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Iron deficiency and *NRAMP1* polymorphisms (INT4, D543N and 3′UTR) do not contribute to severity of anaemia in tuberculosis in the Indonesian population

Edhyana Sahiratmadja[1,2,3], Frank T. Wieringa[4], Reinout van Crevel[4], Adriëtte W. de Visser[3], Iskandar Adnan[1], Bachti Alisjahbana[5], Eline Slagboom[6], Sangkot Marzuki[1], Tom H. M. Ottenhoff[2,3], Esther van de Vosse[3] and Joannes J. M. Marx[7]*

[1]*Eijkman Institute for Molecular Biology, Jakarta, Indonesia*
[2]*Department of Immunohematology and Bloodtransfusion, Leiden University Medical Center, Leiden, The Netherlands*
[3]*Department of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands*
[4]*Department of Internal Medicine, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands*
[5]*Division of Tropical and Infection Diseases, Department of Internal Medicine, Medicine Faculty of University of Padjadjaran, Bandung, Indonesia*
[6]*Department of Molecular Epidemiology, Leiden University Medical Center, Leiden, The Netherlands*
[7]*Eijkman Winkler Institute for Microbiology, Infectious Diseases and Inflammation, University Medical Centre Utrecht, postnr. G04.614, PO Box 85500, 3508, GA Utrecht, The Netherlands*

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Fe-deficiency anaemia is the most common cause of anaemia in developing countries. In these settings, many chronic infections, including tuberculosis (TB), are highly prevalent. Fe is an essential nutrient for both host and mycobacteria that play a pivotal role in host immunity and mycobacterial growth. A case–control study was performed in a TB-endemic region in Jakarta, Indonesia, among 378 pulmonary TB patients and 436 healthy controls from the same neighbourhood with the same socio-economic status. In a number of these subjects the Fe status could be explored. The distribution of three polymorphisms in the natural resistance-associated macrophage protein gene (*NRAMP1*) including INT4, D543N and 3′UTR was examined for a possible association with susceptibility to TB. Anaemia (corrected for sex) was present in 63·2 % of active TB compared with 6·8 % of controls, with female patients more often affected. Anaemia was more pronounced in advanced TB as diagnosed by chest radiography. Lower Hb concentrations in TB patients were accompanied by lower plasma Fe concentrations, lower Fe-binding capacity and higher plasma ferritin. After successful TB therapy, Fe parameters improved towards control values and Hb levels normalised, even without Fe supplementation. *NRAMP1* gene polymorphisms were not associated with TB susceptibility, TB severity or anaemia. In conclusion, most active TB patients had anaemia, which was probably due to inflammation and not to Fe deficiency since TB treatment without Fe supplementation was sufficient to restore Hb concentration.

Tuberculosis: C-reactive protein: Anaemia: Iron: *NRAMP1*

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*MTB*), is a global health threat, with 8 million active new cases and 2–3 million deaths annually. The majority of TB cases reside in developing countries, among others in Indonesia, which harbours more than 10 % of TB cases worldwide[1]. *MTB* is an intracellular pathogen that targets host phagocytes, and consequently an effective host defence is required to coordinate cellular immune response[2]. Following phagocytosis, *MTB* lives within phagosomes of host macrophages and competes with the host to acquire Fe in order to survive and replicate[3]. For the host, Fe is an essential component of Hb, as Fe binds and transports O_2. Fe is also needed for electron transport, DNA synthesis and immune function, for example, for the formation of oxygen radicals[4].

It remains unclear how *MTB* accumulates Fe in macrophages. An excess of Fe supply will result in *MTB* growth, and Fe overload is a known risk factor for infections, as this may worsen the disease. Fe overload, for example from dietary Fe, causes individuals to be more susceptible to TB[5]. Interestingly, the *MTB* growth within macrophages from individuals with hereditary haemochromatosis, a genetic disease which leads to Fe overload, is reduced[6]. This can be explained by the fact that, even in severe Fe overload,

Abbreviations: ACD, anaemia of chronic disease; CRP, C-reactive protein; CXR, chest X-ray radiography; ESR, erythrocyte sedimentation rate; MCH, mean corpuscular Hb; MCV, mean corpuscular volume; *MTB*, *Mycobacterium tuberculosis*; NRAMP1, natural resistance-associated macrophage protein; TB, tuberculosis.

