

# HIV Infection Disrupts the Sympatric Host–Pathogen Relationship in Human Tuberculosis

Lukas Fenner<sup>1</sup>, Matthias Egger<sup>1</sup>, Thomas Bodmer<sup>2</sup>, Hansjakob Furrer<sup>3</sup>, Marie Ballif<sup>1</sup>, Manuel Battegay<sup>4</sup>, Peter Helbling<sup>5</sup>, Jan Fehr<sup>6,7</sup>, Thomas Gsponer<sup>1</sup>, Hans L. Rieder<sup>8,9</sup>, Marcel Zwahlen<sup>1</sup>, Matthias Hoffmann<sup>10</sup>, Enos Bernasconi<sup>11</sup>, Matthias Cavassini<sup>12</sup>, Alexandra Calmy<sup>13</sup>, Marisa Dolina<sup>14</sup>, Reno Frei<sup>15</sup>, Jean-Paul Janssens<sup>16</sup>, Sonia Borrell<sup>17,18</sup>, David Stucki<sup>17,18</sup>, Jacques Schrenzel<sup>19</sup>, Erik C. Böttger<sup>20</sup>, Sebastien Gagneux<sup>17,18\*</sup>, for the Swiss HIV Cohort and Molecular Epidemiology of Tuberculosis Study Groups<sup>¶</sup>

**1** Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland, **2** Mycobacteriology Unit, Institute for Infectious Diseases, University of Bern, Bern, Switzerland, **3** Department of Infectious Diseases, Bern University Hospital and University of Bern, Bern, Switzerland, **4** Division of Infectious Diseases and Hospital Epidemiology, University Hospital of Basel, Basel, Switzerland, **5** Division of Communicable Diseases, Federal Office of Public Health, Bern, Switzerland, **6** Division of Infectious Diseases, University Hospital Zurich, Zurich, Switzerland, **7** University of Zurich, Zurich, Switzerland, **8** Institute of Social and Preventive Medicine, University of Zurich, Zurich, Switzerland, **9** The Union, Paris, France, **10** Division of Infectious Diseases, Kantonsspital St. Gallen, St. Gallen, Switzerland, **11** Division of Infectious Diseases, Ospedale Regionale Lugano, Lugano, Switzerland, **12** Division of Infectious Diseases, University Hospital Lausanne, Lausanne, Switzerland, **13** Division of Infectious Diseases, University Hospital Geneva, Geneva, Switzerland, **14** Cantonal Institute of Microbiology, Bellinzona, Switzerland, **15** Department of Clinical Microbiology, University Hospital of Basel, Basel, Switzerland, **16** Division of Pneumology, University Hospital Geneva, Geneva, Switzerland, **17** Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Basel, Switzerland, **18** University of Basel, Basel, Switzerland, **19** Laboratory of Bacteriology, University Hospital of Geneva, Geneva, Switzerland, **20** Institute of Medical Microbiology, National Center for Mycobacteria, University of Zurich, Zurich, Switzerland

## Abstract

The phylogeographic population structure of *Mycobacterium tuberculosis* suggests local adaptation to sympatric human populations. We hypothesized that HIV infection, which induces immunodeficiency, will alter the sympatric relationship between *M. tuberculosis* and its human host. To test this hypothesis, we performed a nine-year nation-wide molecular-epidemiological study of HIV-infected and HIV-negative patients with tuberculosis (TB) between 2000 and 2008 in Switzerland. We analyzed 518 TB patients of whom 112 (21.6%) were HIV-infected and 233 (45.0%) were born in Europe. We found that among European-born TB patients, recent transmission was more likely to occur in sympatric compared to allopatric host–pathogen combinations (adjusted odds ratio [OR] 7.5, 95% confidence interval [95% CI] 1.21–infinity,  $p = 0.03$ ). HIV infection was significantly associated with TB caused by an allopatric (as opposed to sympatric) *M. tuberculosis* lineage (OR 7.0, 95% CI 2.5–19.1,  $p < 0.0001$ ). This association remained when adjusting for frequent travelling, contact with foreigners, age, sex, and country of birth (adjusted OR 5.6, 95% CI 1.5–20.8,  $p = 0.01$ ). Moreover, it became stronger with greater immunosuppression as defined by CD4 T-cell depletion and was not the result of increased social mixing in HIV-infected patients. Our observation was replicated in a second independent panel of 440 *M. tuberculosis* strains collected during a population-based study in the Canton of Bern between 1991 and 2011. In summary, these findings support a model for TB in which the stable relationship between the human host and its locally adapted *M. tuberculosis* is disrupted by HIV infection.

**Citation:** Fenner L, Egger M, Bodmer T, Furrer H, Ballif M, et al. (2013) HIV Infection Disrupts the Sympatric Host–Pathogen Relationship in Human Tuberculosis. *PLoS Genet* 9(3): e1003318. doi:10.1371/journal.pgen.1003318

**Editor:** Greg Gibson, Georgia Institute of Technology, United States of America

**Received:** November 13, 2012; **Accepted:** December 6, 2012; **Published:** March 7, 2013

**Copyright:** © 2013 Fenner et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** This work was supported by the Swiss National Science Foundation (grant number 324730-12544), the Swiss HIV Cohort Study (grant number 588), and the Federal Office of Public Health (grant number 09.007368 and the National Center for Mycobacteria). L Fenner and M Egger are supported by the National Institute of Allergy and Infectious Diseases (leDEA Southern Africa, grant number 5U01-AI069924-5) and S Gagneux by the National Institutes of Health (grant number R01-AI090928-01 and HHSN266200700022C) and the Swiss National Science Foundation (grant number PP0033-119205). EC Böttger was supported by the University of Zurich, the European Community (PAR, FP7-HEALTH-2009-241476) and the Federal Office of Public Health (National Center for Mycobacteria). The Swiss HIV Cohort Study is supported by the Swiss National Science Foundation (grant no. 33CS30-134277). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

\* E-mail: sebastien.gagneux@unibas.ch

¶ Memberships of the Swiss HIV Cohort and Molecular Epidemiology of Tuberculosis Study Groups are provided in the Acknowledgments.

## Introduction

Host–pathogen co-evolution plays an important role in the biology of infectious diseases [1]. Coevolution between interacting host and pathogen species is difficult to demonstrate formally, but

indirect evidence can be obtained by studying geographical patterns, which can indicate local adaptation of particular pathogen variants to geographically matched host variants [2–4]. Local adaptation is often studied using so-called reciprocal transplant experiments, in which the fitness of locally adapted

## Author Summary

Human tuberculosis (TB) caused by *Mycobacterium tuberculosis* kills 1.5 million people each year. *M. tuberculosis* has been affecting humans for millennia, suggesting that different strain lineages may be adapted to specific human populations. The combination of a particular strain lineage and its corresponding patient population can be classified as sympatric (e.g. Euro-American lineage in Europeans) or allopatric (e.g. East-Asian lineage in Europeans). We hypothesized that infection with the human immunodeficiency virus (HIV), which impairs the human immune system, will interfere with this host-pathogen relationship. We performed a nation-wide molecular-epidemiological study of HIV-infected and HIV-negative TB patients between 2000 and 2008 in Switzerland. We found that HIV infection was associated with the less adapted allopatric lineages among patients born in Europe, and this was not explained by social or other patient factors such as increased social mixing in HIV-infected individuals. Strikingly, the association between HIV infection and less adapted *M. tuberculosis* lineages was stronger in patients with more pronounced immunodeficiency. Our observation was replicated in a second independent panel of *M. tuberculosis* strains collected during a population-based study in the Canton of Bern. In summary, our study provides evidence that the sympatric host-pathogen relationship in TB is disrupted by HIV infection.

(sympatric) pathogen variants is compared to the performance of allopatric pathogen variants [2]. Studies in several invertebrate systems have shown that sympatric pathogens (infection with a phylogeographically concordant strain) tend to outperform allopatric pathogens (infection with a phylogeographically discordant strain) in the corresponding host variants [1,5–7].

*Mycobacterium tuberculosis*, the agent causing human tuberculosis (TB) is an obligate human pathogen, which has been affecting humankind for millennia [8–13]. Contrary to previous beliefs linking the origin of TB to animal domestication ~10,000 years ago [14], more recent data suggest that *M. tuberculosis* evolved as a human pathogen in Africa, and might have co-existed with anatomically modern humans since their origins ~200,000 years ago [8,10,12,13,15]. Analyses of multiple global strain collections have shown that *M. tuberculosis* exhibits a phylogeographic population structure consisting of six main phylogenetic lineages associated with different geographic regions and sympatric human populations [9,11–13,16–20]. Studies in San Francisco, London, and Montreal have shown that these sympatric host-pathogen associations persist in cosmopolitan settings, even under a presumed degree of host and pathogen intermingling [11,18,19]. Moreover, transmission of *M. tuberculosis* has been shown to occur more frequently in sympatric host-pathogen combinations compared to allopatric host-pathogen combinations [9]. Taken together, these observations are consistent with the notion that the different phylogeographic lineages of *M. tuberculosis* have adapted to specific sympatric human populations [21].

