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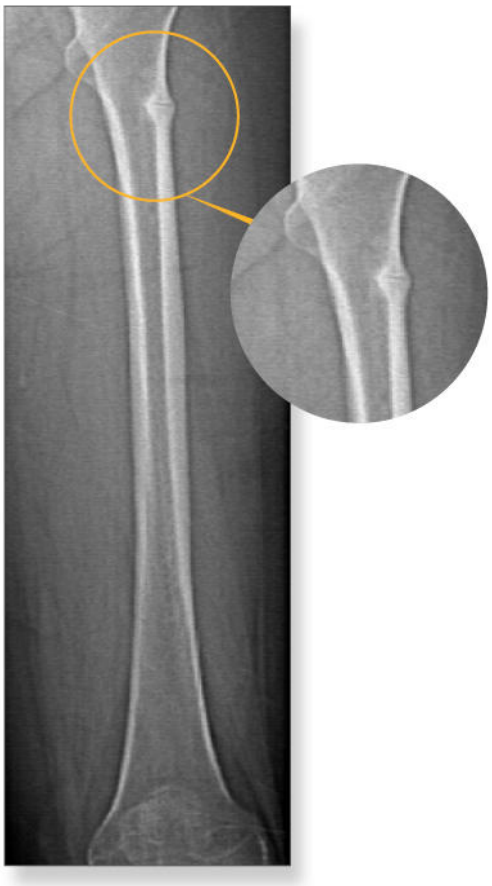

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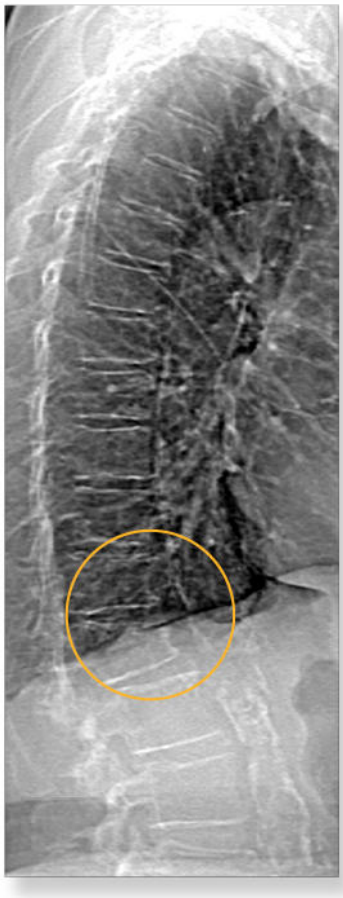

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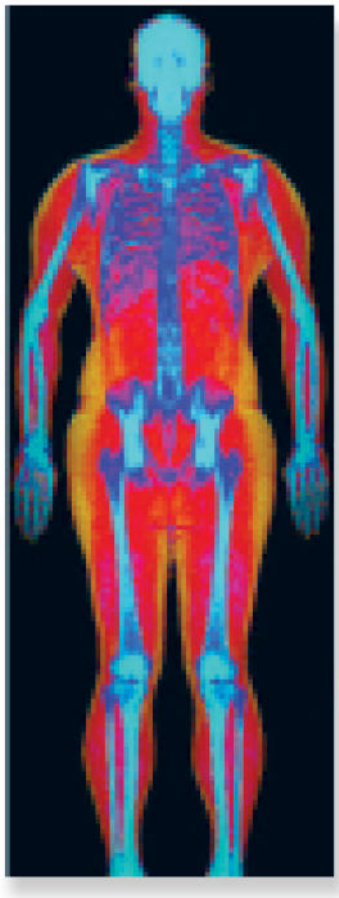

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ORIGINAL ARTICLE

JBMR

Individual Variation in Adaptive Immune Responses and Risk of Hip Fracture—A NOREPOS Population-Based Cohort Study