* **Corresponding author:** Professor J. J. M. Marx, fax +31 30 2541770, email marx@planet.nl

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macrophages in hereditary haemochromatosis provide an Fe-deficient environment due to increased export of Fe from the labile Fe pool to plasma by the export protein ferroportin[7,8].

On the other hand, Fe deficiency can increase susceptibility to various infectious diseases, since macrophages require Fe to function well[3]. Even mild Fe deficiency causes a significant impairment in the immune status and reduces the capacity to control infections. Fe-deficiency anaemia is the most common cause of nutritional deficiency anaemia in developing countries, affecting mostly children and pregnant and lactating women[9]. Therefore, Fe supplementation is often prescribed in developing countries[10].

Several susceptibility-associated genetic polymorphisms have been proposed to explain differential susceptibility to TB. Various studies have reported that subtle variations in the natural resistance-associated macrophage protein gene (*NRAMP1*) result in a higher risk for having TB[11–13]. NRAMP1 is a metal transporter protein localised in late endosomal and lysosomal compartments, and probably plays an important role in transferring diferric Fe across the phagosome membrane[14,15].

In the present study we conducted a case–control study in Indonesia, a country with a high prevalence of both TB and Fe-deficiency anaemia. Fe status of pulmonary TB patients and controls was explored to determine the prevalence of anaemia with or without Fe deficiency in active TB patients. Also, the effect of TB infection on Fe status indicators during the course of TB therapy was investigated, as inflammation affects many indicators of Fe status[16]. Distribution of NRAMP1 alleles and genotypes in Indonesia has not been reported before. In the present study, three commonly investigated polymorphisms in the *NRAMP1* gene, INT4, D543N and 3′UTR, were examined to explore whether such polymorphisms are associated with susceptibility to TB or TB severity.

Materials and methods

Subject recruitment

After written informed consent was obtained from all subjects, 494 newly detected sputum smear-positive pulmonary TB patients aged over 15 years were recruited in a poor setting area at an out-patient TB control clinic, Perkumpulan Pemberantasan Tuberkulosis Indonesia (PPTI), in Jakarta from January 2002 until December 2005. This case–control study was part of a larger TB study in Indonesia. The study design was approved by the Medical Faculty University of Indonesia and the Eijkman Institute Jakarta ethical committees. Pulmonary TB was diagnosed based on the clinical presentation, chest X-ray radiography (CXR), and confirmed by two consecutive acid-fast bacilli-positive sputa. All patients were provided with free anti-TB therapy according to the national TB programme (2HRZE/4H3R3). Patients with seropositive HIV (*n* 7; 1·4 %), diabetes mellitus (*n* 96; 19·4 %), CHD (*n* 3; 0·6 %) or incomplete data (*n* 10; 2·0 %) were excluded from the statistical analyses. For analysis after therapy, only patients with complete medical records after TB therapy (*n* 153) were included.

In the same period, 519 healthy controls from the neighbourhood where the cases lived, with the same socio-economic status, were randomly selected and matched for sex and age (± 10 %). Controls were interviewed using the same standardised questionnaire and underwent the same physical and blood examination and CXR as cases. Control subjects with CXR suspective of TB (*n* 17; 3·3 %) or history of prior anti-TB treatment (*n* 7; 1·4 %), diabetes mellitus (*n* 25; 4·8 %) and incomplete data (*n* 34; 6·5 %) were excluded. Although not all control subjects were tested for HIV status, since informed consent for HIV testing in the control group could only be obtained later in the study, HIV seropositivity was only found in two of 115 (1·7 %) tested controls. Indonesia has a low HIV prevalence in the general population, which was similar to the number found in TB patients and in accordance with earlier reports for Indonesia[1].

Blood samples were obtained by venepuncture. Full blood counts were performed routinely in the clinic for all patients before therapy and all controls using an automated blood analyser (Cell-Dyn 3200, Abbott Laboratories, Abbott Park, IL, USA). Haematology data could be obtained only in sixty-five of 153 patients after therapy since full blood counts are not routinely performed in the clinical setting.