Based on the assumption that *M. tuberculosis* has been coevolving with humans, and that *M. tuberculosis* has locally adapted to sympatric human populations [9], we hypothesized that HIV co-infection will alter this relationship [22]. Specifically, we postulated that because HIV induces immune suppression in humans, and because variation in host immunity will likely play a role in local adaptation, *M. tuberculosis* strains will cause disease in HIV-infected patients, irrespective of their usual sympatric host-pathogen relationship. To test this hypothesis, we performed a

population-based molecular-epidemiological study of HIV-infected and HIV-negative TB patients in Switzerland between 2000 and 2008, a country with a long history of immigration [23].

## Results

### Patient characteristics and phylogeographic distribution of *M. tuberculosis* lineages

A total of 518 patients were included in the study, of whom 112 (21.6%) were HIV-infected. Of these 518 patients, 233 (45.0%) were born in Europe (117 in Switzerland), 131 (25.3%) were born in sub-Saharan Africa, 48 (9.3%) in South-East Asia, 36 (7.0%) in the Indian subcontinent, and 24 (4.6%) in Central and South America. Similar to previous studies [9,18,19], we found an association between the patient's place of birth and the particular *M. tuberculosis* lineages (Figure 1). Lineage 4 (Euro-American lineage) was present in all regions, but particularly common in patients born in Europe and South America. Lineages 5 and 6 (West-African lineages also known as *M. africanum* [24]) were exclusively found in patients originating from West Africa, whereas Lineage 2 (which includes Beijing) and Lineage 1 were mainly seen in patients originating from the Western Pacific and East Asian regions. Patient characteristics are summarized in Table 1.

Because in European-born patients the host-pathogen combinations defined as sympatric (i.e. Lineage 4/Euro-American lineage in European-born patients) or allopatric (i.e. all other lineages in European-born patients) have been well established [9,18,19,25], we focused on this patient group (n = 233) for the remaining of our analyses.

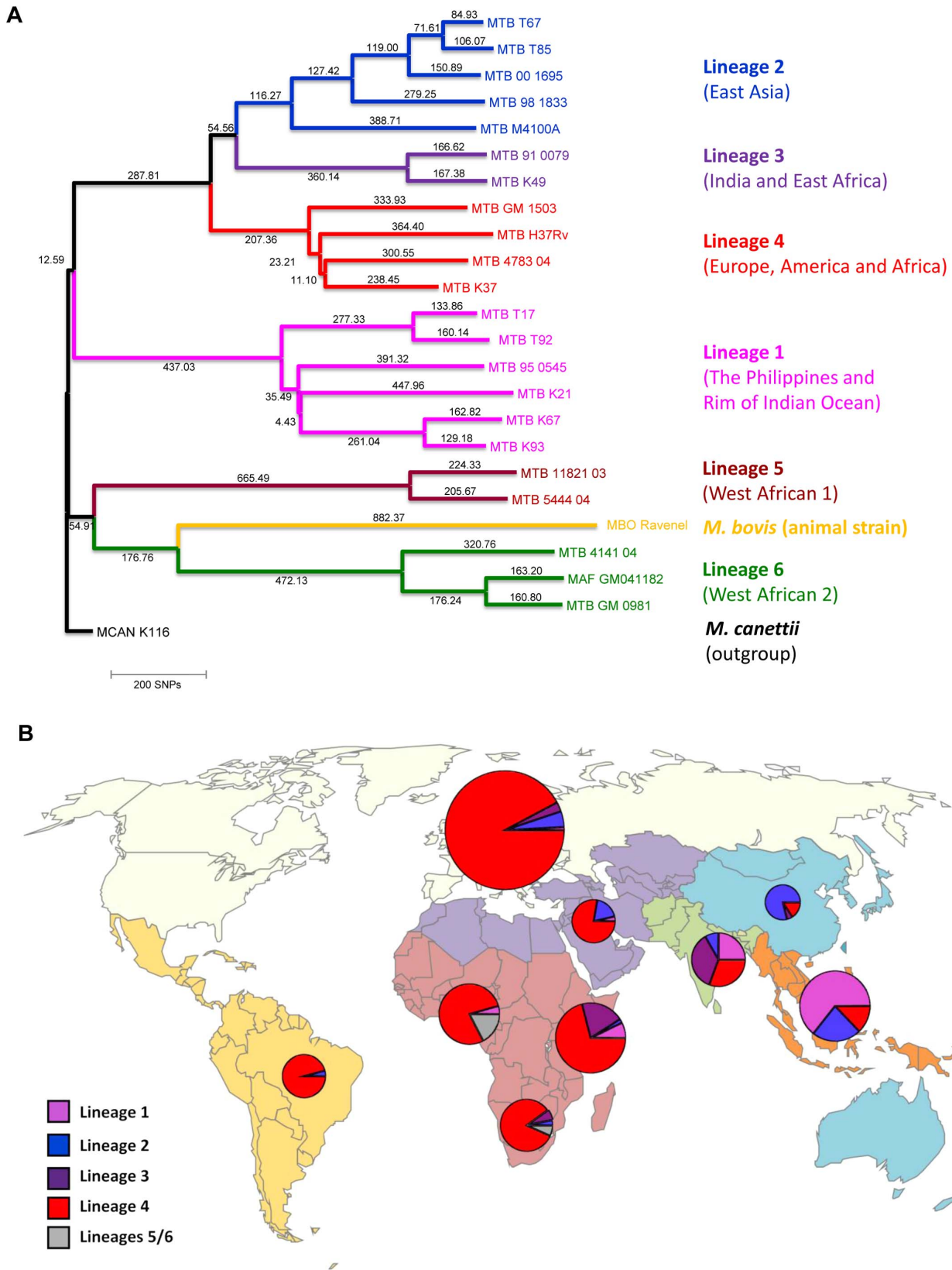
### *M. tuberculosis* transmission occurs primarily in sympatric host-pathogen combinations

*M. tuberculosis* transmission was more likely among patients in a sympatric host-pathogen relationship compared to patients in an allopatric host-pathogen relationship (adjusted odds ratio [OR] 7.5, 95% confidence interval [95% CI] 1.2-infinity, p = 0.03, Table 2). Of note, there was no molecular clustering among European-born TB patients infected with an allopatric *M. tuberculosis* strain. Moreover, we found that only the sympatric Lineage 4 (Euro-American lineage) was detected in European-born clusters as well as in mixed clusters (Table S1), suggesting that sympatric host-pathogen combinations in TB favor transmission.

### Impact of HIV infection on the sympatric host-pathogen combination of *M. tuberculosis* among Europeans

Overall, we found that HIV infection was strongly associated with allopatric *M. tuberculosis* lineages among European-born TB patients (unadjusted OR 7.0, 95% CI 2.5–19.1, p < 0.0001; Table 3). Among the allopatric lineages, we found that Lineages 1, 2 and 3 were more likely to be found in HIV-infected compared to HIV-negative patients when taking the sympatric Lineage 4 (Euro-American lineage) as the reference (Table S2). When investigating the ancestry of the nine HIV-infected patients with an allopatric *M. tuberculosis* strain, seven patients were confirmed to be of Swiss ancestry over the last three generations, and two patients had Swiss and Italian ancestors in the previous generations (Italian father in the previous generation, or emigrating from Italy in the previous generation).

Several factors could contribute to the association between HIV infection and allopatric lineages. We found that patients with an allopatric *M. tuberculosis* lineage were younger (median age 41.5 versus 50 years), and had more often a history of frequent



**Figure 1. Phylogeography of the six main *Mycobacterium tuberculosis* lineages.** A: Phylogenetic tree of the main *M. tuberculosis* lineages described in our study based on the neighbor-joining phylogeny across 23 *M. tuberculosis* complex whole-genome sequences (from Ref. [72]). Numbers on branches refer to the corresponding number of single nucleotide polymorphisms inferred. B: Distribution of the main phylogenetic *M. tuberculosis* lineages among Swiss tuberculosis cases included in the study (n=518), by geographic origin of the patients. In (A) *Mycobacterium canettii* was used as the outgroup. SNPs, single nucleotide polymorphisms. In (B) the sizes of the pie charts correspond to the number of patients

included in the study: European region (233 patients), Middle-East/North Africa (27), Indian subcontinent (36), Western Pacific (19), Central/South America (24), South-East Asia (48), and Eastern (55), Western (46) and Southern region (30) of sub-Saharan Africa. Lineage 1: Indo-Oceanic lineage; Lineage 2: East-Asian lineage (includes Beijing strains); Lineage 3: Delhi/CAS; Lineage 4: Euro-American lineage; Lineages 5 and 6: West African lineages.

doi:10.1371/journal.pgen.1003318.g001

travelling (38.9% versus 4.2%,  $p < 0.0001$ ). Therefore, we developed a model (Figure 2) to take these and other putative confounding variables into account. These variables included age, sex, country of birth, frequent travelling, contact with the foreign-born population, and non-HIV associated immunosuppression. We considered “TB with an allopatric strain” as the outcome because disease is the only measurable outcome with a sympatric or allopatric *M. tuberculosis* strain (only diseased individuals can yield a positive mycobacterial culture). Our multivariate analyses revealed that the association between HIV infection and allopatry remained statistically significant after adjustment for all social and patient factors included in our model (OR 5.5, 95% CI 1.5–20.6,  $p = 0.01$ , Table 3). Age, sex, being Swiss-born, and non-HIV associated immunosuppression had only a minor effect on the association between HIV infection and TB with an allopatric strain (Table 3). In contrast, a history of repeated travelling to low-income countries had a stronger effect, decreasing the OR to 4.50 (95% CI 1.5–13.6,  $p = 0.008$ , Table 3) when adjusting for this variable.

