Development of tuberculin reactivity and sensitization to *M. Scrofulaceum* and *M. Fortuitum* in children BCG-vaccinated at birth

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ABSTRACT: Since the incidence of tuberculosis is steadily declining in Finland and infections by environmental mycobacteria may be increasing, the aim of the present study was to evaluate the development of tuberculin reactivity and sensitization to environmental mycobacteria.

Healthy Finnish schoolchildren aged 10.4–12.4 yrs (n=201) were tested with tuberculin purified protein derivative RT23, *Mycobacterium scrofulaceum* RS95 and *M. fortuitum* RS20 sensitins. The same children had been previously tested with the same antigens and methods at the age of 4–6 yrs in 1989. Rapid waning of tuberculin reactivity and decrease in sensitization to environmental mycobacteria were observed between 4–6 yrs.

Both tuberculin and sensitin skin reaction sizes decreased significantly over the 6-yrs period. The mean tuberculin skin reaction size was 3.2 mm in diameter, which was significantly (p<0.001) smaller than the mean induration size (4.8 mm) at the age of 4–6 yrs. Similarly, the mean skin reaction sizes to *M. scrofulaceum* and *M. fortuitum* sensitins were 3.4 and 1.7 mm, respectively, which were significantly (p<0.001) smaller than 6 yrs earlier (mean 4.5 and 3.1 mm). The number of zero reactions to all antigens increased significantly during the follow-up period. Contacts with pets or farm animals were associated with larger reactions. In contrast, children suffering from allergic symptoms had smaller reactions.

Contacts with mycobacteria, either with *Mycobacterium tuberculosis* or environmental mycobacteria, seem to be too rare to maintain tuberculin responsiveness and a high sensitivity to other mycobacteria. Different bacille Calmette-Guérin vaccine products and dosages used, the declining incidence of tuberculosis and geographical factors, which can influence environmental mycobacterial exposure, may explain the disparity between the present and previous Finnish studies.
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As a result of effective tuberculosis control measures, the incidence of pulmonary tuberculosis has steadily declined in Finland. In 1996, it was estimated to be only 8.4 per 100,000 population [1]. In contrast to in many other parts of the world, the new cases of tuberculosis have been concentrated in the oldest, non-bacille Calmette-Guérin (BCG)-vaccinated, age groups [1, 2]. In addition, the incidence of human immunodeficiency virus (HIV) is low and HIV infections have been very rare in young infants and children (no reported cases in 1996) [1]. Since the 1950s, BCG vaccination has been routinely offered to all newborn babies, and the vaccination coverage has been invariably close to 100% [3].

Environmental mycobacteria, though low in virulence, may cause suppurative cervical adenitis in healthy children at an early age [4]. Although these infections are rare, there is evidence suggesting that their incidence is increasing throughout the world [5–7]. Sensitization to environmental mycobacteria in non-BCG-vaccinated child populations measured by skin testing seems also to be increasing [8]. These data indicate an increase in environmental exposure to mycobacteria [8, 9]. Although previous studies have suggested that sensitization increases with age, the incidence of mycobacterioses due to environmental mycobacteria in children is highest at the age of 1–6 yrs [4, 10, 11]. There is also evidence that sensitization may vary according to geographical location, even within a small country [12–14].

In an earlier cross-sectional study, a rapid decrease in tuberculin reactivity occurring between the ages of 4 and 6 yrs was observed [15]. The sensitization to nontuberculous mycobacteria seemed to be common but also decreased with age [16]. Previous Finnish studies have generally shown higher tuberculin reactivity and higher sensitivity to *Mycobacterium scrofulaceum* in children at the age of 10–13 yrs [17, 18]. The aim of the present study was to examine tuberculin reactivity and follow the degree of sensitization to environmental mycobacteria in a group of BCG-vaccinated children living in the same geographical region during a 6-yr follow-up period.

Subjects and methods

Subjects

In April 1989, all children born in 1983–1984 and living in a defined area of the municipality of Siilinjärvi, Finland, were invited to take part in a study on tuberculin skin test reactivity and sensitization to environmental mycobacteria. It included skin testing with mycobacterial antigens, a questionnaire filled in by the parents and a medical examination. Of the 434 invited children, 353 participated in the initial study [15, 16]. The children were healthy and, according to the medical records, all had been vaccinated as newborns with the Glaxo BCG vaccine (Glaxo Laboratories Ltd, Greenford, Middlesex, UK) at a dose of 0.05 mL.

