

# Low prevalence of latex sensitivity in South African spina bifida children in Cape Town

Johar A, Lim D, Mad Arif SA, Hawarden D, Du Toit G, Weinberg E, Motala C, Fieggen G, Yeang HY, Potter PC. Low prevalence of latex sensitivity in South African spina bifida children in Cape Town. *Pediatr Allergy Immunol* 2005; 16: 165–170. ©2005 Blackwell Munksgaard

Spina bifida children have a high prevalence of latex allergy in studies reported from Europe and the USA. This study investigated the prevalence of latex allergy in a cohort of 24 spina bifida children at the Red Cross Children's Hospital from Cape Town, South Africa. The children were investigated using a detailed questionnaire, skin prick tests (ALK-Abello), ImmunoCap RASTs, Western blotting and ELISA, using the purified latex proteins Hev b1 and Hev b3 and whole latex preparation. A low overall prevalence of latex sensitization of 16.7% was found in the children. Children who were sensitive reacted to water insoluble to Hev b1 and Hev b3 proteins. The low prevalence of latex sensitization in the South African children may not be entirely explained by stringent latex avoidance. The children were from a low socioeconomic social status and 'hygiene' and other factors should be considered.

**Asmah Johar<sup>1,2</sup>, Dawn L. Lim<sup>3</sup>,  
Siti Arija Mad Arif<sup>4</sup>, Di Hawarden<sup>5</sup>,  
George Du Toit<sup>5</sup>,  
Eugene G. Weinberg<sup>5</sup>, Cas Motala<sup>5</sup>,  
G. Fieggen<sup>5</sup>, Hoong Yeet Yeang<sup>4</sup> and  
Paul C. Potter<sup>2</sup>**

<sup>1</sup>Department of Dermatology, Hospital Kuala Lumpur, Malaysia, <sup>2</sup>Allergy Diagnostic and Clinical Research Unit, University of Cape Town Lung Institute, Cape Town, South Africa, <sup>3</sup>National University of Singapore, Singapore, <sup>4</sup>Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia, <sup>5</sup>Red Cross Children's Hospital, Cape Town, South Africa

Key words: spina bifida; children; latex allergy; Hev b1; Hev b3

Prof. Paul C. Potter, Allergy Diagnostic & Clinical Research Unit, University of Cape Town Lung Institute, P.O. Box 34560, Groote Schuur, Cape Town 7937, South Africa  
Tel.: +27 21 406 6889  
Fax: +27 21 406 6888  
E-mail: ppotter@uctgsh1.uct.ac.za

Accepted 27 September 2004

Latex allergy affects approximately 0.1% of the general population (1). Health care workers are frequently exposed to latex, especially surgical gloves. Therefore the prevalence among them is expected to be higher. Reports from Europe and the USA among health care workers give a prevalence of between 3% and 11% for latex allergy (1). A survey among 2316 staff members of Groote Schuur Hospital, South Africa showed a prevalence of clinical allergy in 9.2% (2). Nevertheless, high latex allergy incidence is not necessarily an inevitable consequence of constant and repeated contact with latex. In contrast to what is observed in the West, prevalences of latex allergy in South East Asia are relatively low even among groups that are occupationally exposed to latex. Two Malaysian surveys among rubber tappers and glove factory workers gave prevalence figures of only 3% and 2%, respectively (3, 4), while only 1.3% of rubber tappers and 1.7%

of latex glove factory workers in Thailand were allergic to latex (5).

Spina bifida patients are frequently exposed to latex as they are in contact with latex products very early in life (gloves, catheters) and undergo multiple surgical procedures from an early age. A high latex allergy prevalence of 29–64% had been found in Spina Bifida studies in the USA and Europe (6–11). The prevalence reported in one European country, Italy was substantially lower at 15% (12), while a study from Venezuela put the figure at only 4% (13). No reports are available as yet in any Asian countries or South Africa.

It has been also observed (14, 15) that children with spina bifida in US and Europe react to the water insoluble Hev b1 (14.6 kDa) and Hev b3 (22 kDa) proteins to which health care workers are less commonly sensitized.

The aim of this study was to determine whether the high prevalence of latex allergy

(and particularly sensitization to the rubber particle proteins, Hev b1 and Hev b3) among spina bifida patients in the West was also evident in children with spina bifida living in Africa. Towards this end, we studied sensitivity to latex using a panel of latex skin prick tests (SPT), ImmunoCap (Pharmacia) levels to latex (k82), ELISA and Western blot techniques using whole latex extracts and purified Hev b1 and Hev b3 proteins.