Plasma from heparinised blood was collected and stored at −80°C for further analysis. Fe status indicators including plasma Fe, plasma Fe-binding capacity, Fe saturation and plasma ferritin were measured from patients for whom haematology data were available (*n* 65) and in a set of randomised controls (*n* 76). Total plasma Fe was measured using an ascorbate/FerroZine colorimetric method (Abbott Laboratories, Abbott Park, IL, USA). The plasma ferritin was measured by a solid-phase, two-site chemiluminescent immunometric assay (Immulite 2000; Diagnostic Product Corporation, Los Angeles, CA, USA). Fe status indicators could, however, only be measured in the plasma of thirty-three patients after therapy due to limited plasma availability. Erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP) examination were measured as indicators of the inflammatory response.

Genotyping of NRAMP1 single nucleotide polymorphisms

Genomic DNA was isolated from EDTA blood of patients and controls. Two single nucleotide polymorphisms in the gene *NRAMP1*, D543N (1703G > A in exon 15 leading to an aspartate to asparagine substitution at codon 543) and INT4 (a single nucleotide change in intron 4; 469 + 14G > C), were analysed[11]. Multiplex assays were designed using Assay Design software (Sequenom Inc., San Diego, CA, USA). Genotyping was performed using the MassArray platform according to the manufacturer's protocols (Sequenom Inc.). In brief, after PCR on 2·5 ng DNA a primer extension reaction was performed to introduce mass differences between alleles and, after removing salts by adding a resin, about 15 nl of the product was spotted onto a target chip with 384 patches containing matrix. Mass differences were detected using a Bruker Autoflex matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometer (Sequenom Inc., San Diego, CA, USA) and genotypes were assigned real-time using Typer 3.1 software (Sequenom Inc.). As quality control, 10 % of samples were genotyped in duplicate and

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no inconsistencies were observed. Primer sequences are available upon request.

Genotyping using fragment length analyses

PCR for a TGTG deletion polymorphism in the 3′ untranslated region (1729 + 55del4), denoted as 3′UTR, were performed using 100 ng genomic DNA, 200 μM of each dNTP, 10 pmol of each primer, 50 mM-KCl, 10 mM-tri(hydroxymethyl)-aminomethane-HCl (pH 9·0), 0·1 % Triton X-100, 1·5 mM-$MgCl_2$ and 0·5 U Taq DNA polymerase (Biolabs, Beverly, MA, USA) in a total volume of 25 μl. Forward primers were 5′-labelled with tetrachloro-6-carboxy-fluorescein (TET); primer sequences and cycle conditions are available on request. PCR products and a 400 HD-ROX size standard (Applied Biosystems, Foster City, CA, USA) in HiDi formamide were run on an ABI Prism 3700 DNA Analyzer (Applied Biosystems), and results were analysed using GeneScan Analysis and Genotyper software (Applied Biosystems). Several homozygous alleles were sequenced to verify allele lengths.

Statistical analysis

Data from the questionnaires, physical examinations, laboratory analyses and genotypings were analysed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Data were checked for normality using the Kolmogorov–Smirnov test. Independent and paired *t* tests were used to compare means. Ferritin concentrations were transformed to natural logarithms to obtain normality. Analysis of covariance was used to compare indicators of Fe status.

The Hardy–Weinberg equilibrium of each polymorphism was checked using the program HWE[17]. The program CONTING was used to calculate χ^2 and associated values for a contingency table[17]. All statistical analyses were two-sided and *P* values $<0{\cdot}05$ were considered as statistically significant.

Results

A total number of 378 newly detected sputum-positive pulmonary TB patients (median age 29 (range 15–67) years) and 436 community healthy controls (median age 33 (range 15–70) years) were included. Clinical characteristics of all included TB patients and controls are presented in Table 1. In active TB, CRP concentrations and ESR were both elevated and correlated highly in the active TB patients at recruitment (Spearman's rank *r* 0·52; $P<0{\cdot}001$). Both CRP concentrations and ESR returned to normal levels after successful TB therapy (paired *t* test).