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ABSTRACT

Immune-mediated bone loss significantly impacts fracture risk in patients with autoimmune disease, but to what extent individual variations in immune responses affect fracture risk on a population level is unknown. To examine how immune responses relate to risk of hip fracture, we looked at the individual variation in a post-vaccination skin test response that involves some of the immune pathways that also drive bone loss. From 1963 to 1975, the vast majority of the Norwegian adult population was examined as part of the compulsory nationwide Norwegian mass tuberculosis screening. These examinations included standardized tuberculin skin tests (TSTs). Our study population included young individuals (born 1940 to 1960 and aged 14 to 30 years at examination) who had all received Bacille Calmette-Guerin (BCG) vaccination after a negative TST at least 1 year prior and had no signs of tuberculosis upon clinical examination. The study population ultimately included 244,607 individuals, whose data were linked with a national database of all hospitalized hip fractures in Norway from 1994 to 2013. There were 3517 incident hip fractures during follow-up. Using a predefined Cox model, we found that men with a positive or a strong positive TST result had a 20% (hazard ratio [HR] = 1.20, 95% confidence interval [CI] 1.01–1.44) and 24% (HR = 1.24, 95% CI 1.03–1.49) increased risk of hip fracture, respectively, compared with men with a negative TST. This association was strengthened in sensitivity analyses. Total hip bone mineral density (BMD) was available for a limited subsample and similarly revealed a non-significantly reduced BMD among men with a positive TST. Interestingly, no such clear association was observed in women. An increased immune response after vaccination is associated with an increased risk of hip fracture decades later among men, possibly because of increased immune-mediated bone loss. © 2020 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: GENERAL POPULATION STUDIES; EPIDEMIOLOGY; OSTEOPOROSIS; OSTEOIMMUNOLOGY

Introduction

The term osteoimmunology was coined in 2000[1] and has become a shorthand for the interplay between bone and immune system. A large part of this interplay is facilitated by cytokines released from activated T cells, which affect bone remodeling in different ways. Most activated T cells release cytokines that inhibit rather than stimulate bone loss.[2,3] T-helper cell 17 (Th17) is an exception to this because the exhibited cytokines are mainly stimulatory on osteoclastogenesis.[4] Th17 activity is

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also linked to the development of multiple inflammatory disorders.[5–8] These Th17-mediated immune responses are known to impact bone health in inflammatory conditions such as rheumatoid arthritis,[9] but it is still unclear to what extent they impact bone health in the general population.

Vaccines have presented an opportune way to investigate immune system variation[10] because they are a standardized and well-documented form of exposure. There are, however, few examples of post-vaccination responses routinely being measured. The tuberculin skin test (TST) has been employed since the early 20th century primarily to detect infection with tuberculosis[11] but also to document the immunological reaction to the Bacille Calmette-Guerin (BCG) vaccine. The test is conducted by intracutaneously applying antigens derived from tubercle bacilli. This produces a skin induration if the individual has undergone a prior sensitization toward the injected antigens, commonly from a BCG vaccination or much less common from environmental mycobacteriae or tuberculosis infection.

Because the test predates many of the discoveries that define modern immunology, such as the identification of B and T cells, our understanding of it has changed over time. After the characterization of Th17 during the early 2000s,[12,13] it has been shown that Th17 activity could be crucial to establish a post-BCG-vaccination response[14,15] and also that a lack of Th17 activity severely inhibits the delayed-type hypersensitivity response measured by TST.[16]

From 1948 to 1975, 80% to 85% of the Norwegian adult population were routinely examined as part of a mandatory mass tuberculosis screening.[17] These examinations included chest X-rays, TST, documentation of BCG vaccination status, and vaccination of previously unvaccinated persons with a negative TST. Even those who had a previously documented BCG vaccination, commonly as part of the national school vaccination program at age 12 to 14, underwent testing with TST. Since the incidence of tuberculosis in Norway declined rapidly from the 1930s onward,[18,19] the majority of positive TSTs among those previously BCG vaccinated reflected prior vaccination rather than exposure to environmental mycobacteria or infection with tuberculosis.

Given a prior BCG vaccination and subsequent testing with TST under standardized conditions, the variation in TST results among younger individuals should reflect individual variation in immune responses, rather than environmental exposure to mycobacteria. We therefore aimed to answer the following questions:

Is an increased post-vaccination immune response, as quantified by TST after BCG vaccination in young adulthood, associated with:

a. an increased risk of hip fracture three to four decades later?
b. lower bone mineral density three to four decades later?