Six years later, in May 1995, a follow-up study was undertaken with the purpose of examining the same children again. During the 6-yr period, 70 children had moved away from the Siilinjärvi area. A further 77 children either refused, were acutely ill during the time of testing or were otherwise inaccessible. Thus, the final number of children retested was 205.

The Ethics Committee of Kuopio University Hospital approved the study. A letter was sent to the parents explaining the reason for the study and testing was only performed if the parents had provided written informed consent.

Skin tests

In 1989, the test antigens used were tuberculin purified protein derivative (PPD) RT23, *M. avium* sensitin RS10, *M. scrofulaceum* sensitin RS95 and *M. fortuitum* sensitin RS20. Owing to the high cost of the antigens, the number of antigens was reduced to three in the retesting. *M. avium* was omitted and *M. scrofulaceum* was chosen to represent slowly growing environmental mycobacteria, because it had been widely used in other similar studies [9, 12, 13, 18, 19]. In the initial study, the skin test results were rather similar with *M. avium* and *M. scrofulaceum* antigens [16]. *M. fortuitum* was included as a representative of rapidly growing species commonly present in the environment, that are potentially pathogenic to man [20]. The sensitins (0.1 mL) were used at a dilution of 1 $\mu g \cdot mL^{-1}$ and 2 tuberculin units (TU) tuberculin PPD RT 23 were administered. The antigens were obtained from the Statens Serum Institut (Copenhagen, Denmark). The Mantoux technique was performed according to the World Health Organization (WHO) recommendations [21]. All three injections were performed concomitantly on the dorsal aspects of the forearms, two on the left and one on the right. All test injections as well as the reading of the reactions after 3 days were performed by the same personnel as in the previous study. The skin test results were measured by recording the transverse diameter of the induration in millimetres. The test antigens were coded and the code was broken after measuring the skin test results.

Questionnaire

The parents filled in a questionnaire, which contained questions about contacts with persons with tuberculosis, previous tuberculin testing, chronic diseases such as asthma or allergies, long-term medications and contacts with pets and farm animals. Recent use of antihistamines and topical corticosteroids were also recorded. The questionnaire was checked by the test personnel at the time of the testing.

Statistical methods

Statistical analysis was performed using SPSS for Windows 8.01 software (SPSS, Chicago, IL, USA). The non-parametric Mann-Whitney U-test, Wilcoxon matched-pair signed-rank test and Chi-squared test were used. A p-value of <0.05 was considered significant.

Results

The number of children retested was 205. Since four children were absent from the reading due to an acute illness, the final number was 201 (100 females and 101 males). The mean age of the children was 11.3 yrs (range 10.4–12.4 yrs). The mean diameter of the induration to tuberculin PPD RT23 was 3.2 mm (range 0–28 mm), to *M. scrofulaceum* 3.4 mm (range 0–26 mm) and to *M. fortuitum* 1.7 mm (range 0–14 mm) (fig. 1). The age of the child had no influence on mean skin reaction sizes. Tuberculin reaction sizes were slightly smaller in females (2.9 mm) than in males (3.5 mm). Conversely, reactions to *M. fortuitum* sensitin were larger in females than in males (2.0 *versus* 1.4 mm, p<0.05) (Table 1).

None of the children showed any clinical manifestation of a mycobacteriosis. No cases of tuberculosis had been diagnosed among the study group during the follow-up period. Seven children had been in tuberculosis contact and therefore had also been tested for tuberculin reactivity. In all cases, the testing had been done >1 yrs prior to the present study. There was no difference in their skin test reactivity compared to the other children.

There were 74 children with a 0 mm reaction to tuberculin. If there was a zero reaction to tuberculin, the mean reaction sizes to the two sensitins were also small: 1.1 mm to *M. fortuitum* and 1.3 mm to *M. scrofulaceum* sensitin. In this group, only five (6%) children showed a positive (≥5 mm) reaction to *M. scrofulaceum* sensitin and only three (4%) to *M. fortuitum* sensitin, all of whom expressed strong positive (≥10 mm) skin reactions.