## Methods

Twenty-four patients between the ages of 6 months and 18 yr who attended the spina bifida clinic at the Red Cross Children's Hospital, Cape Town, South Africa, were studied.

Ten samples from adult patients with allergy to latex and 10 samples from non-allergic children were used as positive and negative controls. A questionnaire was used to obtain details of medical and surgical history, latex exposure symptoms and cross-reaction to fruits. Each patient underwent titrated SPT with the commercial latex preparations from ALK-Abello, and with purified Hev b1 and Hev b3 extracts at different dilutions of 1/1000, 1/100, 1/10 and an undiluted sample obtained from Malaysian Rubber Research Institute of Malaysia.

A positive reaction was a wheal of 3 mm or more greater than the saline control, and a wheal of 2 mm was regarded as borderline. Patient sera were tested using the Pharmacia ImmunoCap RAST (k82) test. A value of  $>0.35$  kU/l was regarded as positive.

Preparation of *Hevea brasiliensis* latex, latex fractions and the purified latex allergens Hev b1 and Hev b3

Fresh natural rubber latex from *Hevea brasiliensis* trees (clone RRIM 600) was collected into chilled containers at the Rubber Research Institute of Malaysia Experiment Station, Sungei Buloh. After centrifugation at 43,000 *g* for 1 h at 4–7°C on a Sorvall RC 5C high-speed centrifuge, three main fractions were recovered: the rubber cream, the C-serum and the bottom fraction. Latex B-serum was prepared based on the method of Hsia (16). The latex bottom fraction from centrifuged latex was washed by re-suspension in 0.4 M mannitol and recovered by centrifugation. The washed bottom fraction was then subjected to repeated freezing and thawing to rupture the lutoids that were its main constituents. The lutoidic fluid, the B-serum, was recovered as the supernatant after re-centrifugation. The rubber cream from centrifuged latex was

sampled at the intersection between Moir's Zone 1 and Zone 2 (17) so as to obtain rubber particles belonging to both the large and small size classes (14). The rubber particles were washed by re-suspension in 30% sucrose and proteins on the rubber particle surfaces were then solubilised and extracted using a detergent mixture comprising 0.1% Triton-X 100 and 1% sodium dodecylsulphate (SDS). The two main proteins obtained were Hev b1 and Hev b3. These two proteins were separated and purified by preparative electrophoresis on 15% SDS-polyacrylamide gel using the *Prep Cell* (BioRad, Hercules, CA, USA) according to the manufacturer's instructions.

Analyses of allergen-IgE binding by Western immunoblot

The rubber particle proteins extracted with SDS-Triton X-100 were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 15% gels according to the method of Laemmli (18) and transferred electrophoretically to a nitrocellulose membrane. The nitrocellulose membrane was blocked with 5% non-fat milk in phosphate buffered saline (PBS-milk), pH 7.4, for 30 min and then incubated overnight with patient serum (diluted 1:5.25 in PBS-milk and 0.05% sodium azide) as the primary antibody. After three cycles of washing with PBS-milk, the nitrocellulose membrane was incubated for 1 h with the secondary antibody, anti-human IgE conjugated to alkaline phosphatase. After a further three cycles of washing with PBS-milk, the nitrocellulose membrane was incubated for 10 min in Tris-buffered saline (TBS) before being immersed in 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) substrate to generate the coloured alkaline phosphatase reaction product. The results were recorded semi-quantitatively based on the intensity of the protein bands on the immunoblot. Results were reported in a semi-quantitative form (0, +, ++, +++).

Analyses of allergen-IgE binding by ELISA

The binding of proteins to IgE from latex-allergic and control patients was quantitated by ELISA. The proteins used in the assays were purified Hev b1, purified Hev b3 and a mixture of latex B-serum and C-serum (2:5 v/v) representing whole latex. ELISA plates were coated with the test proteins diluted in PBS, pH 7.4 and left overnight. The next day, the plates were washed thrice in PBS containing 0.05% Tween-20 (PBS-T) and then they were blocked

with 0.5% BSA in PBS-T for 1 h before washing thrice again with PBS-T. Patient serum diluted to 16% with PBS-T was used as the primary antibody source, with incubation carried out for 3 h. After washing thrice with PBS-T, biotinylated anti-IgE was added as the secondary antibody and incubated for 1 h. The plates were washed once with PBS-T and then twice with Tris-buffered saline containing 0.05% Tween 20, pH 7.5 (TBS-T). Following this, 100  $\mu$ l of streptavidin-conjugated alkaline phosphatase in TBS, pH 8.0, containing 0.2 mM magnesium chloride was added. The plates were incubated at room temperature in the dark for 30 min. The plates were then washed twice with TBS-T and once with TBS, pH 9.5, containing 50 mM magnesium chloride. Colour development was initiated by adding *p*-nitrophenyl phosphate in 10% diethanolamine buffer and the absorbance read at 405 nm using an ELISA plate reader. Blank values for each individual patient were subtracted from the ELISA plate absorbance readings.