Table 1. Clinical characteristics of pulmonary tuberculosis (TB) patients before and after TB therapy compared with healthy community controls*

(Mean values and standard deviations)

	Pulmonary TB					
	Before therapy (*n* 378)		After therapy (*n* 153)		Controls (*n* 436)	
	Mean	SD	Mean	SD	Mean	SD
Males (*n*)	225		82		245	
Females (*n*)	153		71		191	
BMI (kg/m^2)	17·6	2·7	19·7	2·6	22·6	4·5
Inflammatory indicators						
ESR (mm/h)	84·0	32·8	18·2	16·2	17·2	13·5
CRP (mg/l)	62·6†	43·5	7·7	8·9	6·6‡	15·9
Haematological indicators						
Anaemia (*n*)§	239		5‖		30	
Anaemia (%)§	63·2		7·6‖		6·8	
Hb (g/l)¶	119	17	139‖	15	142	20
MCV (fl)	76·9	8·1	82·2‖	7·2	84·4	6·5
MCH (pg)	25·9	4·4	28·1‖	2·7	28·8	2·5
Fe status indicators						
Ferritin (μmol/l)¶	313·7‖	289·7	71·9**	90·1	97·9††	65·6
Serum Fe (μmol/l)	8·2‖	5·3	16·0**	6·5	16·8††	5·8
Fe saturation (%)	19·6‖	13·4	32·3**	15·8	30·1††	11·1
Fe-binding capacity (μmol/l)	42·8‖	10·1	52·4**	11·2	56·7††	8·6

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; MCV, mean corpuscular volume; MCH, mean corpuscular Hb.

* All values before TB therapy were significantly different compared with controls (*t* test). All values before TB therapy were also increased or decreased significantly ($P<0{\cdot}05$) at the end of the therapy (paired *t* test, tested only in individuals with two time points).

† Data were collected from 240 individuals.

‡ Data were collected from 295 individuals.

§ Criteria for anaemia: males Hb < 130, females Hb < 120 g/l.

‖ Data were collected from sixty-five individuals.

¶ Male and female values were taken together.

** Data were collected from thirty-three individuals.

†† Data were collected from seventy-six individuals.

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Anaemia in active tuberculosis and its association with clinical presentation

Active TB was a strong predictor of lower Hb concentrations. In the patients, Hb concentrations were significantly correlated to CRP concentrations ($P=0{\cdot}013$), but not to ferritin concentration ($P>0{\cdot}2$; analysis of covariance controlling for age and sex). Anaemia was found in 239 active TB patients (63·2 %) compared with only thirty controls (6·8 %). Females were more often affected by anaemia than males, both among TB patients (74·5 *v.* 55·6 %) and among controls (10·9 *v.* 3·6 %). At the end of successful TB therapy, anaemia had been corrected without any Fe therapy or dietary supplementation in almost all patients. Hb concentrations were 20 g/l higher after treatment (paired *t* test; $P<0{\cdot}001$). The increase of Hb concentrations between recruitment and end of TB treatment was associated with a decrease of the inflammatory indicators such as ESR and CRP. Also mean corpuscular volume (MCV) of erythrocytes and mean corpuscular Hb (MCH) levels increased (Table 1). Only five patients (7·6 % of sixty-five measured post-therapy) remained anaemic but their Hb concentrations increased significantly.

TB patients had coughing (98 %) as their main complaint. As TB is a chronic disease, the duration of the main complaint before TB patients presented themselves in the clinic might be of importance. Anaemia was more prevalent in TB patients with coughing for more than 1 month as compared with patients with a recent complaint (< 1 month) ($P=0{\cdot}041$; data not shown), probably reflecting long-term effects of immune activation on Hb concentrations[16]. Blood coughing was present in 46 % of the cases. Although a trend could be observed, there was no significant correlation between occurrence of anaemia and blood coughing ($P=0{\cdot}054$) (Table 2). Furthermore, anaemia was present more frequently in advanced TB (ninety-two of 118 known CXR results; 77·9 %) compared with mild or moderate TB (forty-eight of eighty-eight cases; 54·5 %) ($P<0{\cdot}001$) as assessed by CXR abnormalities (Table 2). CXR abnormalities were classified as mild or moderate TB (*n* 88) or advanced TB (*n* 118), based on the extent of lesions on CXR as described elsewhere[18]. Anaemia was also negatively associated with smoking habits. TB patients who were currently smoking or had ever smoked in the past (designated as 'ever') were surprisingly less frequently anaemic compared with those who never smoked ($P=0{\cdot}003$) (Table 2). In a more extended study in our group we found that smoking was not associated with TB (OR 0·99 (95 % CI 0·76, 1·31); data not shown)