When compared with the results from the initial study in 1989, the average diameter of reaction sizes to tuberculin and both sensitins had decreased. At the age of 4–6 yrs, the mean skin reaction sizes to tuberculin PPD and *M. scrofulaceum* and *M. fortuitum* sensitins were 4.8 mm, 4.5 mm and 3.1 mm, respectively. Six years later, at the age of 10–12 yrs, the values were significantly (p<0.01) smaller, *i.e.* 3.2 mm, 3.4 mm and 1.7 mm, respectively. Subgroup analysis revealed that, in children initially <5.5 yrs old, the decrease in mean reaction size was significant (p<0.05) but no change was found in children initially >5.5 yrs (fig. 2). The proportion of children with zero skin test reaction sizes increased significantly between the baseline and the follow-up studies. The mean reaction sizes in children with skin reactions of ≥1 mm were also significantly smaller at the follow-up survey (table 2).
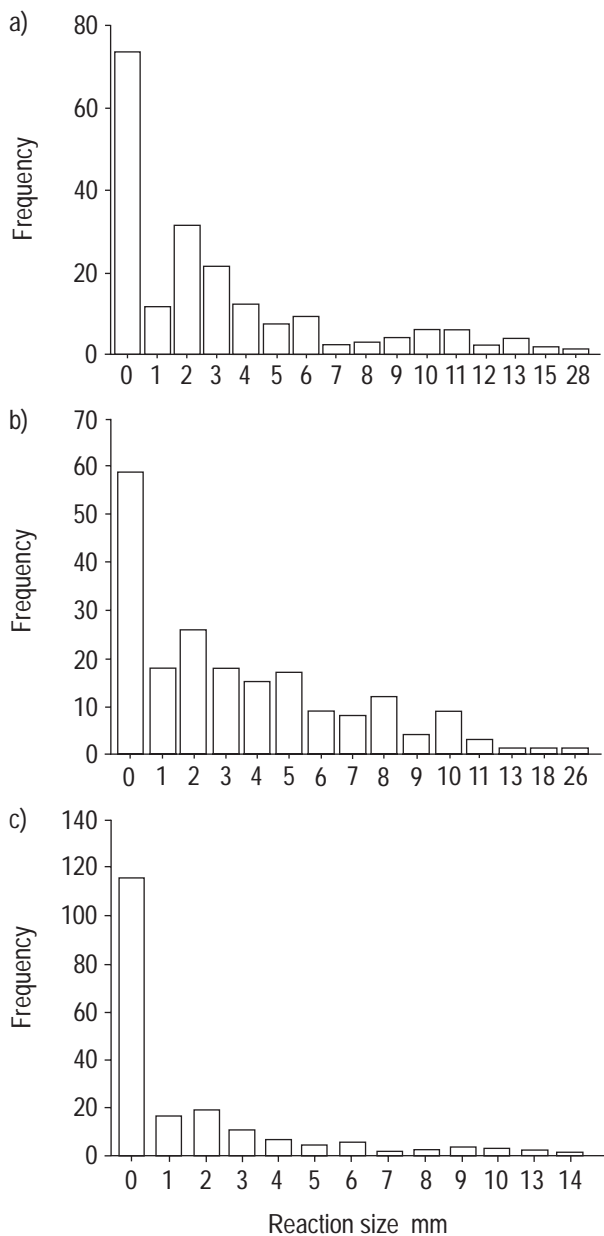
Fig. 1. – Frequencies of the different skin reaction sizes to a) tuberculin; and b) *Mycobacterium scrofulaceum*; and c) *M. fortuitum* sensitins.

Twelve (6%) children expressed an increase in reaction size to *M. scrofulaceum* and eight (4%) to *M. fortuitum* sensitin, the greatest increase being 12 mm. In eight children, tuberculin reaction size was ≥4 mm greater than at baseline, the greatest difference being 13 mm. None of the children who previously gave negative skin test reactions and who subsequently gave positive tuberculin reactions showed any clinical sign of tuberculosis.

Allergic diseases and the use of antihistamines were associated with lower tuberculin and sensitin reactivity. In children suffering from allergic diseases, the mean tuberculin induration size was smaller (2.5 mm) than in nonallergic children (3.4 mm), but the difference was not statistically significant. The reactions to *M. scrofulaceum* sensitin were significantly ($p<0.05$) smaller in children with allergic disorders (2.5 mm *versus* 3.8 mm). The same

tendency was observed in reactions to *M. fortuitum* sensitin, although the differences were not statistically significant (table 1).