Statistical analyses for serologic tests

As ELISA absorbance readings do not normally vary linearly with IgE binding, a non-parametric analysis, the Kruskal-Wallis test was adopted. ELISA readings from the different experimental treatments (18). Comparisons of latex allergy prevalences between different studies was by 2  $\times$  2 contingency tables followed by Fisher's exact probability test (19).

Ethical approval

Ethical approval was obtained from the Ethics and Research Committee of the University of Cape Town.

## Results

Patient characteristics

Clinical characteristics and results of the serological evaluations of the latex sensitive South African children are shown in Table 1. The 24 children from South Africa consisted of 10 males (41.7%) and 14 females (58.3%). Their age ranged between 14 months and 15-yr-old. The mean age was 8.34 yr. All were diagnosed at birth except for one patient with lipomyelomeningocele who was diagnosed at the age of 2-yr-old. One patient had transverse myelitis at the age of 3-yr-old and one patient had possible sinus pilonidal with a hypoplastic right thumb.

Table 1. Characteristics of the South African patients with positive or borderline latex allergy tests

Patients	Sex	Age (Yr)	Personal history of atopy	Relatives with Atopy	Disturbing nasal symptoms	No. of operations	Skin prick diameter (mm)			Overall skin prick reaction to latex†	IgE-Cap RAST (kUA/l)	ELISA‡			Western blot		
							ALK 100Hep*	Hep b1*	Hep b3*			Whole latex	Hep b1	Hep b3	Hep b1	Hep b3	
1	M	10	No	Yes	No	3	0	2	3	Pos	<0.35	0.009	0.009	0	0	0	0
2	M	6	No	Yes	No	4	4	3	0	Pos	37.9	1.258	1.377	+++	0	0	0
3	M	2	No	Yes	No	3	0	1	1	BL	<0.35	0.009	0.012	0	0	0	0
4	M	3	Yes	Yes	Yes	2	3	0	2	Pos	<0.35	0.101	-0.009	0	0	0	0
5	F	6	Yes	No	No	3	0	2	0	BL	<0.35	0.101	-0.017	0	0	0	0
6	F	11	Yes	Yes	No	11	4	3	4	Pos	>100	>3.4	>3.4	+++	+++	+++	+++
7	F	3	Yes	Yes	No	8	0	0	0	Neg	1.5	0.027	0.722	+	+	+	+

\*Results shown for reaction to highest concentrations tested.

†Overall evaluation based on reactions to multiple dilutions of latex, purified Hep b1 and Hep b3. Pos, Positive; Neg, Negative; BL, Borderline

‡Readings above 0.030 (which is equal to 3x the standard deviation of readings from non-latex allergic pediatric controls) are regarded as positive.

There was a personal history of atopy in six patients (16.7%); four (16.7%) had allergic rhinitis, two had asthma (8.3%) and one with atopic dermatitis (4.2%). Ten patients had a family history of atopy: four (16.7%) had relatives with allergic rhinitis, nine (37.5%) had relatives with asthma, two (8.3%) had relatives with allergic conjunctivitis and two (8.3%) had relatives with atopic dermatitis. None of these patients were diagnosed to have latex allergy previously.

Of these patients, only one presented with symptoms of allergic rhinitis such as itchiness of nose and sneezing. However symptoms were not aggravated either at home or in the hospital by any known latex exposure. All patients were exposed to latex at home either to rubber toys, balloons, erasers, rubber lining splints, disposable nappies or rubber wheelchair cushions. None of the patients were having problems eating avocado, banana or kiwi, though a few were never exposed to any of the fruits.

All the patients had undergone operations and a third of them had had up to three operations. Those patients who had undergone more than three operations were those with complications of their ventriculo-peritoneal shunt. The common procedures done during operations were closure of myelomeningocele, insertion of ventriculo-peritoneal shunt and correction of ankle deformity mainly for clubbed feet. All patients had been exposed to latex gloves during operations, but only one had a latex-indwelling catheter.