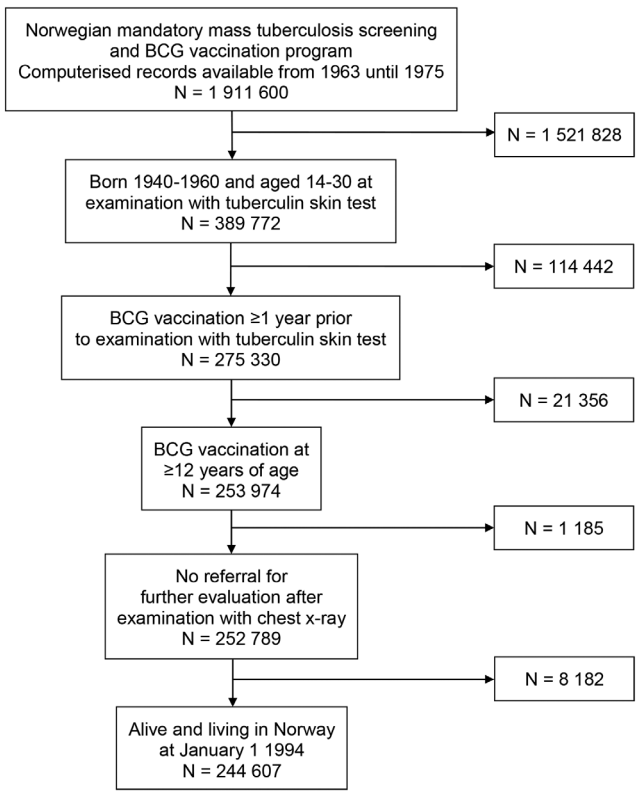

Fig 1. Study population.

Materials and Methods

Study population

Norwegian mandatory mass tuberculosis screening and BCG vaccination program

The nationwide Norwegian mandatory mass tuberculosis screening and BCG vaccination program (from here on referred to as the screening program) was conducted in the period 1948 to 1975. It aimed to reduce the risk of tuberculosis through examination of all individuals above school age with chest X-ray and TST. Unvaccinated persons with a negative TST were offered BCG vaccination. BCG was produced at the Bergen State BCG Laboratory (Bergen, Norway) using the Swedish Gothenburg strain until 1973.[20] From then it was provided by Statens Serum Institute (Copenhagen, Denmark). Liquid BCG was gradually replaced by freeze-dried BCG between 1959 and 1973.[21]

Computerized records of these examinations are available from 1963 to 1975, including 1,911,600 individuals. The screening program covered all counties in Norway except the capital Oslo, where inhabitants were screened in a separate program.

Selection of the study population for our study is described in Fig. 1. We aimed to include young, vaccinated individuals with an available TST result.

There were 389,772 individuals that matched our predefined criteria of being born 1940 to 1960 and aged 14 to 30 years at examination with TST. Only calendar year for vaccination and TST were available. Therefore, to ensure that vaccination had preceded TST, the sample was limited to individuals with a previously documented BCG vaccination at least 1 year before examination with TST (275,330 individuals; 70.6%).

The national school vaccination program in Norway was gradually implemented from the early 1950s, during 6th to 8th grade (ages 12 to 14 years) with very high coverage. According to expectations, 208,743 (75.8%) of the considered individuals had received a vaccination during ages 12 to 14. Only children with a previously confirmed positive TST after suspected infection with tuberculosis were to be exempted from vaccination. The final study population did also include individuals who had received vaccination at age >14 years.

We also excluded 21,356 (7.8%) individuals who were vaccinated before 12 years of age (ie, before the national school vaccination program), since it was likely that most of them had been in close contact with tuberculosis patients and had a high probability of being infected with tuberculosis. We also excluded 1185 individuals who for any reason were referred for further evaluation after examination with chest X-ray. Of the remaining 252,789 individuals, 244,607 were alive and living in Norway on January 1, 1994.

Exposure

Tuberculin skin test - adrenaline Pirquet

For TST, the adrenaline Pirquet (aP) method was used with Old Tuberculin, measuring infiltrates according to strict national guidelines during the screening program, which is described by Waaler and colleagues.[22] All aP/TST measurements used in the following analysis were conducted between 1954 and 1975 (99% between 1963 and 1975). Two skin scratches of 5 mm length were applied to the volar side of the distal left arm. After 48 hours (maximum 72 hours), the largest of the two infiltrates were recorded in mm.

During the Norwegian mandatory mass tuberculosis screening and BCG vaccination program, the aP test was categorized as negative (<4 mm) or positive (≥4 mm),[22] although positive reactions of ≥8 mm were sometimes referred to as strong positive reactions. A previous study defined a strong positive reaction as ≥10 mm,[23] whereas Bjartveit briefly mentions a strong reaction as ≥8 mm.[24] In our analyses, we accordingly categorized the aP tests as negative (<4 mm), positive (≥4 mm), or strong positive (≥8 mm), and performed sensitivity analyses with infiltrates of ≥10 mm being considered strong positive rather than ≥8 mm. All mentions of TST in Results refer to aP measurements.

Outcomes

Hip fracture – NORHip

Data on all cervical, trochanteric, or subtrochanteric hip fractures treated in Norwegian hospitals from January 1, 1994, through December 31, 2013, were retrieved from the NORHip database compiled by the Norwegian Epidemiologic Osteoporosis Studies (NOREPOS) research network.[25] We did not have access to data regarding potential hip fractures before 1994, as this was the first year all hospitals used electronic patient administrative systems. Only the first hip fracture for each individual was included in the analysis. We could not differentiate between high- and low-energy hip fractures. Information on definitions, classification, quality assurance, and validation of data collection for the NORHip database is available at www.norepos.no/documentation.