### Impact of HIV infection on the sympatric host-pathogen association is a function of the degree of HIV-induced immunosuppression

We also tested if the degree of immunodeficiency as measured by the nadir CD4 T cell count (defined as the lowest CD4 T cell

count ever measured in a patient) would have an impact on the association between host population and *M. tuberculosis* lineage. Among Europeans, the strength of the association between HIV infection and allopatric lineages increased with a decreasing nadir CD4 T cell count in a dose-dependent manner: from an OR of 4.6 (95% CI 0.9–24.7) in patients with a nadir CD4 T cell count of  $>200$  cells/ $\mu\text{L}$  to an OR of 12.5 (95% CI 2.6–60.8) in patients with nadir CD4 T cell counts  $<50$  cells/ $\mu\text{L}$  (test for trend  $p < 0.0001$ ; HIV-negative patients as reference). This trend remained statistically significant when adjusting for age, sex, being born in Switzerland, frequent travelling, contact with the foreign-born population, and non-HIV associated immunosuppression (Table 4).

### Impact of social mixing on the sympatric host-pathogen association

Increased contact with foreigners originating from high TB burdened countries, who have a higher risk of TB [26] and are more likely to have TB caused by an allopatric *M. tuberculosis* strain, could also lead to an allopatric host-pathogen relationship in European-born patients. However, the association between HIV and allopatry remained statistically significant when adjusting for this variable (Table 3). Furthermore, we examined molecular clusters defined by standard bacterial genotyping [27,28], to test

**Table 1.** Patient characteristics of tuberculosis (TB) patients born in Europe, by presence of allopatric and sympatric *Mycobacterium tuberculosis* strains.

Characteristic	All (n = 233)	Allopatric (n = 18)	Sympatric (n = 215)	P value
Age, median (IQR), years	49 (36–71)	41.5 (32–45)	50 (37–73)	0.0029
Male sex, n (%)	126 (54.1)	10 (55.6)	116 (54.0)	0.90
Origin of birth, n (%)				0.46
Switzerland	118 (50.6)	11 (61.1)	107 (49.9)	
Europe (without Switzerland)	115 (49.4)	7 (38.9)	108 (50.2)	
Cavitary disease, n (%)	55 (23.6)	4 (22.2)	51 (23.7)	0.99 <sup>1</sup>
Clinical manifestation, n (%)				0.54 <sup>1</sup>
Pulmonary	191 (82.0)	16 (88.9)	175 (81.4)	
Extrapulmonary	42 (18.0)	2 (11.1)	40 (18.6)	
Recent TB within families/social surroundings	15 (6.4)	0 (0)	15 (7.0)	0.61 <sup>1</sup>
Frequent travelling	16 (6.9)	7 (38.9)	9 (4.2)	$<0.0001$ <sup>1</sup>
HIV infection, n (%)	36 (15.5)	9 (50.0)	27 (12.6)	$<0.0001$ <sup>1</sup>
Immunosuppression other than HIV infection <sup>2</sup> , n (%)	24 (10.3)	1 (5.6)	23 (10.7)	0.70
Most likely source of HIV infection <sup>3</sup> , n (%)				0.94 <sup>1</sup>
Heterosexual	15 (41.7)	5 (55.6)	10 (37.0)	
Injecting drug user	9 (25.0)	2 (22.2)	7 (25.9)	
Men having sex with men	7 (19.4)	2 (22.2)	5 (18.5)	
Others/unknown	5 (13.9)	0 (0)	5 (18.5)	

<sup>1</sup>Fisher's exact test.

<sup>2</sup>Use of TNF-alpha inhibitors, malignancy, organ transplantation, use of steroids, or methotrexate.

<sup>3</sup>Among HIV-infected patients (n = 36).

95% CI, 95% confidence interval; IQR, interquartile range.

doi:10.1371/journal.pgen.1003318.t001

**Table 2.** Recent transmission of *Mycobacterium tuberculosis* among tuberculosis (TB) cases born in the European region, according to sympatric and allopatric lineages.

Lineages	n (%) cases		Association of transmission with lineages				
	Clustered	Unclassified	P value <sup>1</sup>	OR (95% CI)	P value	Adjusted OR (95% CI)	P value
			0.024		0.048		0.027
Sympatric	42 (19.5)	173 (80.5)		6.09 (1.01–∞)		7.48 (1.21–∞)	
Allopatric	0 (0)	18 (100)		1 (Ref)	-	1 (Ref)	-

Recent transmission was determined by spoligotyping and MIRU-VNTR genotyping which is based on repetitive sequences. Clustered cases were defined as cases belonging to a molecular cluster of TB transmission based on isolates showing an identical genotyping pattern, and unclustered as cases with a unique genotyping pattern. Sympatric was defined as a strain belonging to Lineage 4 (Euro-American lineage), allopatric as a strain belonging to a lineage other than Lineage 4.

<sup>1</sup>Fisher's exact test (1-sided).

95% CI, 95% confidence interval; ND, not defined; OR, Odds Ratio.

Odds ratios were derived from exact logistic models. Model was adjusted for age group (45 years and younger), being born in Switzerland and recent TB in families or social surroundings.

doi:10.1371/journal.pgen.1003318.t002

the hypothesis that HIV-infected patients were more frequently seen among ethnically mixed clusters where transmission occurred between non-European and European-born cases [29]. We found that the prevalence of HIV infection was similar among TB cases in mixed clusters (5 HIV-infected cases out of 26 cases, 19.2%) and among cases in clusters involving only European-born cases (4 out of 26 cases, 15.4%, see Table S1).

### Sensitivity analyses

When restricting the main analysis (n = 233) to European-born patients without a history of frequent travelling, we found that the association between HIV infection and allopatric TB remained statistically significant (adjusted OR 6.96, 95% CI 1.25–38.88, p = 0.027). Furthermore, we explored associations of socio-demographic and clinical factors with TB with an allopatric *M. tuberculosis* strain in a model focusing on HIV-infected European patients only (Figure S1, Table S3): frequent travelling was confirmed to be an important factor, and patients with a low nadir CD4 T cell count tended to be associated with an allopatric TB although the associations did not reach statistical significance (Table S3). Finally, we obtained very similar results for the association between HIV infection and allopatric TB (Table S4), and for the association between the degree of immunodeficiency and allopatric TB (Table S5) when repeating

analyses using a Bayesian approach [30], which is more robust when numbers are small.

### Other supporting information

The birth origin of HIV-infected and non-infected patients is shown on a map in Figure S2. The main phylogenetic *M. tuberculosis* lineages stratified by place of birth and HIV status are presented in Table S6.

### Replication in a second panel of *M. tuberculosis* strains

To replicate our main finding, we investigated a second panel of *M. tuberculosis* strains from an ongoing population-based TB study in the Canton of Bern, Switzerland, between 1991 and 2011. Of the 1,642 *M. tuberculosis* isolates analyzed, 1,350 (82.2%) belonged to Lineage 4 (Euro-American lineage), and 292 (17.8%) to non-Euro-American lineages (Lineages 1, 2, 3, 5 or 6). We compared all 40 European-born patients with an allopatric strain (non-Lineage 4) with 400 randomly selected European-born patients with a sympatric strain (Lineage 4). We found that the proportion of HIV infection was 4.5 (95% CI 1.6–11.9) times higher in patients with an allopatric strain compared to patients with a sympatric strain (12.5% versus 2.8%, p = 0.010, Table 5).

**Table 3.** Unadjusted and adjusted associations between HIV infection and tuberculosis (TB) with an allopatric *Mycobacterium tuberculosis* strain among European patients (n = 233), in the context of other potential factors influencing the risk for an allopatric TB.