### Skin prick test and serology results

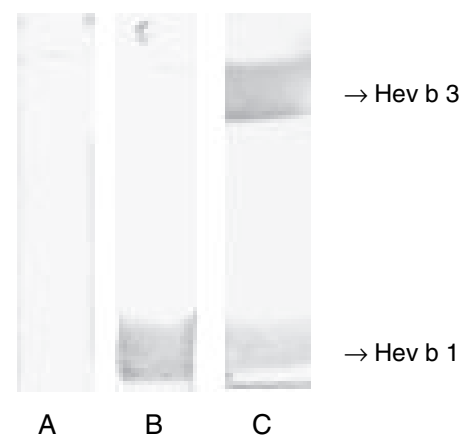
#### Skin prick test

The SPT was positive in four patients (Patients 1, 2, 4 and 6) ( $n = 2416.6\%$ ) and two patients had borderline results (Table 1). Three out of four patients reacted to the commercially available Alk 100 Hep latex allergen. SPT with purified rubber protein particle, Hev b1 and Hev b3 was positive in three patients (patient number 1 and 3), one of them not reacting to Alk 100 Hep. Two patients had borderline reactions to Hev b1 and Hev b3 of which one was only to Hev b1. The maximum wheal size diameter was only 4 mm.

For the patients who were 7 yr and older (median age = 10), only one patient out of 12 tested positive.

IgE-Cap RAST to latex and ELISA to purified rubber protein particle

IgE-Cap RAST was positive in three patients (Patients 2, 6 and 7) with one patient having a



*Fig. 1.* Western immunoblots of the rubber particle proteins, Hev b 1 and Hev b 3, incubated with patient sera. Representative immunoblots are presented, showing no protein-IgE binding (A), IgE binding to Hev b 1 (B) and IgE binding to Hev b 1 and Hev b 3.

level greater than 100 kUA/l. This patient had undergone 11 operations because of the complications of her ventriculo-peritoneal shunt. One of these patients (Patient 7) was negative to SPT with low level of IgE level (1.5 kU/l) despite having had eight operations. ELISA testing was also positive for these three patients.

The pediatric controls were negative for both tests.

The latex positive children were positive to the whole latex extract in the ELISA. One patient had a slightly raised reading of 0.101 and was also positive to SPT, despite being negative IgE-Cap RAST. Latex-positive adult controls had readings that were significantly above the whole latex ELISA pediatric controls (Table 1).

Western blots were positive to Hev b1 in 3 of the children and only one was positive to Hev b3 (Fig. 1). None of the 10 adult control latex positive patients were positive to Hev b1 or Hev b3 in the Western blots.

### Discussion

Spina bifida patients (20, 21) and adults undergoing multiple operations (22) are at risk of developing latex allergy. These patients may not be symptomatic even though they are sensitized to latex allergens. Testing for latex sensitization is important for those requiring more than two operations to avoid clinical reactions. Two patients with positive serologic tests had a history of allergic rhinitis. They were however not symptomatic in the hospital environment. Latex gloves have not been used in the spina bifida clinic for the last 6 yr and this probably this could explain

Table 2. Latex allergy prevalence among spina bifida patients as determined by skin prick tests

Study	Country	Sample size	Latex allergy prevalence (%)	Comparison with latex allergy prevalence in the present study* (p-Value)	
				All South African patients	South African patients aged 7 and above†
Present study (all patients)	South Africa	24	16.7	—	—
Present study (patients aged 7 and above†)	South Africa	12	16.7	—	—
Yassin et al. (1992) (6)	USA	76	64.5	<0.0001***	0.0032**
Kelly et al. (1993) (7)	USA	86	48.8	0.0050**	0.0600 NS
Niggemann et al. (1996) (9)	Germany	81	44.4	0.0165*	0.1135 NS
Moneret-Vautrin et al. (1993) (8)	France	25	32.0	0.3209 NS	0.4447 NS
Nieto et al. (1996) (10)	Spain	100	29.0	0.3055 NS	0.5054 NS
Bernardini et al. (1998) (11)	Italy	59	15.3	1.0000 NS	1.0000 NS
Capriles-Hulett et al. (1995) (13)	Venezuela	93	4.3	0.0545 NS	0.1388 NS

\*Probability observed difference is because of chance occurrence as determined by the Fisher's exact probability test. NS, not statistically significant.