The National Registry provided dates of emigration and death. Of the 252,789 individuals eligible for inclusion from the screening program, 244,607 (from here on referred to as the study population) were alive and living in Norway on January 1, 1994, and were included in the analyses (Fig. 1). Each individual was followed from January 1, 1994, to the date of his or her first hip fracture, emigration, death, or end of follow-up December 31, 2013, whichever occurred first. An overview of the timeline for exposure and outcome is presented in Fig. 2.

Bone mineral density

Bone mineral density (BMD) in the total hip at mean age 52 years (range 45 to 62 years) was available in a subsample of dual-energy X-ray absorptiometry (DXA) scans performed in the Hordaland Health Study (HUSK) 1997 to 2000 and the fifth Tromsø Study (Tromsø 5) 2001 to 2002. Both studies used GE Lunar densitometers (GE Lunar, Madison, WI, USA): Tromsø 5 used Prodigy, while HUSK used EXPERT-XL. Dual hip scans were performed in Tromsø 5. The left hip was scanned in HUSK, unless there was a history of previous fracture or surgery, whereupon the right hip was scanned. The measurements have been cross-calibrated.[26]

We have included left hip scans where available, and right hip scans if left was missing (n = 25). There were a total of 849 BMD total hip measurements available for the individuals included in our study population, 493 from HUSK and 356 from Tromsø 5.

Statistical methods

Data were analyzed using Stata for Windows (version 15.0, Stata Corporation, College Station, TX, USA). Risk estimates (hazard ratios) of hip fracture according to TST infiltrate were obtained using a multivariable Cox proportional hazards model with time on study scales. TST was both entered as a continuous as well as a categorical variable during analyses. Categories were determined based on Cox regression with cubic splines (5 knots) and hazard estimates from the described Cox model, as well as the size of each group. The results from the spline analysis were in accordance with the previously described clinical categorization.[23,24] We therefore categorized TST infiltrate size into three levels: negative (<4 mm, reference), positive (≥4 mm), and strong positive (≥8 mm). In addition, the predefined Cox models included the following covariates: age at TST (years), time from BCG vaccination to TST (years), time from TST to start of follow-up (years), BMI (categorical, <18.5, <25, <30, ≥30), and county (categorical). The time from BCG vaccination to TST was included in the model to standardize the TST measurements, since this time is known to impact the infiltrate size.[27,28]

In sensitivity analyses, Cox models corresponding to those described above were performed: (i) limited to individuals born 1945 or later; (ii) limited to those who had received BCG vaccination at age 12 to 14 years, which is most likely as part of the school vaccination program; and (iii) defining a strong positive TST response as >10 mm rather than >8 mm.

Differences in total hip BMD between categories of TST response were evaluated using a linear regression model that included the same covariates as the described Cox model, except for "time from TST to start of follow-up," which was substituted for "time from TST to BMD measurement."

The Student's t test was used to compare means for independent samples with normal distribution, whereas the Mann–Whitney U test was used for samples with non-normal distribution. The chi-square test was used to compare frequencies between groups. Pearson's r is reported when measuring linear correlation.

A p value of <0.05 was considered statistically significant. Where deemed appropriate, 95% confidence intervals (CI) are reported.

Ethics

The study and the data linkages have been approved by the Regional Committee for Medical and Health Research Ethics, Statistics Norway, The Norwegian Directorate of Health, and the

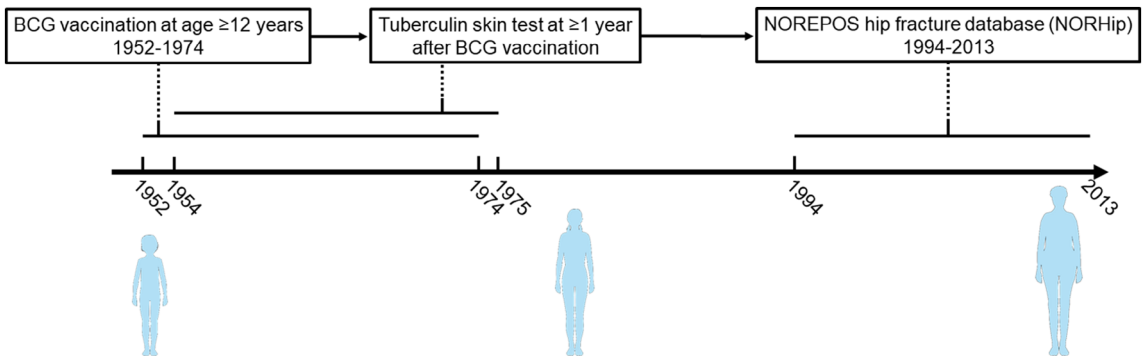

Fig 2. Timeline for exposure (vaccine and skin test) and outcome (hip fracture). Illustrations modified from *Servier Medical Art by Servier* (Creative Commons License).