Variables adjusted for <sup>1</sup>	OR	(95% CI)	P value
Unadjusted	6.96	(2.54–19.08)	<0.0001
Age, sex, Swiss-born	7.54	(2.32–24.55)	0.0010
Frequent travelling	4.50	(1.49–13.61)	0.0080
Age, sex, Swiss-Born, frequent travelling, contact with foreign-born population	5.57	(1.49–20.81)	0.011
Immunosuppression <sup>2</sup>	7.06	(2.57–19.42)	<0.0001
Age, sex, Swiss-born, frequent travelling, contact with foreign-born population, immunosuppression <sup>2</sup>	5.51	(1.47–20.61)	0.011

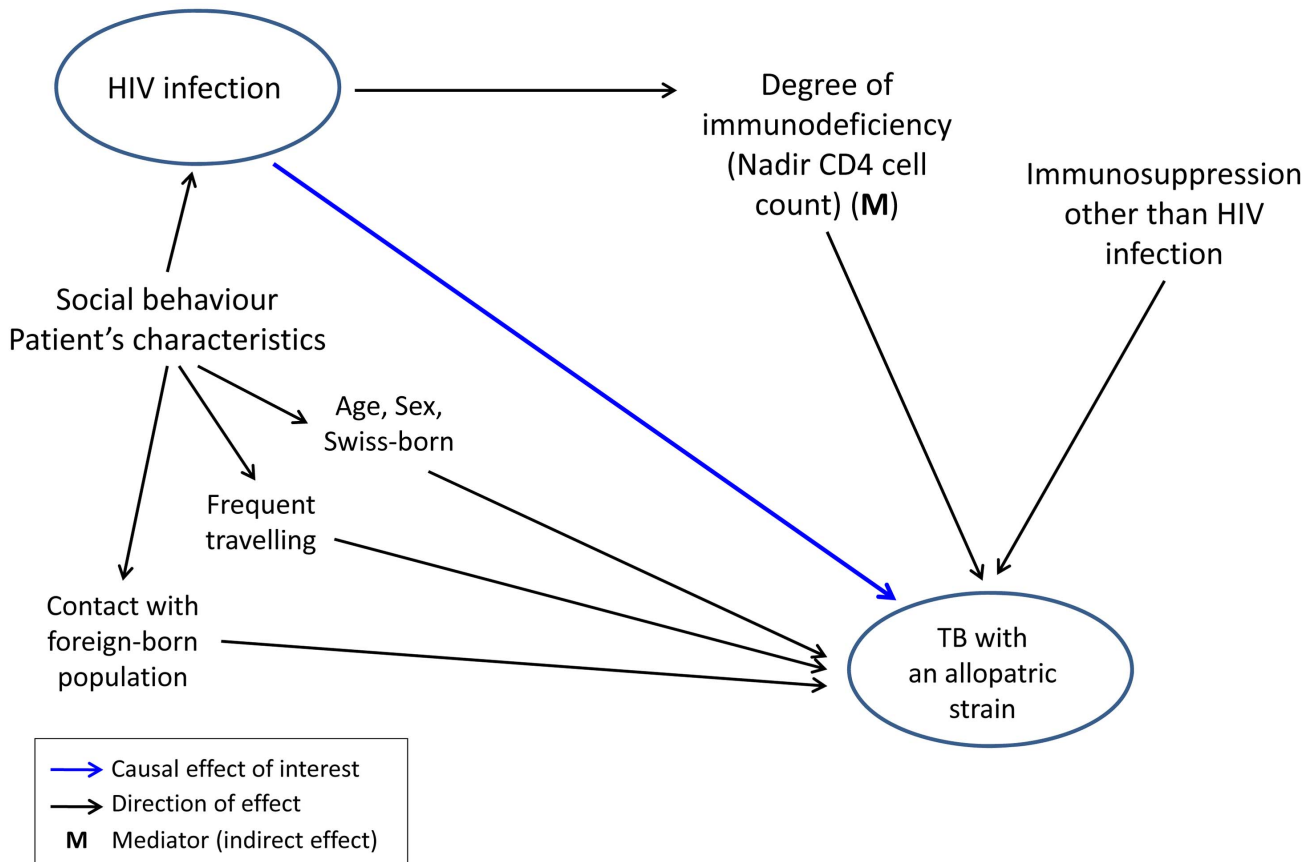
HIV-negative TB patients were used as the reference group.

<sup>1</sup>See Figure 2 for a graphical overview of associations.

<sup>2</sup>Immunosuppression other than HIV infection (use of anti-TNF blockers, malignancy, organ transplantation, use of steroids or methotrexate).

OR, odds ratio; 95% CI, 95% confidence interval.

doi:10.1371/journal.pgen.1003318.t003



**Figure 2. Graphical model showing direct and indirect potential effects of HIV infection on tuberculosis (TB) caused by an allopatric *Mycobacterium tuberculosis* strain, in the context of other potential factors influencing this association.**  
doi:10.1371/journal.pgen.1003318.g002

**Discussion**

The phylogeographic distribution of *M. tuberculosis* lineages observed here suggests local adaptation to sympatric human populations. In contrast, we found that allopatric host-pathogen relationships in European-born TB patients were strongly associated with HIV co-infection. The association with HIV infection became stronger in a ‘dose-dependent’ manner in patients with a history of more pronounced immunodeficiency, and was not explained only by frequent travelling to high TB-incidence

countries or increased social mixing with the foreign-born population. The association of *M. tuberculosis* lineages with sympatric patient populations reported here is in agreement with previous findings [9,11–13,16–19]. Similarly, our finding that recent TB transmission was more likely to occur in sympatric compared to allopatric host-pathogen combinations supports previous work [9]. Taken together, these data are consistent with local adaptation of *M. tuberculosis* to different human populations, which in turn can be viewed as indirect evidence for coevolution between *M. tuberculosis* and its human host [1–4,9–13].

**Table 4. Association between the degree of immunodeficiency and tuberculosis with an allopatric *Mycobacterium tuberculosis* strain among European patients (n = 233).**

Degree of immunodeficiency	n	Unadjusted			Adjusted		
		OR	95% OR	P value	OR	95% OR	P value
Nadir CD4 T cell count (CD4 cells/μl)							
HIV-negative	197	1.0	(ref)	<0.0001	1.0	(ref)	0.0050
≥200	11	4.64	(0.87–24.70)		2.56	(0.37–17.65)	
50–199	17	6.43	(1.74–23.70)		6.96	(1.18–41.11)	
<50	8	12.53	(2.58–60.84)		13.0	(1.54–109.75)	

Model was adjusted for age, sex, Swiss-born, frequent travelling, contact with foreign-born population, and immunosuppression other than HIV infection (see Figure 2 for a graphical overview).

P values of linear tests for trend are shown.

doi:10.1371/journal.pgen.1003318.t004

**Table 5.** HIV status in tuberculosis (TB) patients with an allopatric compared to patients with a sympatric *Mycobacterium tuberculosis* strain among European patients in a second panel.

HIV status	TB cases, n (%)			P value
	All (n = 440)	Allopatric (n = 40)	Sympatric (n = 400)	
				0.010 <sup>1</sup>
HIV-infected	16 (3.6)	5 (12.5)	11 (2.7)	
HIV-negative	424 (96.4)	35 (87.5)	389 (97.3)	
Prevalence ratio (95% CI)		4.55 (1.66–12.43)		

Patient isolates were obtained from a population-based TB study (n = 1,642) in the Canton of Bern, Switzerland, diagnosed between 1991 and 2011.

Sympatric was defined as a strain belonging to Lineage 4 (Euro-American lineage), allopatric as a strain belonging to a lineage other than Lineage 4.

<sup>1</sup>Fisher's exact test.

95% CI, 95% confidence interval.

doi:10.1371/journal.pgen.1003318.t005

We found that TB allopatric host-pathogen combinations were strongly associated with HIV infection in a nation-wide study and a second panel of strains from one Canton of Switzerland. This supports the notion that *M. tuberculosis* lineages have evolved subtle differences in their interaction with different human immune systems. However, in the presence of HIV-induced immunodeficiency, any *M. tuberculosis* lineage seems to cause disease in a given human host. *M. tuberculosis* is an obligate human pathogen which lives in constant interaction with the host immune system [31]. Human populations, however, are known to differ genetically and immunologically [15]. The clinical disease reflects host-dependent immune-pathological processes [31]. In other words, while initially triggered by the pathogen, it is the host immune response which is ultimately responsible for the chronic inflammation and associated tissue destructions. These processes contribute to the successful transmission of *M. tuberculosis* [22,32]. On the other hand, only 5–10% of the 2 billion individuals estimated to be latently infected with *M. tuberculosis* globally will develop active TB during their lifetime [33–35]. Hence most of the time, humans are able to control the infection. In our study, we chose culture-confirmed TB cases as the main endpoint which reflects successful transmission and progression from infection to active disease.