Table 2. Anaemia status of active pulmonary tuberculosis (TB) patients in relation to clinical signs or symptoms

(Frequency and percentage frequency)

	Non-anaemics*		Anaemics*		
	n	%	*n*	%	*P*
Coughing blood					
No	66	17·6	138	36·5	0·054
Yes	73	19·3	101	26·7	
Chest X-ray†					
Mild or moderate TB	40	19·4	48	23·3	<0·001
Advanced TB	26	12·6	92	44·7	
BMI					
No wasting (≥ 18·5 kg/m^2)	62	16·4	59	15·6	<0·001‡
Mild wasting (17 to < 18·5 kg/m^2)	37	9·80	50	13·2	
Moderate wasting (16 to < 17·0 kg/m^2)	19	5·0	50	13·2	
Severe wasting (< 16·0 kg/m^2)	21	5·6	80	21·2	
Smoking					
Ever	96	25·5	128	33·9	0·003
Never	43	11·4	111	29·4	

* Criteria for anaemia: males Hb < 130, females Hb < 120 g/l.
† A chest X-ray was assessed in 206 patients.
‡ Subjects with BMI < 18·5 kg/m^2 were pooled as the wasting group and compared with the no wasting group (χ^2 test).

Anaemia also affected thirty-one control individuals, consisting of twenty-two females (10·0 %) and nine males (5·7 %). Of the female controls with low Hb (69–118 g/l), eleven individuals had a very low MCV (48·6–76·0 fl), and in the male controls with low Hb (103–128 g/l), seven individuals had a very low MCV (59·1–69·3 fl), suggesting that these individuals may have anaemia with Fe deficiency. Furthermore, nine males of the control group had a normal Hb (133–174 g/l) with a very low MCV (58·5–75·7 fl) and six females had a normal Hb (124–140 g/l) with a low MCV (60·4–74·1 fl), which could be related to thalassaemia minor. Thalassaemia (especially minor) must be considered in the differential diagnosis of normal Hb with a low MCV value.

Follow up of thirty-three tuberculosis patients: iron status indicators and anaemia

The Fe status indicators were measured in plasma for the baseline data in a random set of active TB patients before therapy (*n* 65) and after therapy (*n* 33), and compared with controls, matched for sex and age (*n* 76). Many indicators of Fe status were influenced by the inflammatory response. In patients with active TB, plasma Fe concentrations and Fe saturation were lower, whereas ferritin concentration was increased as compared with controls. As was to be expected, there was a strong correlation between these indicators of Fe status and CRP concentrations. Linear regression analysis showed a correlation between ferritin and CRP concentrations (R^2 0·19; $P<0{\cdot}001$) with an unadjusted coefficient of 2·9, meaning that for every 10 g/l increase in CRP concentration, ferritin concentration had increased by almost 30 μg/l. Moreover, at the end of TB therapy, all Fe indicators had returned to normal values with disappearance of the inflammatory response as indicated by normal concentrations of CRP and a normal ESR (Table 1). At the end of successful TB therapy, anaemia without Fe deficiency (ferritin concentrations > 12 μg/l) was found in three patients (all males, Hb range 119–129 g/l, CRP all < 5 g/l). One of those anaemic patients had a very low MCV and MCH value (Hb 128 g/l, MCV 60·2 fl, MCH 20·8 pg), which may suggest thalassaemia minor. On the other hand, Fe deficiency was observed in three female patients who were, however, not anaemic.