Norwegian Institute of Public Health. Use of dates of deaths and emigration from the National Registry was approved by the Norwegian Tax Administration. Data from the Norwegian Patient Registry have been used in this publication. The interpretation and reporting of these data are the sole responsibility of the authors, and no endorsement by the Norwegian Directorate of Health is intended nor should be inferred.

Results

There were 119,693 men (48.9%) and 124,914 women (51.1%) in the study population (Table 1). The mean TST infiltrate was 6.2 mm (range 0 to 60, SD 2.8), with men having a mean infiltrate that was 0.5 mm larger than women ($p < 0.001$). A total of 40,450 individuals (16.5%) were TST negative (<4 mm). Among the 204,157 TST positive, 122,787 (60.1%) had an infiltrate size of 4 to 7 mm, 52,225 (25.6%) had an infiltrate size of 8 to 9 mm, and 29,145 (14.2%) had an infiltrate size of ≥10 mm. The mean time between BCG vaccination and measurement of TST was 6.8 years (SD 4.2 years), and the mean age at the time of examination with TST was 20.4 years (SD 4.4 years). There was a significant positive correlation between the size of the TST infiltrate and the time that elapsed between BCG vaccination and measurement of TST (Pearson's $r = 0.203$, $p < 0.001$).

Table 1. Characteristics of the Study Population ($N = 244,607$)

	Men ($n = 119 693$)	Women ($n = 124 914$)
Age at tuberculin skin test (TST), mean years (SD)	20.3 (4.4)	20.5 (4.4)
Age at BCG vaccination, mean years (SD)	13.6 (2.1)	13.5 (2.0)
Time between BCG vaccination and TST, mean years (SD)	6.7 (4.2)	7.0 (4.2)
TST infiltrate size, mean mm (SD)	6.4 (2.7)	5.9 (2.9)
TST negative individuals, *n* (%)	15 881 (13.3)	24 569 (19.7)
Body mass index (BMI), mean (SD)[a]	22.2 (2.8)	21.9 (3.0)
Hip fracture during follow-up, *n* (%)	1 437 (1.2)	2 080 (1.7)
Age at hip fracture, mean years (IQR)	59.7 (6.9)	61.4 (6.3)

[a]85 men and 230 women missing.

Hip fracture

There were 3517 (1.4%) incident first hip fractures in the study population during follow-up. The mean age at the time of fracture was 59.7 years (SD 6.9 years) among men and 61.4 years (SD 6.3 years) among women. A total of 59% of the fractures occurred among women.

Among the men, there were 4.89, 6.13, and 7.09 fractures per 10,000 person-years in the negative (<4 mm), positive (≥4 mm), and strong positive (≥8 mm) groups, respectively, whereas for women there were 8.74, 8.22, and 9.22.

These unadjusted trends were also reflected in the multivariable Cox proportional hazard model (Table 2). Men with a positive (≥4 mm) or a strong positive (≥8 mm) TST infiltrate had a 20% (hazard ratio [HR] = 1.20, 95% confidence interval [CI] 1.01–1.44) and 24% (HR = 1.24, 95% CI 1.03–1.49) increased risk of hip fracture, respectively, compared with those with a negative reaction (<4 mm). There was no significant difference in risk among women with either a positive (HR = 0.89, 95% CI 0.79–1.00) or strong positive reaction (HR = 0.90, 95% CI 0.79–1.02) compared with those with a negative one. Data are presented in Table 2.

TST as a continuous measure (millimeters) was not significantly associated with risk of hip fracture in any of the Cox models (Table 2).

Sensitivity analyses

Excluding all individuals born before 1945 (birth year range 1940 to 1944) yielded similar but somewhat stronger effect estimates. Men with a positive or a strong positive TST infiltrate had a 25% (HR = 1.25, 95% CI 1.01–1.54) and 30% (HR = 1.30, 95% CI 1.04–1.62) increased risk of hip fracture, respectively, compared with those with a negative reaction. Again, there was no significant difference among women (HR = 0.94, 95% CI 0.80–1.09 and HR = 1.01, 95% CI 0.85–1.19, respectively).