Our study on the association between allopatric TB and HIV was able to control for important cofactors [36,37]. These cofactors included frequent travelling abroad and increased contact to foreign-born populations. A particularly important cofactor for allopatric TB was frequent travelling to high TB burden countries with potential exposure to foreign *M. tuberculosis* strains; HIV-infected individuals may be at a higher risk for travel-related infectious diseases [38]. However, the association between HIV infection and allopatric TB remained even when adjusting for these behavioral and other patient characteristics. A previous study reporting on allopatric TB and HIV was not able to control for these factors [9]. Furthermore, we found no evidence for increased social mixing among HIV-infected individuals, which argues against mere social factors leading to the association between allopatric TB and HIV.

A biological basis for this association is further supported by the striking dose-dependency we observed with increasing immunosuppression as defined by lower nadir CD4 T cell counts. Of note, this trend was also independent of other variables. Low nadir CD4

T cell counts are associated with incomplete immune recovery after starting combination antiretroviral therapy [39,40] and impaired functional immune restoration despite normalization of CD4 T cells [41]. More generally, infection with HIV and *M. tuberculosis* interferes with the immune system in many ways [42,43]. HIV infection disrupts the function of *M. tuberculosis*-infected macrophages [44,45], but also seems to reduce the number and functionality of *M. tuberculosis*-specific T cells over time [46]. On the other hand, *M. tuberculosis* strains have been shown to induce variable immune responses [47]. Based on these observations, it is reasonable to hypothesize that HIV/TB co-infection might impact immune cell functions, intracellular signaling and immune regulation, perhaps leading to an immune response less capable of discriminating between *M. tuberculosis* variants.

Besides *M. tuberculosis*, several other human pathogenic bacteria exhibit phylogeographic population structures, possibly reflecting local adaptation to different human populations. These include *Haemophilus influenzae* [48], *Streptococcus mutans* [49], *M. leprae* [50] and *Helicobacter pylori* [51,52]. Interestingly, like *M. tuberculosis*, all of these microbes are obligate human pathogens. In the case of *H. pylori*, functional studies have shown that strains associated with South America have adapted their adhesins to the human blood group O, which is particularly frequent in native populations of this region [53]. Similarly, a study of the bacterial genome evolution of an asymptomatic *Escherichia coli* bacteriuria strain showed adaptation at the genomic level in distinct human hosts [54]. No similar experimental work has yet been carried out in TB. However, several studies have reported associations between human genetic polymorphisms and particular *M. tuberculosis* lineages [55–59], indicating possible interaction between human and *M. tuberculosis* variation. Whether such variation in pathogen and host genetics can be attributed to co-evolution will be difficult to demonstrate conclusively, but the data presented here support this possibility.

The strength of our study was that we used a nation-wide sample to specifically look at the impact of HIV infection on the host-pathogen relationship in human TB. Yet, our study is limited by the relatively small sample size, and the difficulty to quantify the complex social context through which the host-pathogen relationship is influenced in human TB. In addition, we looked at European-born patients only, because sympatric and allopatric host-pathogen combinations are more easily defined for this patient population [9,18,19,25]. Additional studies in large cosmopolitan cities of Asia and Africa would be required to test whether the association between allopatric TB and HIV holds true in these settings. Ultimately, detailed experimental work is needed to establish the biological basis of the host-pathogen association in human TB.

In conclusion, our data suggest that the phylogeographical host-pathogen relationship in TB influences transmission patterns. Among the studied European-born TB patients, we showed that HIV infection disrupts the sympatric host-pathogen relationship in human TB, and that this effect increased as a function of immunodeficiency. Various interactions between HIV and *M. tuberculosis* at the cellular level make an association biologically plausible [42,43]. Further studies are needed to investigate the impact of HIV on the genetic population structure of *M. tuberculosis* with its consequences for transmission and clinical manifestations in high TB burden countries [36]. This will lead to a better understanding of biological factors that shape the current HIV/TB syndemic [60].

## Methods

### Study setting

The Swiss Molecular Epidemiology of Tuberculosis (SMET) study is a collaborative project between the Swiss HIV Cohort

Study (SHCS), the National Center for Mycobacteria, diagnostic microbiology laboratories, departments of respiratory medicine and public health, and the Federal Office of Public Health (FOPH) [29,61,62]. The overarching aims were to examine the genetic population structure of *M. tuberculosis* and the associations between strain variation, patient origin, and clinical characteristics in HIV–infected and HIV–negative TB patients in Switzerland. Further information on the SMET project is available at [www.tb-network.ch](http://www.tb-network.ch). All participating sites are listed in the Acknowledgements.

The SHCS is a prospective observational study of HIV–infected individuals followed up in HIV outpatient clinics in Switzerland [63]. All HIV–infected patients diagnosed with TB between 2000 and 2008 whose *M. tuberculosis* complex (MTBC) isolate was available were included in the SMET study [29]. Furthermore, we randomly selected 288 from the 4,221 culture-confirmed TB cases reported to the National TB Surveillance Registry during the same period (approximately three cases for one HIV–infected TB case within the SHCS). Finally, all reported drug-resistant TB cases were included. Two *M. bovis* isolates were excluded from this study as they are animal-adapted species within the MTBC and therefore represent a different host–pathogen relationship.

### Clinical data collection and definitions

We obtained clinical data by standardized questionnaires sent to the treating physicians and extracted relevant data from the SHCS database. We collected socio-demographic data (age, sex, origin of birth, citizenship, legal status, immunosuppressive therapy, risk factors for TB such as recent TB within family or immediate social surroundings in the last two years), laboratory parameters (CD4 cell count and plasma HIV RNA in HIV–infected cases) and clinical information (site of disease, radiography findings). Chest radiography parameters were consolidation, cavitations, enlarged intrathoracic lymph nodes and pleural thickening. Any drug resistance was defined as any resistance to isoniazid, rifampicin or ethambutol as reported to the FOPH. Most TB cases in Switzerland are treated under the guidance of experienced infectious and respiratory disease specialists, and the clinical data were of high quality.

Geographic origin of patients was defined as the country of birth, and countries were grouped in seven geographic regions (see Figure 1) according to the current understanding of the phylogeography of *M. tuberculosis* [25]. Birth country was used as a proxy of the ancestry of the study population. Immunosuppression due to other causes than HIV infection was defined as use of TNF-alpha inhibitors, malignancy, solid organ transplantation, use of steroids or methotrexate. Nadir CD4 T cell count was defined as the lowest CD4 T cell count (cells/ $\mu$ L) ever measured in a patient. Nadir CD4 T cell count is a predictor of poor immune recovery after ART [39]. Travel history was extracted from the free text field “Risk factor for TB” and defined as repeated travelling of longer duration ( $>30$  days) to low-income countries with a high TB burden and a relevant exposure to *M. tuberculosis* according to the physician’s judgment. Belonging to a molecular cluster involving Swiss-born and foreign-born TB cases was used as a proxy for contact with the foreign-born population.

### Molecular analyses

Mycobacterial isolates were cultured and DNA extracted according to standard laboratory procedures. We used spacer oligonucleotide typing (spoligotyping) and 24-loci mycobacterial interspersed repetitive units (MIRU–VNTR) which are based on repetitive DNA sequences as genotyping tools with high discriminatory power to identify recent TB transmission [29,64–66]. Data were analyzed with the MIRU–VNTRplus online tool ([http://](http://www.miru-vntrplus.org)

[www.miru-vntrplus.org](http://www.miru-vntrplus.org)). Molecular clusters were defined as a group of completely identical isolates in the spoligotyping and MIRU–VNTR pattern indicating a chain of TB transmission. In addition, we used single nucleotide polymorphisms (SNPs) as stable genetic markers to define the main phylogenetic *M. tuberculosis* lineages [67]. Lineages were determined by SNPs using multiplex real-time PCR with fluorescence-labeled probes (Taqman, Applied Biosystems, USA) adopted from previous studies [9,12,67,68]. The SNP used to define Lineage 4 was originally described by Sreevatsan et al. [69] and shown to be specific to this lineage [9].

### Graphical models

Graphical models were built using the principles of directed acyclic graphs [70]. Our model considered infection and disease as a combined outcome (“TB with an allopatric strain”). Our hypothesis that HIV infection causes TB with an allopatric strain is shown as a potentially causal direct effect, and risk factors potentially influencing this effect are shown in the hypothetical direction. Mediators represent variables that are caused by the independent variable and, in turn, have a direct effect on the outcome variable. We included age and sex in our model as risk factors for infection and disease [37]. We also considered contact with the foreign-born population who have a higher risk for TB compared to the native Swiss population [26] and who have a higher risk of exposure to “foreign” *M. tuberculosis* strains. Finally, we included frequent traveling to countries with a high TB burden, which increases exposure risk and thus potentially infection risk with “foreign” *M. tuberculosis* strains (Figure 2).