†Young patients who received treatment in a mainly latex-free environment excluded.

why they were not symptomatic. The third positive patient, Patient 6 who had undergone 11 operations is much older (11-yr-old) and had high levels to all the serologic tests. This patient would have been significantly exposed to latex gloves in the first few years of life.

It is possible that the low prevalence of latex sensitization in the small cohort we have studied in spina bifida patients from the Cape Town area of South Africa might have been because of the fact that the younger patients were exposed to much less latex, during the past 6 yr, when latex avoidance measures have become more stringent. A comparison was made between the 12 patients in the sample who were 7 yr or older, and who therefore had been exposed to latex with those who were younger than seven. The results (Table 2) showed that latex sensitization prevalence was not significantly increased in the older group of patients who had greater exposure to latex in our hospital before more stringent latex avoidance measures were adopted.

We have also recently studied a smaller group of seven Singaporean children (data not shown) with spina bifida and found a prevalence of latex allergy to be 42%. A large study would be necessary to determine the true prevalence of latex allergy in this Asian region.

We have confirmed that rubber elongation factor (Hev b1) and small rubber particle protein (Hev b3) play an important role in the development of latex allergy in spina bifida patients using a ELISA, Western blot and SPT. The negative results on ELISA and Western blot among the 'control' confirmed latex sensitive adult health care workers confirms that they are not commonly sensitized to Hev b1 and Hev b3 (10). This observation was consistent with previous reports of European and American patients.

The major finding in this study is that the prevalence of sensitization to latex and to the rubber particle proteins among spina bifida patients in South Africa (16.7%) is significantly lower than observed in Europe and the US (6–10, 12), and similar to the prevalences reported from Italy (11) and Venezuela (13).

In the study from Venezuela (13) it was suggested that the low prevalence was because of strict avoidance of latex exposure in this children and a low frequency of surgical procedures.

The low prevalence of latex sensitization in Red Cross Children's Hospital is not necessarily the result of a policy to avoid using latex gloves in the spina bifida clinic in the past 6 yr, as the prevalence is the same as in the children who were exposed to latex. Airborne latex allergens may be a route of sensitization, especially in those with allergic rhinitis or asthma. It is likely though that repeated and prolonged mucosal exposure is the major route of sensitization. However, other factors such as population prevalences of allergy, 'hygiene' factors and 'Western life style' may also influence the prevalence of latex sensitization in spina bifida patients. All of the South African children in this study were of mixed ethnicity and from a poor community of low socioeconomic status. None of the children in the cohort were from black African ethnic groups (i.e. Xhosa) in the region. We have recently found the prevalence of atopic sensitization to aeroallergens to be 42.3% in urbanized Xhosa children and 45% in other urbanized children in the Cape Town area (23).

High prevalences of latex allergy in spina bifida children have been reported from more affluent countries and only in the northern hemisphere.

Detection of latex allergy is important in spina bifida patients as they may not be clinically symptomatic, but may still be at risk of severe clinical reactions if inadvertently exposed. Most hospitals in developing countries are not latex free.

Although the prevalence of latex sensitization in the South African spina bifida children is low, it is probable that in countries where latex avoidance has more recently been strictly implemented that the high prevalence of latex allergy in spina bifida levels previously reported in the USA (6, 7) and Europe (8, 9) should have decreased. Studies of the current prevalence of infants born in the past 4 yr in these regions would be of great interest.

### Acknowledgments

The authors thank Mrs. L. Terblanche, Ms. Magda Schinkel, Sr. S. Baker and Mrs. B. Fenemore for assistance with clinical testing of the patients.

The part of the research carried out at the Rubber Research Institute of Malaysia was supported by IRPA Grant 06-04-04-0001 from the Ministry of Science, Technology and the Environment, Malaysia. We thank Chew Nyu Ping and Loke Yin Ho for laboratory assistance.