Restricting the analysis to those who had received BCG vaccination during ages 12 to 14 (most likely as part of the school vaccination program) also yielded similar and somewhat stronger

Table 2. Hazard Ratios for Hip Fracture by Categories of Tuberculin Skin Test Result[a]

	Men				Women			
	n (%)	Fractures (%)	HR	95% CI	*n* (%)	Fractures (%)	HR	95% CI
Tuberculin skin test (TST)								
<4 mm (negative, ref.)	15,869 (13.3)	148 (10.3)	1.00	(ref.)	24,533 (19.7)	413 (19.9)	1.00	(ref.)
4–7 mm (positive)	60,471 (50.6)	708 (49.3)	1.20	1.01–1.44	62,155 (49.9)	987 (47.5)	0.89	0.79–1.00
≥8 mm (strongly positive)	43,268 (36.2)	580 (40.4)	1.24	1.03–1.49	37,996 (30.5)	676 (32.6)	0.90	0.79–1.02
HR per 1 mm increase in infiltrate size			1.01	0.99–1.03			0.99	0.98–1.01

[a]Estimated using a Cox regression model including the following covariates: age at TST (years), time between BCG vaccination and TST (years), time between TST and start of follow-up (years), BMI (categorical), and county (categorical).

effect estimates. Men with a positive or a strong positive TST infiltrate had a 26% (HR = 1.26, 95% CI 1.02–1.55) and 29% (HR = 1.29, 95% CI 1.04–1.61) increased risk of hip fracture, respectively, compared with those with a negative reaction, whereas there was no significant difference in risk for women (HR = 0.92, 95% CI 0.81–1.05 and HR = 0.92, 0.80–1.06, respectively).

