Genes & Immunity
https://doi.org/10.1038/s41435-019-0080-1

ARTICLE

A SNP upstream of the *cyclic GMP-AMP synthase (cGAS)* gene protects from relapse and extra-pulmonary TB and relates to BCG vaccination status in an Indian cohort

Shruthi Thada[1,2] · Sanne Burkert[1] · Ramya Sivangala[2] · Abid Hussain[3] · Saubashya Sur[1] · Nickel Dittrich[1] · Melanie L. Conrad[1,4] · Hortense Slevogt[1,5] · Suman Latha Gaddam[2,6] · Ralf R. Schumann[1]

Received: 14 December 2018 / Revised: 20 March 2019 / Accepted: 1 April 2019
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Abstract

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*M.tb*) is a major health care threat worldwide causing over a million deaths annually. Host-pathogen interaction is complex, and a strong genetic contribution to disease susceptibility has been proposed. We have investigated single-nucleotide polymorphisms (SNPs) within cGAS/STING in Indian TB patients and healthy cohorts from India and Germany by Lightcycler®480 genotyping technique. The cGAS/STING pathway is an essential defense pathway within the cytosol after *M.tb* is internalized and mycobacterial DNA is released inducing the production of type I IFNs. We found that the rs311686 SNP upstream of cGAS provides protection from getting TB overall and is differently distributed in pulmonary TB patients compared with extra-pulmonary and particularly relapse cases. This SNP furthermore differs in distribution when comparing individuals with respect to BCG vaccination status. Taken together, our results show that the presence of the rs311686 SNP influences the course of TB significantly. However, structural conformation changes were found only for the cGAS rs610913 SNP. These findings underscore the importance of *M.tb* DNA recognition for TB pathogenesis and may eventually help in risk stratification of individuals. This may ultimately help in prevention of disease and aid in developing new vaccination and treatment strategies.

Introduction

According to the World Health Organization (WHO) report in 2017, tuberculosis (TB) is the ninth leading cause of death worldwide with an estimated range of 10.4 million new TB cases registered globally and 1.3 million deaths having occurred in 2016. India alone accounts for one fourth of the global incidents of TB cases every year. Approximately 7.7% of the notified cases were TB relapse cases worldwide. WHO estimates the rate of treatment completion to be between 83% and 86% and the risk of TB relapse after successful treatment 2% annually (World Health Organization. Global TB database. Geneva, Switzerland: WHO, 2017. http://www.who.int/tb/data/en/ accessed November 2017).

TB is caused by *Mycobacterium tuberculosis* (*M.tb*), an acid-fast bacterium that can elude the host's conventional combat strategies to promote intracellular life within the endosomal compartments of macrophages and antigen-presenting cells (APCs). Recognition of *M.tb* by pattern recognition receptors (PRRs) in APCs triggers a Th1-response crucial for eliminating the disease. Besides toll-like receptors (TLRs), complement receptors, C-type

Supplementary information The online version of this article (https://doi.org/10.1038/s41435-019-0080-1) contains supplementary material, which is available to authorized users.

✉ Ralf R. Schumann
ralf.schumann@charite.de

1 Institute for Microbiology and Hygiene, Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Berlin, Germany

2 Bhagwan Mahavir Medical Research Centre, Hyderabad, India

3 Department of Biotechnology and Bioinformatics, University of Hyderabad, Hyderabad, India

4 Division of Psychosomatic Medicine, Department of Internal Medicine and Dermatology, Charite-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Berlin, Germany

5 ZIK Septomics, Jena University Hospital, Jena, Germany

6 Department of Genetics, Osmania University, Hyderabad, India

Published online: 23 May 2019

lectins, dectin 1 & 2, MINCLE, DC_SIGN, and other PRRs restrict maturation of mycobacteria-containing phagosomes by inducing synthesis and release of inflammatory and regulatory cytokines [1–4]. Intracellular pathogens like *M.tb* can escape the endosome and translocate to the cytosol during infection thereby initiating cytosolic recognition pathways [5–7]. The cytosolic translocation ability depends on an intact *region of difference (RD) 1* locus of *M.tb* encoding components of a type seven secretion system (ESX-1) and essential secreted effectors. These have been shown to be involved in arrest of phagosome maturation, necrosis, granuloma formation, and dissemination of mycobacteria [8, 9]. The subcellular compartmentalization of *M.tb* thus critically affects inflammatory patterns of infected macrophages and bacillary spread.