In contrast, contact with pets or farm animals was associated with larger reactions to tuberculin and sensitins (table 1). As many as 151 (74%) children had pets at home, and 16 (8%) were living on a farm. The mean skin reaction sizes in children with pets at home were 3.5 mm to tuberculin, 3.8 mm to *M. scrofulaceum* sensitin and 1.9 mm to *M. fortuitum* sensitin, which were significantly larger than in children without contacts with animals. In children living on a farm (n=16), mean skin reaction sizes to tuberculin and *M. scrofulaceum* and *M. fortuitum* sensitins were 3.9, 5.2 and 1.8 mm, respectively. The reactions to *M. fortuitum* sensitin were significantly ($p<0.05$) larger in children living on a farm.

Sixty-four of the children had been given a booster polio vaccine (Salk) during the preceding 4 months (according to the Finnish vaccination programme). There were no significant differences between revaccinated and nonrevaccinated groups in reaction sizes (table 1).

Discussion

No follow-up studies on tuberculin reactivity have previously been carried out in Finnish children. Earlier cross-sectional studies have indicated an increase in tuberculin reactivity from early childhood to adolescence. A study in the 1970s, performed in children aged 3 months, 11–12 yrs and 18–19 yrs, showed reaction sizes of 6.8, 6.9 and 9.2 mm, respectively [17]. In a more recent study, performed in children 11–13 yrs of age, the mean tuberculin reaction size was 8.2 mm [18]. Compared to these earlier Finnish studies, the present study in 4–6-yr-old children revealed an unexpectedly small mean induration size (4.8 mm) with a significant age-related decline from 4–6 yrs [15]. Six years later, the same children yielded only half of the mean reaction size of those studied in the earlier Finnish study of children of the same age [18]. Thus the present results were unexpected.

As far as the authors are aware, earlier studies on sensitization to environmental mycobacteria have all been cross-sectional. In the present study, the same children were prospectively followed for changes in their reactivity to mycobacterial antigens. Only children living continuously in the same community were included in the testing to avoid the potential confounding effect caused by change of environment, which is the likeliest source of exposure to environmental mycobacteria [22]. The initial study showed a decrease in mycobacterial sensitization with increasing age from 4 to 6 yrs [16]. An earlier Finnish study on mycobacterial sensitivity in BCG-vaccinated children aged 11–13 yrs had shown significantly larger skin test reaction sizes than detected in the authors' initial study [17]. It was therefore expected that there would be an increase in reactions size as the children aged from 6 yrs to 11–13 yrs of age. However, this was not the case. In fact, a further decrease in mean skin reaction sizes and an increase in the number of zero reactions were observed.

It has been postulated that tuberculin sensitivity may wane more rapidly in children to whom BCG vaccination was given shortly after birth compared to those in whom

Table 1. – Mean tuberculin and sensitin reaction size in subgroups according to sex, allergic disease, use of anti-histamines or topical corticosteroids and recent polio vaccination

	Subjects n	Reaction size mm		
		Tuberculin	*Mycobacterium scrofulaceum*	*Mycobacterium fortuitum*
Sex				
Male	101	3.5±0.4	3.4±0.4	1.4±0.2[+]
Female	100	2.9±0.4	3.5±0.4	2.0±0.3
Allergy				
Allergic symptoms	57	2.5±0.4	2.5±0.4[#]	1.4±0.4
No allergy	144	3.4±0.4	3.8±0.3	1.8±0.2
Peroral antihistamines	15	2.1±0.6	2.1±0.6	0.9±0.4
Topical corticosteroids	13	2.0±0.8	3.0±0.8	1.9±0.7
Animal contacts				
Pets at home	151	3.5±0.3***	3.8±0.3[‡]	1.9±0.2***
Contact with farm animals	16	3.9±0.3	5.2±1.1	1.8±0.7*
No pets/farm animals	48	2.4±0.7	2.3±0.6	1.0±0.2
Polio vaccine				
Revaccinated	64	2.7±0.4	3.0±0.4	1.7±0.4
Nonrevaccinated	137	3.3±0.4	3.6±0.3	1.7±0.4

Data are presented as mean±SEM. [+]: p<0.05 compared to females. [#]: p<0.05 compared with children with no allergic symptoms; *: p<0.05; ***: p<0.001; [‡]: p<0.0001 compared with children with no contacts with pets or farm animals.