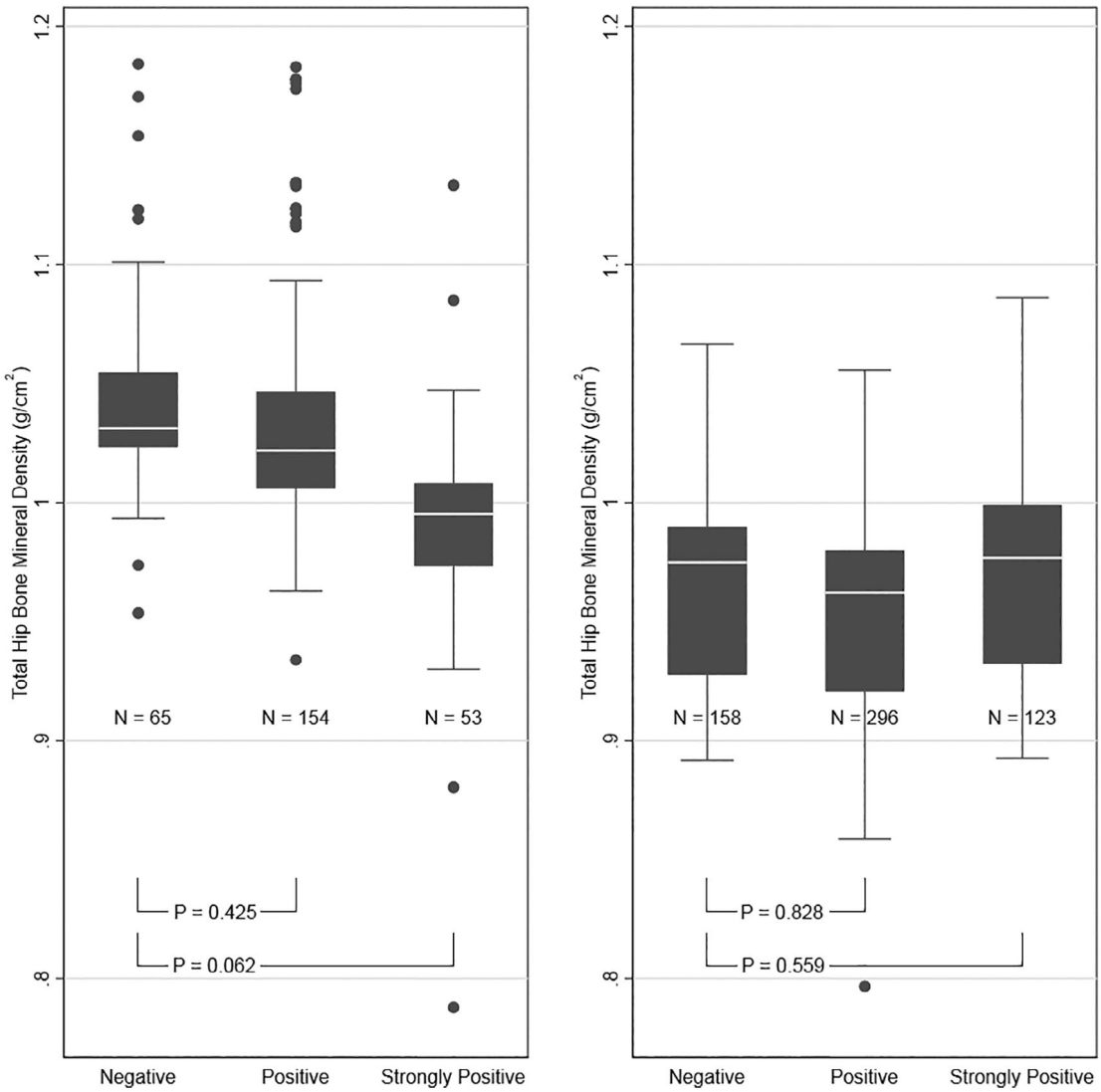

Fig 3. Adjusted total hip bone mineral density by categories of tuberculin skin test (TST) result among a subsample of men (left, *n* = 272) and women (right, *n* = 577). Median with interquartile range (IQR; box) and ±1.5 x IQR (whiskers). Estimated using a linear regression model including the following covariates: age TST (years), time from BCG vaccination to TST (years), time from TST to bone mineral density measurement (years), body mass index (categorical), and county (categorical).

Changing the definition of a strong positive response from ≥8 mm to ≥10 mm produced similar risk estimates as in the main model (born 1940 to 1960, BCG at 12 years or older), with a 21% (HR = 1.21, 95% CI 1.02–1.44) and 25% (HR = 1.25, 95% CI 1.01–1.55) increased risk of hip fracture among men with a positive or strong positive reaction, respectively, compared with a negative reaction. There was, however, a slight change among women, with a statistically significant 11% reduced risk among the positive group (HR = 0.89, 95% CI 0.79–0.99). The difference between women with a strong positive reaction versus negative remained non-significant (HR = 0.95, 95% CI 0.81–1.11).

TST as a continuous measure (millimeters) was not significantly associated with risk of hip fracture in any of the sensitivity analyses.

Bone mineral density

In the subsample with available DXA measurements of total hip BMD (272 men and 577 women), mean age at the time of DXA measurement was 52.3 years (range 45 to 62, SD 5.1). In men, total hip BMD was lowest among those with a strong positive TST reaction, although no differences between any groups were statistically significant. There was no clear trend among women. Data are presented in Fig. 3 and Supplemental Table S1.

Discussion

Our results showed that men with a positive TST after BCG vaccination at a young age had a slightly increased risk of hip fracture later in life, potentially mediated through increased immune-mediated bone loss. This notion was also supported by a non-significantly lower total hip BMD several decades after the screening among TST positive men, although this analysis had insufficient statistical power to make clear inferences.

The association between TST measurement and risk of hip fracture was not present among women. A similar sex-specific difference has previously been described for C-reactive protein (CRP) and BMD,[29] and there are also indications that men and women have differing immune responses toward tuberculin/tuberculosis.[30] The impact of female reproductive health on immune activity may also be important to consider. Immune activity differs depending on the menstrual cycle,[31] and pregnancy has been shown to reduce Th17 activity.[32] A change in the presence of sex steroid hormones will also significantly affect adaptive immune responses,[33] for example after menopause. Most of the women included in the study population would likely have undergone menopause during follow-up. Since there are multiple factors throughout life that both impact immune activity and are exclusive to women, it could be that a single measure of immune activity obtained during young adulthood is not representative of individual variation in the same way among women as in men. There are also fewer inherent risk factors for hip fracture among younger men than among women. Although the loss of hormones after menopause comprises a major risk factor among women and may override smaller influences such as immune-mediated factors, there is no single comparable factor among men.

By focusing on younger adults, we aimed to reduce the impact of differences in environmental exposure on TST and also to ensure that BCG vaccination had happened under similar circumstances across the study population. The majority (around 76%) were vaccinated as part of the strictly standardized national school vaccination program. There was also less variation in lag-time between vaccination and the TST among the younger participants, and both age at vaccination and time since vaccination have been shown to be important factors for the TST reaction after BCG vaccination.[27,28] Results from the sensitivity analyses, which revealed that both a narrower range of birth year (1945 to 1960) as well as age at vaccination (12 to 14 years) produced stronger effect estimates, even though statistical power was reduced, further support the importance of a young population with standardized exposure.