### Statistical analyses

We used  $\chi^2$  tests or Fisher’s exact tests to assess differences between groups in binary variables, and the Wilcoxon rank sum test for continuous variables (Table 1, Table 2). Univariate and multivariate exact logistic regression models were fitted to estimate the association between transmission as defined by molecular clustering and patients with sympatric *M. tuberculosis* lineages (patients with allopatric lineages were used as the reference, Table 2). Results were presented as ORs unadjusted and adjusted for age group, being born in Switzerland and recent TB in families or social surroundings. To assess the association of HIV infection with allopatric TB, we fitted univariate and multivariate logistic models (Table 3), and presented ORs unadjusted and adjusted for age, sex, Swiss-born, frequent travelling, contact with foreign born populations, and/or immunosuppression. We used univariate and multivariate logistic models to estimate the association between the degree of immunodeficiency and allopatric TB (Table 4), and presented ORs unadjusted and adjusted for age, sex, Swiss-born, frequent travelling, contact with foreign-born populations, and immunosuppression other than HIV infection. Finally, we determined statistical significance of HIV prevalence in patients with allopatric *M. tuberculosis* lineages compared to patients with sympatric lineages using Fisher’s exact tests (Table 5). All analyses were performed in Stata version 11.1 (Stata Corporation, College Station, TX, USA).

### Sensitivity analyses

In sensitivity analyses, we excluded patients with a history of frequent travelling to remove its influence on the association between HIV infection and allopatric lineages. In addition, we repeated the analyses using fully probabilistic Bayesian methods using weakly informative prior distributions [71]. The CIs reported from these analyses are 95% credible intervals and correspond to tail probabilities of the coefficient’s posterior



distributions. Bayesian statistics are less sensitive to errors when calculating estimators and CIs in small datasets.

### Second panel of *M. tuberculosis* strains

We obtained 1,642 *M. tuberculosis* isolates from all TB cases ( $n = 1,940$ , 84.6%) notified in the Canton of Bern, Switzerland, between 1991 and 2011. For all patient isolates, we determined the main phylogenetic *M. tuberculosis* lineages. Of these, we included all patient isolates belonging to a non-Euro-American lineage (Lineage 1, 2, 3, 5 or 6) from European-born TB patients (40 of a total of 292 isolates belonging to lineages other than Lineage 4). Furthermore, we randomly selected control strains belonging to the Euro-American lineage (Lineage 4) from European-born TB patients (400 of a total of 1,350 isolates belonging to Lineage 4). European ancestry was confirmed in HIV-infected patients with an allopatric *M. tuberculosis* strain. Finally, we determined the HIV status in these patients using the same procedures as in the main sample.

### Ethics approval

The study was approved by the ethics committee of the Canton of Bern, Switzerland. Written informed consent was obtained from all patients enrolled in the SHCS. For patients outside the SHCS, written informed consent was obtained by the treating physicians. In some cases informed consent could not be obtained from the patient because he or she could not be located or was known to have died. For these cases we obtained permission from the Federal expert commission on confidentiality in medical research to use the data provided by the treating physician.

### Supporting Information

**Figure S1** Graphical model showing direct potential effects on tuberculosis (TB) with an allopatric *Mycobacterium tuberculosis* strain among HIV-infected patients. (PDF)

**Figure S2** Distribution of tuberculosis (TB) cases included in the study, by origin of birth and HIV status. (PDF)

**Table S1** Distribution of the main phylogenetic *Mycobacterium tuberculosis* lineages according to molecular clusters involving either non-European-born tuberculosis (TB) cases only, European-born cases, or mixed clusters involving both non-European-born and European-born TB cases. (PDF)

**Table S2** Crude and adjusted analysis comparing HIV-infected and HIV-negative tuberculosis (TB) patients born in Europe ( $n = 233$ ) across the four most frequent *Mycobacterium tuberculosis* lineages. (PDF)

**Table S3** Associations of socio-demographic and clinical factors with tuberculosis (TB) with an allopatric *Mycobacterium tuberculosis* strain among HIV-infected European patients ( $n = 36$ ). (PDF)

**Table S4** Associations between HIV infection and tuberculosis (TB) with an allopatric *Mycobacterium tuberculosis* strain among European patients ( $n = 233$ ) in the context of other potential factors influencing this association, using Bayesian statistics and presented as unadjusted or adjusted odds ratios. (PDF)

**Table S5** Association between the degree of immunodeficiency and tuberculosis (TB) with an allopatric *Mycobacterium tuberculosis* among European patients ( $n = 233$ ) using Bayesian statistics. (PDF)

**Table S6** Comparing the main phylogenetic *Mycobacterium tuberculosis* lineages, by HIV status and birth region. (PDF)

### Acknowledgments

We thank all tuberculosis patients included in this study. We are grateful to the Swiss HIV Cohort Study and their cohort centers, to the treating physicians for providing clinical information, and to the Microbiology Laboratories for providing strains. We are indebted to the National TB Surveillance Registry at the Federal Office of Public Health and the Bernese Lung Association (Christa Butz and Yvonne Bongni). We also thank Joel Ernst, Douglas Young, and Peter Small for critical review of the manuscript.

The members of the study groups:

#### Swiss Molecular Epidemiology of Tuberculosis Study Group

**Central coordinating team:** Lukas Fenner and Matthias Egger, Institute of Social and Preventive Medicine, Bern; Sebastian Gagneux and Marcel Tanner, Swiss Tropical and Public Health Institute, Basel; Hansjakob Furrer, Inselspital Bern. **National Center for Mycobacteria:** Erik C. Böttger, Institute of Medical Microbiology, University of Zurich, Switzerland. **Microbiology laboratories:** Reno Frei, Clinical Microbiology, University Hospital Basel; Thomas Bodmer, Institute for Infectious Diseases, University of Bern; Beatrice Ninet, Jacques Schrenzel, Central Laboratory of Bacteriology, University Hospital Geneva; Katia Jatou, Amalio Telenti, Institute of Microbiology, University Hospital of Lausanne; Hans H. Siegrist, ADMed Microbiology, La Chaux-de-Fonds; Gaby E. Pfyffer, Department of Medical Microbiology, Luzerner Kantonsspital, Luzern; Thomas Bruderer, Centre for Laboratory Medicine, St.Gallen; Marisa Dolina, Cantonal Institute of Microbiology, Medical Bacteriology, Bellinzona; Olivier Dubuis, Viollier AG Switzerland, Allschwil. **Swiss HIV Cohort Study:** Manuel Battegay, University Hospital Basel; Enos Bernasconi, Andrea Parini Lugano; Matthias Hoffmann, St.Gallen; Hansjakob Furrer, Inselspital Bern; Matthias Cavassini, University Hospital of Lausanne; Bernard Hirschel, Alexandra Calmy, University Hospital of Geneva; Jan Fehr, University Hospital of Zurich. **Respiratory clinics:** Jean-Paul Janssens, University Hospital of Geneva; Jessica Mazza Stalder, University Hospital of Lausanne. **Federal Office of Public Health:** Peter Helbling and Ekkehardt Altpeter, Division of Communicable Diseases. **The Union:** Hans L. Rieder, Institute of Social and Preventive Medicine, University of Zürich, Switzerland; The Union, Paris, France.

#### Swiss HIV Cohort Study

Barth J, Battegay M, Bernasconi E, Böni J, Bucher HC, Burton-Jeangros C, Calmy A, Cavassini M, Cellerai C, Egger M, Elzi L, Fehr J, Fellay J, Flepp M, Francioli P (President of the SHCS), Furrer H (Chairman of the Clinical and Laboratory Committee), Fux CA, Gorgievski M, Günthard H (Chairman of the Scientific Board), Haerry D (deputy of "Positive Council"), Hasse B, Hirsch HH, Hirschel B, Hösli I, Kahlert C, Kaiser L, Keiser O, Kind C, Klimkait T, Kovari H, Ledergerber B, Martinetti G, Martinez de Tejada B, Metzner K, Müller N, Nadal D, Pantaleo G, Rauch A, Regenass S, Rickenbach M (Head of Data Center), Rudin C (Chairman of the Mother & Child Substudy), Schmid P, Schultze D, Schöni-Affolter F, Schüpbach J, Speck R, Taffé P, Tarr P, Telenti A, Trkola A, Vernazza P, Weber R, Yerly S.

