unknown Rh+ Rh− Unknown	Yes No	Yes No	1 2 3 4 5 6 7 8 9	Alive deceased
4.	../../..	A B AB 0 Unknown Rh+ Rh− Unknown	Yes No	Yes No	1 2 3 4 5 6 7 8 9	Alive deceased
5.	../../..	A B AB 0 Unknown Rh+ Rh− Unknown	Yes No	Yes No	1 2 3 4 5 6 7 8 9	Alive deceased
6.	../../..	A B AB 0 Unknown Rh+ Rh− Unknown	Yes No	Yes No	1 2 3 4 5 6 7 8 9	Alive deceased
7.	../../..	A B AB 0 Unknown Rh+ Rh− Unknown	Yes No	Yes No	1 2 3 4 5 6 7 8 9	Alive deceased

1. Pilonidal Sinus or Cyst; 2. Spina Bifida Occulta; 3. Meningocele; 4. Myelomeningocele (spina bifida cystica); 5. Syringomyelocele; 6. Encephalocele; 7. Hydrocephaly; 8. Other (please specify); 9. None.

exception of Alaska, Washington, and Wyoming from which no replies were received. Of the 738 replies that were received, 475 knew the Rh-ABO blood type of the mother, 419 knew the Rh-ABO blood type of the father, and 375 knew the Rh-ABO blood type of both the mother and father.

When compared to the expected frequency of occurrence of Rh blood type in a random sample of the population as reported by Wiener and Socha, there is a significant increase in the incidence of Rh− blood type in the population of mothers of children with spina bifida ($\chi^2 = 117.59$ $p < 0.001$). There were approximately 2.15 times as many mothers with Rh− blood type in this population than would be expected with a random sample of the general population. There was no significant difference in the birth order of the child with spina bifida, when comparing the mothers with Rh− and Rh+ phenotype. There were no reports of perinatal jaundice and/or prenatal or perinatal transfusions. The frequency of occurrence of the fathers' Rh and ABO blood types and the mothers' ABO blood types did not differ significantly from that expected in a random sample of the general population. There was also not a significant increase in spina bifida children in ABO incompatible pregnancies (i.e. mother A, father B or 0; mother B, father A or 0; mother AB, father A, B, or 0).

Discussion. It seems clear that, at least for the population sampled by the questionnaire, there is a significant relationship between maternal RH phenotype and the birth of a baby with spina bifida. Unfortunately, we were not able to obtain any information about anti-Rh antibodies in the mothers with Rh-blood type. However, since birth order of the child with spina bifida is not a significant factor and since there are no reports of perinatal jaundice and/or prenatal or postnatal transfusions, it seems unlikely that this relationship is based on an immunological response to an Rh blood type incompatibility between the mother and father. Clearly, since Rh genotypes are complex, it would be important to determine if there are one or more genotypes that are prevalent in this group of mothers. We are currently attempting to determine the genotype of a selected sample of mothers in our experimental population.

Frequency of occurrence of Rh and ABO blood types amoung parents of children with spina bifida

	Mother Observed	Theoretical	Father Observed	Theoretical
A+	131	122	28	21
B+	43	43	6	8
AB+	18	17	3	3
0+	138	148	21	26
A−	51	53	124	117
B−	17	19	37	41
AB−	7	7	24	16
0−	70	65	132	143
Rh+*	343**	430	354	356
Rh−*	163**	76	65	63

* Based on Rh blood type without regard for ABO blood type.
** Significant difference between observed and expected values, $p < 0.01$.

1 G.D. Stark, Spina Bifida: Problems and Management. Blackwell Scientific Publications, Oxford 1977.
2 H. Kitchen, R.E. Murray and Y. Beverly Cockrell, Am. J. Path. *68,* 203 (1972).
3 H. Gruneberg, J. Genet. *52,* 52 (1954).
4 J. Gillman, C. Gilbert, T. Gillman and I. Spence, S. Afr. J. med. Sci. *13,* 47 (1948).
5 A.S. Wiener, Am. J. clin. Path. *16,* 319 (1946).
6 B. MacMahon, T.F. Pugh and T.H. Ingalls, Br. J. prov. Soc. *7,* 211 (1953).
7 R.D. Collmann and A. Stroller, Aust. N. Z. J. Obstet. Gynec. *2,* 38 (1962).
8 D.A. Baker and C.J. Sherry, Archs Dis. Childhood *54,* 567 (1979).
9 A.S. Wiener and W.W. Socha, A-B-0 blood groups and Lewis type. N.Y. Stratton Intercontinental Medical Book Corp., New York 1976.
10 F.E. Croxton, Elementary statistics: with applications in medicine and the biological sciences. Dover, New York 1953.
11 E.T. Engle, ed. Pregnancy Wastage. Thomas, Springfield, Ill. 1973.

The quantitative analysis of constitutive heterochromatic regions of human chromosomes 1, 9, and 16 in relation to size and inversion heteromorphisms in East Indians

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Summary. 100 normal East Indians were studied by C-banding technique to estimate the frequency of size and inversion heteromorphisms of the secondary constriction regions (h) of human chromosomes 1, 9, and 16, and the data were compared to those of Caucasians and Black Americans.

The heteromorphic nature of the constitutive heterochromatin of the secondary constriction regions (h) of human chromosomes 1, 9 and 16 has been recognized since the early 1960s[1], but the extent of the variation has been explored only recently using various banding techniques[2]. CBG (C-bands by barium hydroxide using Giemsa) technique[3] is most commonly used to examine such variation[4], because the h region stains very darkly and contains primarily repetitive DNA[5,6]. There are also some other selective techniques which are being used to examine the extent of such variation[7-9]. The h regions are heteromorphic in size as well as in position (inversion heteromorphisms)[10]. A large number of family studies indicate that these heteromorphisms are inherited in a Mendelian fashion; nevertheless several studies have implied that apparent non-inherited variation between parent and offspring, somatic mosaicism[11,12] and preferential segregation occur[13,14]. Further, an association of increased size of h