It is hard to reach any definitive conclusion as to what the TST response specifically represents, as there are multiple immunological mechanisms involved. However, this complexity is also an advantage in that it reflects a complete immune response as it happens in vivo, rather than a single inflammatory marker. The conventional interpretation of a negative TST after BCG vaccination is that the individual did not induce an adequate cell-mediated immune reaction post vaccination, and thereby lack memory CD4+ T cells to respond to the tuberculin. The reaction is generally considered to be Th1-mediated, but Th17 has also been shown to be an important promoter of both the post-vaccine response,[14,15] as well as the delayed-type hypersensitivity reaction measured during TST.[16] A possible involvement of Th17 activity is in line with our observation of a reduced fracture risk among the negative group, as Th17 is one of the few CD4+ T cells with a cytokine profile that is net stimulatory on osteoclastogenesis.[4] This would also fit with previous reports of positive associations between levels of the inflammatory cytokines IL-6/TNF and fracture risk.[34–37] Still, the specific immunologic mechanisms involved in the TST could not be determined in the current study and must be investigated using other study designs.

There is also an inherent variability in the tuberculin skin test that adds uncertainty to our measurements. Although most participants were exposed to the same form of BCG vaccine and testing procedure, there is still an intra-individual variation in the TST results with the same individual producing slightly different induration sizes when tested multiple times.[38] The possibility also remains that some of the positive test results may have been caused by exposure to environmental mycobacteriae or tubercle bacilli.[22]

Another question is whether the immunological tendencies we have measured are persistent over time, as we followed the participants up to 59 years. Recent studies of human immune system variation using post-vaccination responses found little intra-individual variation over time,[39,40] and even temporal stability for immunological markers that vary significantly between individuals.[41] This could imply that the human immune phenotype remains stable for large parts of our life, although it is important to note that the data points from these studies are separated by months or years and not decades as in our study.

Several health and lifestyle-related variables occurring through the life span could influence the association between immune response and subsequent fracture risk. We have adjusted for BMI at screening, age, and county. Smoking is well known to affect both the innate and adaptive immune system,[42] but given the young age of our participants at the time of TST measurements, this impact would likely have been limited. It is also unlikely that the presence of autoimmune disorders should have significantly altered the described risk estimates, given the low prevalence of these conditions during early life. Individuals with autoimmune disorders are, however, also more likely to take medication with a detrimental effects on bone integrity, such as corticosteroids.

We did not have access to information on hip fractures that could have occurred between the time of measurement of TST

and the start of follow-up in 1994. It is unlikely that this comprised a substantial proportion of all hip fractures, since our population was relatively young. Only an estimated 6.8% of all hip fractures among men, and 1.7% among women, occur before 55 years of age.[43] Median age at start of follow-up in 1994 was 46 years. The oldest individual in the cohort was 54 years old at the start of follow-up and 74 years old at the end of follow-up. The median age at hip fracture was therefore low at 60/62 years (men/women). This compares to a mean age at hip fracture of 79/82 years (men/women) for the Norwegian population as a whole,[44] which means that there likely was a different profile of risk factors present among our cohort compared with that in the background population. This was an advantage of our study design, as prevalent risk factors among older persons could override any immune-mediated effect. However, this relatively young age of the study population could also imply that there are several high-energy level fractures present, which we could not account for. On the other hand, it can also be argued that such high-energy fractures should be included as outcomes in observational studies on osteoporosis, as BMD has been shown to be similarly inversely associated with both high-trauma and low-trauma nonspine fractures in at least the elderly.[45]

In summary, data from our nationwide cohort showed a consistent trend of increased risk of hip fracture later in life in men with an increased post-vaccination immune response. This may be due to an increased immune-mediated bone loss, but there are several uncertainties as to which immunological mechanisms the measured immune response actually represents. This hypothesis of immune-mediated bone loss was supported by a non-significant inverse relationship between the immune response and total hip BMD among a subsample of the study population, although this analysis was severely underpowered. There was no similar trend among women. We have speculated that there are factors unique to women that we could not account for, which may have affected any potential association.

An increased post-vaccination immune response is associated with an increased risk of hip fracture decades later among men, possibly due to increased immune-mediated bone loss. A similar association was not found among women.

Disclosures

All authors state that they have no conflicts of interest.

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AUTHOR CONTRIBUTIONS

JD: Conceptualization; formal analysis; methodology; validation; visualization; writing-original draft; writing-review and editing. **KH:** Conceptualization; data curation; funding acquisition; methodology; project administration; writing-review and editing. **EH:** Conceptualization; methodology; writing-review and editing. **GG:** Conceptualization; writing-review and editing. **MH:** Conceptualization; writing-review and editing. **TF:** Conceptualization; writing-review and editing. **EA:** Conceptualization; writing-review and editing. **HM:** Conceptualization; funding acquisition; methodology; project administration; supervision; writing-review and editing.

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