### Author Contributions

Conceived and designed the experiments: L Fenner, M Egger, T Bodmer, P Helbling, H Furrer, HL Rieder, EC Böttger, S Gagneux. Performed the experiments: L Fenner, M Egger, T Bodmer, H Furrer, M Ballif, M Battegay, P Helbling, J Fehr, T Gsponer, HL Rieder, M Zwahlen, M Hoffmann, E Bernasconi, M Cavassini, A Calmy, M Dolina, R Frei, J-P Janssens, S Borrell, D Stucki, J Schrenzel, EC Böttger, S Gagneux. Analyzed the data: L Fenner, M Egger, M Ballif, M Zwahlen, T Gsponer, S Gagneux. Contributed reagents/materials/analysis tools: L Fenner, M Egger, T Bodmer, H Furrer, M Ballif, M Battegay, P Helbling, J Fehr, T

Gsponer, HL Rieder, M Zwahlen, M Hoffmann, E Bernasconi, M Cavassini, A Calmy, M Dolina, R Frei, J-P Janssens, S Borrell, D Stucki, J Schrenzel, EC Böttger, S Gagneux. Wrote the paper: L Fenner, M Egger, T Bodmer, H Furrer, M Ballif, M Battagay, P Helbling, J Fehr, T Gsponer,

HL Rieder, M Zwahlen, M Hoffmann, E Bernasconi, M Cavassini, A Calmy, M Dolina, R Frei, J-P Janssens, S Borrell, D Stucki, J Schrenzel, EC Böttger, S Gagneux. All authors met the criteria of the ICMJE and approved the final version of the manuscript.

## References

- Woolhouse ME, Webster JP, Domingo E, Charlesworth B, Levin BR (2002) Biological and biomedical implications of the co-evolution of pathogens and their hosts. *Nat Genet* 32: 569–577.
- Gandon S, Van Zandt PA (1998) Local adaptation and host-parasite interactions. *Trends Ecol Evol* 13: 214–216.
- Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecology Letters* 7: 1225–1241.
- Schulte RD, Makus C, Hasert B, Michiels NK, Schulenburg H (2011) Host-parasite local adaptation after experimental coevolution of *Caenorhabditis elegans* and its microparasite *Bacillus thuringiensis*. *Proc Biol Sci* 278: 2832–2839.
- Agnew P, Koella C, Michalakis Y (2000) Host life history responses to parasitism. *Microbes Infect* 2: 891–896.
- Gandon S, Agnew P, Michalakis Y (2002) Coevolution between parasite virulence and host life-history traits. *Am Nat* 160: 374–388.
- Lively CM, Dybdahl MF (2000) Parasite adaptation to locally common host genotypes. *Nature* 405: 679–681.
- Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, et al. (2002) A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proc Natl Acad Sci U S A* 99: 3684–3689.
- Gagneux S, DeRiemer K, Van T, Kato-Maeda M, de Jong BC, et al. (2006) Variable host-pathogen compatibility in *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U S A* 103: 2869–2873.
- Gutierrez MC, Brisse S, Brosch R, Fabre M, Omais B, et al. (2005) Ancient origin and gene mosaicism of the progenitor of *Mycobacterium tuberculosis*. *PLoS Pathog* 1: e5. doi:10.1371/journal.ppat.0010005
- Hirsch AE, Tsolaki AG, DeRiemer K, Feldman MW, Small PM (2004) Stable association between strains of *Mycobacterium tuberculosis* and their human host populations. *Proc Natl Acad Sci U S A* 101: 4871–4876.
- Hershberg R, Lipatov M, Small PM, Sheffer H, Niemann S, et al. (2008) High functional diversity in *Mycobacterium tuberculosis* driven by genetic drift and human demography. *PLoS Biol* 6: e311. doi:10.1371/journal.pbio.0060311
- Wirth T, Hildebrand F, Allix-Beguec C, Wolbeling F, Kubica T, et al. (2008) Origin, spread and demography of the *Mycobacterium tuberculosis* complex. *PLoS Pathog* 4: e1000160. doi:10.1371/journal.ppat.1000160
- Pearce-Duvel JM (2006) The origin of human pathogens: evaluating the role of agriculture and domestic animals in the evolution of human disease. *Biol Rev Camb Philos Soc* 81: 369–382.
- Vina MA, Hollenbach JA, Lyke KE, Szein MB, Maiers M, et al. (2012) Tracking human migrations by the analysis of the distribution of HLA alleles, lineages and haplotypes in closed and open populations. *Philos Trans R Soc Lond B Biol Sci* 367: 820–829.
- Brudey K, Driscoll JR, Rigouts L, Prodinger WM, Gori A, et al. (2006) *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol* 6: 23.
- Filliol I, Motiwala AS, Cavatore M, Qi W, Hazbon MH, et al. (2006) Global phylogeny of *Mycobacterium tuberculosis* based on single nucleotide polymorphism (SNP) analysis: insights into tuberculosis evolution, phylogenetic accuracy of other DNA fingerprinting systems, and recommendations for a minimal standard SNP set. *J Bacteriol* 188: 759–772.
- Baker L, Brown T, Maiden MC, Drobniewski F (2004) Silent nucleotide polymorphisms and a phylogeny for *Mycobacterium tuberculosis*. *Emerg Infect Dis* 10: 1568–1577.
- Reed MB, Pichler VK, McIntosh F, Mattia A, Fallow A, et al. (2009) Major *Mycobacterium tuberculosis* lineages associate with patient country of origin. *J Clin Microbiol* 47: 1119–1128.
- Gutacker MM, Mathema B, Soini H, Shashkina E, Kreiswirth BN, et al. (2006) Single-nucleotide polymorphism-based population genetic analysis of *Mycobacterium tuberculosis* strains from 4 geographic sites. *J Infect Dis* 193: 121–128.
- Gagneux S (2012) Host-pathogen coevolution in human tuberculosis. *Philos Trans R Soc Lond B Biol Sci* 367: 850–859.
- Brites D, Gagneux S (2012) Old and new selective pressures on *Mycobacterium tuberculosis*. *Infect Genet Evol* 12: 678–85.
- Wicker HR, Fibbi R, Haug W (2004) “Ergebnisse des Nationalen Forschungsprogramms ‘Migration und interkulturelle Beziehungen’”. Seismo publishing, Zürich, Switzerland.
- de Jong BC, Antonio M, Gagneux S (2010) *Mycobacterium africanum*—review of an important cause of human tuberculosis in West Africa. *PLoS Negl Trop Dis* 4: e744. doi:10.1371/journal.pntd.0000744
- Gagneux S, Small PM (2007) Global phylogeography of *Mycobacterium tuberculosis* and implications for tuberculosis product development. *Lancet Infect Dis* 7: 328–337.
- Federal Office of Public Health (2011) Tuberkulose in der Schweiz 2005–2009. [Erratum appears in *Bull Bundesamt für Gesundheit* (Switzerland) 2011;(no 13):277]. *Bull BAG* (no 10): 205–213.
- Borgdorff MW, Nagelkerke N, Van Soolingen D, de Haas PE, Veen J, et al. (1998) Analysis of tuberculosis transmission between nationalities in the Netherlands in the period 1993–1995 using DNA fingerprinting. *Am J Epidemiol* 147: 187–195.
- Lillebaek T, Andersen AB, Bauer J, Dirksen A, Glismann S, et al. (2001) Risk of *Mycobacterium tuberculosis* transmission in a low-incidence country due to immigration from high-incidence areas. *J Clin Microbiol* 39: 855–861.
- Fenner L, Gagneux S, Helbling P, Battagay M, Rieder HL, et al. (2012) *Mycobacterium tuberculosis* transmission in a country with low tuberculosis incidence: role of immigration and HIV infection. *J Clin Microbiol* 50: 388–395.
- Spiegelhalter DJ, Myles JP, Jones DR, Abrams KR (1999) Methods in health service research. An introduction to bayesian methods in health technology assessment. *BMJ* 319: 508–512.
- Lawn SD, Zumla AI (2011) Tuberculosis. *Lancet* 378: 57–72.
- Rodrigo T, Caylà JA, Garcia de Olalla P, Galdós-Tangüis H, Jansà JM, et al. (1997) Characteristics of tuberculosis patients who generate secondary cases. *Int J Tuberc Lung Dis* 1: 352–357.
- Comstock GW, Livesay VT, Woolpert SF (1974) The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol* 99: 131–138.
- Vynnycky E, Fine PEM (2000) Life time risks, incubation period, and serial interval of tuberculosis. *Am J Epidemiol* 152: 247–263.
- Horsburgh CR, Jr. (2004) Priorities for the treatment of latent tuberculosis infection in the United States. *N Engl J Med* 350: 2060–2067.
- Fenner L, Egger M, Gagneux S (2009) Annie Darwin’s death, the evolution of tuberculosis and the need for systems epidemiology. *Int J Epidemiol* 38: 1425–1428.
- Rieder HL (1999) Epidemiologic basis of tuberculosis control. International Union Against Tuberculosis and Lung Disease, Paris: 1999.
- Fenner L, Weber R, Steffen R, Schlagenhaut P (2007) Imported infectious disease and purpose of travel, Switzerland. *Emerg Infect Dis* 13: 217–222.
- Negredo E, Massanella M, Puig J, Perez-Alvarez N, Gallego-Escudero JM, et al. (2010) Nadir CD4 T cell count as predictor and high CD4 T cell intrinsic apoptosis as final mechanism of poor CD4 T cell recovery in virologically suppressed HIV-infected patients: clinical implications. *Clin Infect Dis* 50: 1300–1308.
- Kelley CF, Kitchen CM, Hunt PW, Rodriguez B, Hecht FM, et al. (2009) Incomplete peripheral CD4+ cell count restoration in HIV-infected patients receiving long-term antiretroviral treatment. *Clin Infect Dis* 48: 787–794.
- Lange CG, Lederman MM, Medvik K, Asaad R, Wild M, et al. (2003) Nadir CD4+ T-cell count and numbers of CD28+ CD4+ T-cells predict functional responses to immunizations in chronic HIV-1 infection. *AIDS* 17: 2015–2023.
- Diedrich CR, Flynn JL (2011) HIV-1/*Mycobacterium tuberculosis* coinfection immunology: how does HIV-1 exacerbate tuberculosis? *Infect Immun* 79: 1407–1417.
- Falvo JV, Ranjbar S, Jasenosky LD, Goldfeld AE (2011) Arc of a vicious circle: pathways activated by *Mycobacterium tuberculosis* that target the HIV-1 LTR. *Am J Respir Cell Mol Biol* 45: 1116–24.
- Hoshino Y, Nakata K, Hoshino S, Honda Y, Tse DB, et al. (2002) Maximal HIV-1 replication in alveolar macrophages during tuberculosis requires both lymphocyte contact and cytokines. *J Exp Med* 195: 495–505.
- Patel NR, Zhu J, Tachado SD, Zhang J, Wan Z et al. (2007) HIV impairs TNF-alpha mediated macrophage apoptotic response to *Mycobacterium tuberculosis*. *J Immunol* 179: 6973–6980.
- Geldmacher C, Schuetz A, Ngwenyama N, Casazza JP, Sanga E, et al. (2008) Early depletion of *Mycobacterium tuberculosis*-specific T helper 1 cell responses after HIV-1 infection. *J Infect Dis* 198: 1590–1598.
- Portevin D, Gagneux S, Comas I, Young D (2011) Human macrophage responses to clinical isolates from the *Mycobacterium tuberculosis* complex discriminate between ancient and modern lineages. *PLoS Pathog* 7: e1001307. doi: 10.1371/journal.ppat.1001307
- Musser JM, Kroll JS, Granoff DM, Moxon ER, Brodeur BR, et al. (1990) Global genetic structure and molecular epidemiology of encapsulated *Haemophilus influenzae*. *Rev Infect Dis* 12: 75–111.
- Caufield PW (2009) Tracking human migration patterns through the oral bacterial flora. *Clin Microbiol Infect* 15 Suppl 1: 37–39.
- Monot M, Honore N, Garnier T, Zidane N, Sherafi D, et al. (2009) Comparative genomic and phylogeographic analysis of *Mycobacterium leprae*. *Nat Genet* 41: 1282–1289.
- Falush D, Wirth T, Linz B, Pritchard JK, Stephens M, et al. (2003) Traces of human migrations in *Helicobacter pylori* populations. *Science* 299: 1582–1585.
- Linz B, Balloux F, Moodley Y, Manica A, Liu H, et al. (2007) An African origin for the intimate association between humans and *Helicobacter pylori*. *Nature* 445: 915–918.
- Aspholm-Hurtig M, Dailide G, Lahmann M, Kalia A, Ilver D, et al. (2004) Functional adaptation of BabA, the *H. pylori* ABO blood group antigen binding adhesin. *Science* 305: 519–522.