vaccination was given at a later age [23–25]. The policy of vaccinating newborns has remained unaltered in Finland since the early 1950s and in this respect, the present and previously examined child populations are identical. Although both child cohorts were BCG-vaccinated at birth, children in the earlier Finnish studies had received a different BCG vaccine at a higher dose (0.1 mL), which also caused more post-vaccination complications [26]. In contrast, the present cohort had been vaccinated with the Glaxo BCG vaccine, which replaced earlier vaccines made from the Swedish BCG strain Gothenburg, and at half of the previous dosage (0.05 mL) [26]. Another possible explanation for the decrease in tuberculin reaction size is the steadily decreasing tuberculosis morbidity in Finland. Tuberculosis incidence has declined from 53.6–49.2 per 100,000 population in the 1970s to 13 per 100,000 population in 1996 [2, 3]. Thus, the present child population has less frequently been at risk of natural exposure to *M. tuberculosis* than the children from the 1970s.

There can be wide geographical variation in the occurrence of environmental mycobacteria. A recent Finnish study revealed that the occurrence of mycobacteria in water and soils varies geographically and shows significant correlations with environmental factors such as pH and concentrations of metals and minerals in surface water [27]. Similarly, environmental studies in different countries as well as studies concerning sensitin skin test reactivity in children in Sweden, Norway and Greece have indicated that exposure to environmental mycobacteria varies according to geographical location [12, 13, 28, 29]. In Sweden, sensitization was significantly lower in children living in rural inland areas compared to those living in urban areas in the coastal region [12, 13]. The present study area was an inland lake area with partly urban and partly rural conditions/environment. In contrast, the earlier Finnish study cohort demonstrating higher levels of sensitization was conducted in the western coastal region, 500 km from the present study area. The authors believe that this is another important factor, potentially
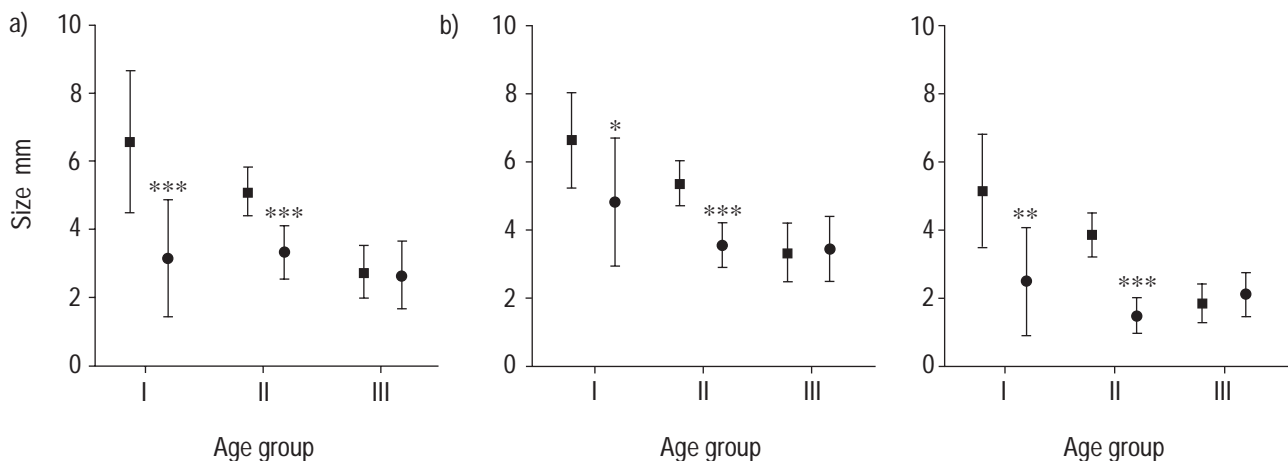

Fig. 2. – Mean skin reaction sizes to a) tuberculin; and b) *Mycobacterium scrofulaceum*; and c) *M. fortuitum* sensitins at baseline (■) and at follow-up (●) in the baseline age groups of <4.5 yrs (I), 4.5–5.5 yrs (II) and >5.5 yrs (III). Data are presented as mean±SD. *: p<0.05; **: p<0.01; ***: p<0.001 between baseline and follow-up reaction sizes. (Wilcoxon sigened-rank test).