In the control group, three anaemic individuals (all female) showed a normal plasma ferritin level (35–130 μg/l). One of these controls had very low MCV and MCH values (Hb 118 g/l, MCV 63·4 fl, MCH 22·1 pg), again suggesting thalassaemia minor. A plasma ferritin value ≤ 12 μg/l was observed in one female control with a normal Hb value (122 g/l).

NRAMP1 gene polymorphisms and susceptibility to tuberculosis

In the Indonesian population, the INT4 polymorphism proved to be rare and was not further analysed. The genotypes of the *NRAMP1* D543N and the 3′UTR polymorphisms were in Hardy–Weinberg equilibrium in the total group of individuals as well as in the healthy controls and patients. No significant differences could be observed between healthy controls and TB patients, suggesting that *NRAMP1* polymorphisms in our population are not associated with TB susceptibility. The distribution of the alleles and genotypes of the *NRAMP1* polymorphisms in the Indonesian population is presented in Table 3. Furthermore, *NRAMP1* polymorphisms are not associated with TB severity, as evidenced by CXR, or by anaemia in active TB (data not shown).

Discussion

Fe-deficiency anaemia has been reported in many developing countries[19,20]. In these countries many chronic infectious diseases are present at high rates, including pulmonary TB. The prevalence of anaemia in our healthy control group, living in a poor and TB-endemic area, was surprisingly low (6·7 %), females being more affected than males. In the present study children aged less than 15 years and pregnant women, individuals at high risk of anaemia, were not included. Like others we observed that in developing countries Fe deficiency is becoming a less important cause of anaemia compared with infection[21,22].

It is well known that most patients with active pulmonary TB have anaemia[23–25], but the precise mechanism remains unclear. Blood loss in the sputum (haemoptysis) has been mentioned in textbooks as one of the causes. However, original studies were never performed and haemoptysis was not associated with anaemia in the present study population. Furthermore, deficiencies of Fe and other nutrients, caused by loss of appetite and fever, are associated with a low BMI[24,26,27].

Anaemia of chronic disease (ACD), also in active TB, is associated with a low serum Fe, Fe saturation and Fe-binding capacity, and with a high serum ferritin[5,28], while in uncomplicated Fe deficiency serum ferritin is always low[29]. During inflammation ferritin, being an acute-phase reactant, is increased. Hence, the presence of inflammation in ACD can be estimated by increased concentrations of acute-phase protein such as CRP or by ESR[16,24]. In our population of TB patients, anaemia was mostly due to ACD and not to Fe deficiency, as shown by the comparison of haematological and Fe parameters within the same patients before and at 6 months after TB therapy, as these subjects received no Fe treatment. In ACD the decrease of Fe in the plasma compartment and the increase of ferritin, mainly in macrophages, is due to cytokine-mediated up regulation of ferritin, and reduced Fe export due to increased hepcidin production[30]. All such modifications in Fe status may be a protective response against the invading microbes[4,31].

As the patients in the present study did not receive Fe supplementation, an increase of the Hb concentration over 6 months is mainly due to the normalisation of the inflammatory response. Alternatively, the increase of Hb concentration could be influenced by a better nutrition and a better appetite; patients gained weight as evidenced by a higher BMI after successful TB therapy[26]. Fe supplementation in developing countries, where Fe deficiency is highly prevalent, should therefore not be routinely prescribed when Fe status is unknown, as this may exacerbate infection, not only TB[32], but also malaria[33] or helminth infection[34]. In children living in malaria endemic areas, Fe supplementation appears to have beneficial effects in Fe-deficient children, but harmful effects in Fe-replete children[35]. In contrast, a study in Malawi reported that Fe supplementation in developing countries with a high prevalence of both HIV infection and Fe deficiency is not contraindicated[36]. Fe is believed to

Table 3. Distribution of *NRAMP1* alleles and genotypes*
(Frequency and percentage frequency)