### References

1. TURJANMAA K, ALENIUS H, MAKINEN-KILJUNEN S, RUNALA T, PALSOVO T. Natural rubber latex allergy. *Allergy* 1996; 51: 593–602.
2. POTTER PC, CROMBIE I, MARION A, KOSHEVA O, MAQUILA B, SCHINKEL M. Latex allergy at Groote Schuur Hospital: prevalence, clinical features and the outcome. *S Afr Med J* 2001; 91: 760–5.
3. HASMA H, SHAHNAZ M, YIP E, AZIZAH M, MOK KL, NASARUDDIN BA. Binding patterns of IgE antibodies in sera of rubber tappers to fresh Hevea latex serum proteins. *J Rubb Res* 1998; 1: 146–53.
4. NASARUDDIN BA, SHAHNAZ M, AZIZAH MR, HASMAH H, MOK KL, HIP E. Prevalence Study of Type I Latex Hypersensitivity Among High Risk Groups in the Malaysian Population. A Preliminary Report. Kuala Lumpur, Malaysia: Workshop on Latex Protein Allergy, 1994.
5. CHAIEAR N, SADRJA S, JONES M, CULLINAN P, FOULDS IS, BURGE PS. Sensitization to natural rubber latex: an epidemiological study of workers exposed during tapping and glove manufacture in Thailand. *Occup Environ Med* 2001; 58: 386–91.
6. YASSIN M, SANYURAH S, LIERL MB, et al. Evaluation of latex allergy in patients with meningomyelocele. *Ann Allergy* 1992; 69: 207–11.
7. KELLY KJ, KURUP V, ZACHARISEN M, RESNICK A, FINK JN. Skin and serologic testing in the diagnosis of latex allergy. *J Allergy Clin Immunol* 1993; 91: 1140–5.
8. MONERET-VAUTRIN DA, BEAUDOUIN E, WIDMER S, et al. Prospective study of risk factors in natural rubber latex hypersensitivity. *J Allergy Clin Immunol* 1993; 92: 668–77.
9. NIGGEMANN MT, MOERS A, SEIDEL U, WAHN U, SCHENER D. Risk factors for latex allergy in patients with spina bifida. *Clin Exp Allergy* 1996; 26: 934–9.
10. NIETO A, ESTORNELL F, MAZON A, REIG C, GARCIA-IBARRA F. Allergy to latex in spina bifida: a multivariate study of associated factors in 100 consecutive patients. *J Allergy Clin Immunol* 1996; 100: 501–7.
11. FRANKLAND AW. Latex allergy in children. *Paediatr Allergy Immunol* 1999; 10: 152–9.
12. BENARDINI R, NOVEMBRE E, LOMBARDI E, et al. Prevalence and risk factors for latex sensitization in patients with spina bifida. *J Urol* 1998; 160: 1775–8.
13. CAPRILES-HULETT A, SANCHEZ-BORGES M, VON-SCANZONI C, MEDINA JR. Very low frequency of latex and fruit allergy in patients with spina bifida from Venezuela: influence of socioeconomic factors. *Ann Allergy Asthma Immunol* 1995; 75: 62–4.
14. YEANG HY, CHEONG KF, SUNDERASAN E, et al. The 14.6 kd rubber elongation factor (Hev b1) and the 24 kd (Hev b3) rubber particle proteins are recognized by IgE from patients with spina bifida and latex allergy. *J Allergy Clin Immunol* 1996; 98: 628–39.
15. RUE F, KIENITZ A, SCHOPF P, et al. Frequency of natural rubber latex allergy in adults is increase in those with multiple operative procedures. *Allergy* 2001; 56: 889–94.
16. HSIA RCH. Oxygen absorption by *Hevea brasiliensis* latex. *Institution of the Rubber Industry: Transactions and Proceedings*, 1958; 34: 267–90.
17. MOIR GFJ. Ultracentrifugation and staining of Hevea latex. *Nature (Lond)* 1959; 184: 626–8.
18. LAEMLIH UK. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 1970; 227: 680–5.
19. MOTULSKY H. *Intuitive Biostatistics*. New York, Oxford: Oxford University Press, 1995.
20. CREMER R, KLEINE-DIEPENBRUCK U, BLAKER F. Latex allergy in spina bifida patients. Prevention by primary prophylaxis. *Allergy* 1998; 53: 709–71.
21. BOWMAN RM, MC LORE DG, GRANT JA, TOMITA T, ITO JA. Spina bifida outcome: a 25 year prospective. *Pediatr Neurosurg* 2001; 34: 141–20.
22. BENARDINI R, NOVEMBRE E, LOMBARDI E, et al. Risk factors for latex allergy in patients with spina bifida and latex sensitization. *Clin Exp Allergy* 1999; 29: 681–6.
23. HEINMAN HA, DONSAN H, KAWALSKI M, TOERIEN A, POTTER PC. Bronchial hyper-responsiveness and atopy in urban, peri urban and rural South African children. *Paediatr Allergy Immunol* 2003; 14: 383–93.