Table 2. – Proportion of children with zero reactions to tuberculin and *Mycobacterium scrofulaceum* and *M. fortuitum* sensitins at the age of 4–6 yrs (baseline) and at the age of 10–12 yrs (follow-up) and mean skin reaction sizes among subjects with reaction sizes of ≥ 1 mm

	Baseline	Follow-up	p-value*
Tuberculin			
0-mm reaction size %	21.1	36.2	<0.001
Reaction size (≥ 1 mm) mm	5.7±0.3	3.8±0.3	<0.001
M. scrofulaceum			
0-mm reaction size %	22.0	29.5	0.009
Reaction size (≥ 1 mm) mm	5.7±0.2	3.9±0.3	<0.001
M. fortuitum			
0-mm reaction size %	34.7	57.8	0.014
Reaction size (≥ 1 mm) mm	4.7±0.3	2.2±0.3	<0.001

Data are presented as mean±SEM or percentage. *: Chi-squared or Wilcoxon rank test.

accounting for the discrepancy in results between these two Finnish studies.

The antigens and techniques of testing could have been potential sources of discrepant results between the present and the earlier Finnish study (M. Tala-Heikkilä group) [18]. However, the manufacturer of the antigens and the Mantoux technique used were the same. In addition, the test personnel, who were the same in both parts of the present study were initially trained by the group of M. Tala Heikkilä. There has, however, been discussion regarding the possible loss of potency of tuberculin PPD over the years since it was prepared in 1958 [30]. However, skin test reactivity is influenced by many simultaneously occurring factors whose true nature is difficult to analyse.

It is well known that genetic factors play an important role in responses to BCG vaccination, manifest both in skin-test results and protection against mycobacterial infections [31, 32]. This explanation does not apply to the examined child population because the same children were re-examined after a 6-yr interval. Genetically, this child population was also similar to the populations examined in earlier Finnish studies, of the same nationality and therefore with a very similar genetic background [15–18].

Allergic symptoms are relatively common in children. In this study population, 58 (28%) children had either asthma, allergic rhinitis or dermatitis, which was approximately the same proportion as reported in a recent Finnish epidemiological study of children aged 13–14 yrs (34%) [33]. In a Japanese study, a strong inverse relationship between tuberculin responses and atopic symptoms was found and it was suggested that exposure to *M. tuberculosis* might inhibit atopic disorders [34]. However, early BCG vaccination does not seem to affect the development of atopic diseases [35]. It has been suggested that persons suffering from allergic diseases may have smaller tuberculin reactions [36]. As in the authors′ study, children with allergic symptoms gave smaller mean skin reaction sizes. The use of corticosteroids is a potential cause of depression of delayed cell-mediated immunity [36], but the basic mechanisms behind allergy and the relationship between tuberculin responses and atopic disorders are still partly unknown [37].

Infections with *M. bovis* or *M. tuberculosis* have not been detected in animals in Finland during the last 20 yrs (M.L. Katila, personal communication), but colonization or mycobacterioses caused by *M. avium* and *M. intracellulare* are relatively common in birds and pigs. This could account for the slightly higher reactivity in those children with contacts with pets or farm animals, as has also been indicated by earlier studies [12–14]. In contrast, no differences could be detected in the present study between children with contact with different species of animal or bird, as has been observed previously [12]. However, the degree of exposure may be different in an urban home from that in a rural environment, in which pets are often kept outdoors.

According to the Finnish vaccination programme, 64 children had been revaccinated against polio (Salk vaccine) during the preceding months. In these children, skin test reaction sizes were slightly but not significantly smaller than those in nonrevaccinated children. This was expected because viral disease as well as vaccination with live virus vaccines may have a depressing effect on cell-mediated immunity [36], although contradictory results after vaccination against measles, mumps and rubella have also been reported [38].

The present results also suggest that exposure to environmental mycobacteria is not very common in school-age children living in an inland lake area, and is not sufficient to maintain skin sensitivity. In addition, these results suggest that sensitization to environmental mycobacteria, known to be boosted by cross-reactive BCG vaccination, may widely vary according to geographical location even in a homogenous child population or may significantly be influenced by the BCG vaccine preparation used.

Tuberculosis is a rarely encountered disease in Finland [1, 2], and the need for routine bacile Calmette-Guérin vaccination at birth is being re-evaluated. As there is evidence that bacille Calmette-Guérin vaccination offers protection to children, not only against *M. tuberculosis* but also against mycobacterioses due to environmental mycobacteria [6, 10], changes in bacile Calmette-Guérin vaccination policy may also influence the epidemiology of disease caused by other mycobacteria.

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