Polymorphism	Allele or genotype	Frequency in cases		Frequency in controls		
		n	%	*n*	%	*P*
INT4	G	419	99·3	704	97·8	n.a.
(469+14G > C)	C	3	0·7	16	2·2	
D543N	G	334	81·5	546	78·0	0·17
(1627G > A)	A	76	18·5	154	22·0	
	GG	136	66·3	217	62·0	0·33†
	GA	62	30·2	112	32·0	
	AA	7	3·4	21	6·0	
3′UTR	TGTG +	348	81·3	568	78·2	0·21
	TGTG del	80	16·7	158	21·8	
	TGTG +/+	141	65·9	226	62·3	0·35‡
	TGTG +/del	66	30·8	116	32·0	
	TGTG del/del	7	3·3	21	5·8	

n.a., Not analysed.
* No significant differences were observed in the distribution of *NRAMP1* single nucleotide polymorphism alleles or genotypes between tuberculosis patients and healthy controls (χ^2 tests).
† Genotype GA and AA combined for analysis.
‡ Genotype TGTG +/del and TGTG del/del combined for analysis.

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enhance the activity of the pyrazionic acid-containing first-line TB drug pyrazinamide[37]. The conclusion that some patients with pulmonary TB and mild to moderate anaemia may benefit from Fe supplementation[22] has no practical implication since Fe treatment of TB patients only caused a minimal increase of Hb after the first month, which disappeared after the second month of treatment. In contrast, in Fe-overload patients, Fe chelators may become important drugs for treatment of malaria, TB and HIV, to deprive micro-organisms from Fe as an essential nutrient[4,38]. Fe chelators added to TB therapy should only be given to known overload patients as it may impair the host defence, and reliable investigations are not available[39].

The only effective treatment for ACD is, thus, to cure the underlying disease. Individuals in the present study with a very low MCV and only mild anaemia may have had thalassaemia minor. Haemoglobinopathies, present in 0·1–10 % of individuals of various ethnic groups in Indonesia[40,41], may be underdiagnosed[42]. The risk of these individuals developing TB, however, needs to be further investigated.

Macrophages have several strategies to acquire Fe from their specific environment, including erythrophagocytosis and uptake of transferrin-bound Fe, non-transferrin-bound Fe, haem and Hb. Fe is needed for both host defence and survival of the pathogen[4]. There is a constant competition between host and microbes for this essential but toxic element. It remains unclear which role NRAMP1 plays in the pathogenesis of TB, being a metal transporter that resides in the phagosome membrane[14]. Atkinson & Barton[43] showed that NRAMP1 has a function in Fe efflux from phagosome to cytosol[43], while others found that it promotes the influx of Fe into the phagosome[44]. In a recently published meta-analysis of the influence on TB susceptibility of the four most frequently studied *NRAMP1* polymorphisms[45] including INT4, D543N and the 3′UTR, it was shown that a large difference between populations can be observed. In Europeans none of the polymorphisms were associated with TB[13]. In Africans three of the four polymorphisms (not the 3′UTR variant) were associated with TB[11] and in Asians also three out of four polymorphisms (not the INT4 variant) were associated with TB[46,47]. A striking difference between the present study and the other studies in Asian populations is that the allele frequencies of the polymorphisms are very different. We find the C allele of the INT4 polymorphism in only 2 % of our controls while this is found in 14 % of Japanese and 21 % of Chinese controls. We find the 3′UTR deletion allele in 22 % of our controls while this is found in 8–19 % in five other Asian populations. Similarly we find the A allele of the D543N polymorphism in 22 % of our controls while this is found in 2–15 % of six other Asian populations. It appears that not only the association of TB with *NRAMP1* polymorphisms is different between continents but also the distribution of alleles is also very different between Asian populations, which may reflect a difference in selective pressure in the past. If the *NRAMP1* polymorphisms studied here are functional polymorphisms influencing TB susceptibility directly, we should have observed a similar association. If these polymorphisms are, however, merely in linkage disequilibrium with a functional variant elsewhere in *NRAMP1* (or a neighbouring gene) the association between certain alleles and TB susceptibility can vary greatly between populations as has been shown by Li *et al.*[45]. Further studies into other variations in *NRAMP1* or in other genes involved in susceptibility to TB are needed.

To conclude, the present results support earlier observations that anaemia in active TB is mainly the result of ACD, more than of Fe deficiency. As the Hb concentration increased after successful TB therapy, Fe supplementation was not necessary. Fe supplementation in developing countries should be restricted to children and women of reproductive age, who have the highest prevalence of Fe deficiency.

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