54. Zdziarski J, Brzuszkiewicz E, Wullt B, Liesegang H, Biran D, et al. (2010) Host imprints on bacterial genomes – rapid, divergent evolution in individual patients. *PLoS Pathog* 6: e1001078. doi:10.1371/journal.ppat.1001078
55. Caws M, Thwaites G, Dunstan S, Hawn TR, Lan NT, et al. (2008) The influence of host and bacterial genotype on the development of disseminated disease with *Mycobacterium tuberculosis*. *PLoS Pathog* 4: e1000034. doi:10.1371/journal.ppat.1000034
56. van Crevel R, Parwati I, Sahiratmadja E, Marzuki S, Ottenhoff THM, et al. (2009) Infection with *Mycobacterium tuberculosis* Beijing genotype strains is associated with polymorphisms in *SLC11A1/NRAMP1* in Indonesian patients with tuberculosis. *J Infect Dis* 200: 1671–1674.
57. Herb F, Thye T, Niemann S, Browne EN, Chinbuah MA, et al. (2008) ALOX5 variants associated with susceptibility to human pulmonary tuberculosis. *Hum Mol Genet* 17: 1052–1060.
58. Intemann CD, Thye T, Niemann S, Browne EN, Amanua CM, et al. (2009) Autophagy gene variant IRGM –261T contributes to protection from tuberculosis caused by *Mycobacterium tuberculosis* but not by *M. africanum* strains. *PLoS Pathog* 5: e1000577. doi: 10.1371/journal.ppat.1000577
59. Thye T, Niemann S, Walter K, Homolka S, Intemann CD, et al. (2011) Variant G57E of mannose binding lectin associated with protection against tuberculosis caused by *Mycobacterium africanum* but not by *M. tuberculosis*. *PLoS ONE* 6: e20908. doi:10.1371/journal.pone.0020908
60. Kwan CK, Ernst JD (2011) HIV and tuberculosis: a deadly human syndemic. *Clin Microbiol Rev* 24: 351–376.
61. Fenner L, Gagneux S, Janssens JP, Fehr J, Cavassini M, et al. (2012) Tuberculosis in HIV-negative and HIV-infected patients in a low-incidence country: clinical characteristics and treatment outcomes. *PLoS ONE* 7: e34186. doi:10.1371/journal.pone.0034186
62. Fenner L, Egger M, Bodmer T, Altpeter E, Zwahlen M, et al. (2012) Effect of mutation and genetic background on drug resistance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 56: 3047–3053.
63. Schoeni-Affolter F, Ledergerber B, Rickenbach M, Rudin C, Gunthard HF, et al. (2010) Cohort profile: the Swiss HIV Cohort study. *Int J Epidemiol* 39: 1179–1189.
64. Allix-Beguec C, Fauville-Dufaux M, Supply P (2008) Three-year population-based evaluation of standardized mycobacterial interspersed repetitive-unit-variable-number tandem-repeat typing of *Mycobacterium tuberculosis*. *J Clin Microbiol* 46: 1398–1406.
65. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rusch-Gerdes S, et al. (2006) Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. *J Clin Microbiol* 44: 4498–4510.
66. Oelemann MC, Diel R, Vatin V, Haas W, Rusch-Gerdes S, et al. (2007) Assessment of an optimized mycobacterial interspersed repetitive-unit-variable-number tandem-repeat typing system combined with spoligotyping for population-based molecular epidemiology studies of tuberculosis. *J Clin Microbiol* 45: 691–697.
67. Stucki D, Malla B, Hostettler S, Huna T, Feldmann J, et al. (2012) Two new rapid SNP-typing methods for classifying *Mycobacterium tuberculosis* complex into the main phylogenetic lineages. *PLoS ONE* 7: e41253. doi:10.1371/journal.pone.0041253
68. Fenner L, Malla B, Ninet B, Dubuis O, Stucki D, et al. (2011) “Pseudo-Beijing”: Evidence for convergent evolution in the direct repeat region of *Mycobacterium tuberculosis*. *PLoS ONE* 6: e24737. doi:10.1371/journal.pone.0024737
69. Sreevatsan S, Pan X, Stockbauer KE, Connell ND, Kreiswirth BN, et al. (1997) Restricted structural gene polymorphism in the *Mycobacterium tuberculosis* complex indicates evolutionarily recent global dissemination. *Proc Natl Acad Sci U S A* 94: 9869–9874.
70. Pearl J (2010) An Introduction to Causal Inference. *Int J Biostat* 6: Article 7.
71. Gelman A, Jakulin A, Pittau MG, Su YS (2008) A weakly informative default prior distribution for logistic and other regression models. *Ann Appl Stat* 2: 1360–1383.
72. Bentley SD, Comas I, Bryant JM, Walker D, Smith NH, et al. (2012) The genome of *Mycobacterium africanum* West African 2 reveals a lineage-specific locus and genome erosion common to the *M. tuberculosis* complex. *PLoS Negl Trop Dis* 6: e1552. doi:10.1371/journal.pntd.0001552