The endosomal and cytosolic PRRs (i.e., TLRs and retinoic acid-inducible gene-I-like receptors - RLRs) are crucial to detect nucleic acids from various intracellular pathogens to generate protective immune responses to pathogens. Immunostimulatory activity of foreign nucleic acids have been known for a long time, however, research on cytosolic DNA sensing has been advanced only in the past few years. Upon recognition of cytosolic DNA the *cyclic GMP-AMP synthase (cGAS)* and *stimulator of interferon genes (STING)* pathway leads to the induction of type I interferons [10, 11], whereas activation of the NLRP3/AIM2 inflammasome leads to the release of mature IL-1β and IL-18 [12, 13]. These two pathways counteract each other in restricting or developing the disease depending on the host's immune status and stage of infection [14].

cGAS is a member of the nucleotidyl transferase family and it acts as a sensor for cytosolic dsDNA, which stimulates the endogenous second messenger cGAMP for STING activation in multiple cell types including macrophages [15]. cGAS interaction with DNA precedes the synthesis of cyclic di-GMP-AMP (c-diGAMP) from ATP and GTP activating STING to induce a conformational change allowing it to translocate from the ER to the Golgi [16, 17]. This leads to the availability of the STING carboxyl terminus to subsequent recruitment and activation of TANK-binding kinase 1 (TBK1) and IFN regulatory factor 3 (IRF3) via a phosphorylation-dependent mechanism [18, 19] to produce IFNβ [11, 15, 20]. STING also activates NF-κB, which together with IRF3 activates transcription of type I IFNs and other cytokines [21]. Cytosolic DNA is vital for the induction of autophagy, an important host defense mechanism against *M.tb* [22]. Thus, it is important to understand the role of the *cytosolic DNA surveillance pathway (CSP)* during mycobacterial infection being either protective or detrimental to the host. In vitro experiments with cGAS knockdown/knockout in human and mouse macrophages upon infection with *M.tb* revealed a hampered production of IFNs and induction of autophagy [11, 23, 24] in an ESX-1-dependent manner [25], thus indicating a specific importance of cGAS in the pathogenesis of TB. So far, there have been no reports on the importance of cGAS single-nucleotide polymorphisms (SNPs) for disease susceptibility. Owing to the strong genetic factor in TB, however, we considered it worthwhile looking into the potential role of genetic variations of this important pathway for TB susceptibility.

Apart from the c-diGAMP produced through cGAS, STING also recognizes cyclic di-nucleotides (CDNs). CDNs are characterized by ubiquitous secondary messengers in bacteria maintaining bacterial metabolism, virulence regulation, and biofilm formation [26]. CDN-induced immune responses through STING have been shown to be effective against intracellular pathogens [27]. A recent report suggests that the CDN adjuvant protein subunit of *M.tb* elicits a TH1/TH17 immune response through STING activation, leading to an enhanced protection against TB infection [28]. STING has a prominent role in several infectious diseases and autoimmune disorders [29–32]. There are reports suggesting that variations in the STING gene may lead to conformational changes near the c-terminal region, where the ligand-binding pocket is located [33–35]. This may eventually impact binding efficacy of STING with the respective ligands.

It has been well established that host genome-wide association studies (GWAS) are crucial to understand the genetic role in susceptibility to *M.tb* [36]. However, candidate gene approaches are also needed as so far no clear results from GWAS studies have been obtained. We have reported earlier on the importance of TLR mutations in TB susceptibility in an Indian TB cohort [37–39]. Although there were several mutations identified, there is still a need to understand the complete role of immunological variations within the cohorts that can control TB and vaccine efficacy. Hence, we considered it important to check for a potential role of variations of cGAS/STING genes for individual TB susceptibility, as we still do not know why only 10% of people infected with *M.tb* actually proceed to active disease. The following SNPs were selected to study them in both Indian and German cohorts of TB patients and controls: cGAS rs610913 C/A and rs311686 A/G; STING rs78233829 G/C (aa code G230A), rs1131769 A/G (aa code H232R) and rs7380824 C/T (aa code R293Q).

Results

Baseline characteristics of the patient cohorts and controls

The baseline characteristics of the Indian cohort consisting of TB patients, household controls (HHC, apparently

Table 1 Baseline characteristics of the Indian cohort

	Age (mean ± SD)	Gender		BCG
		F (%)	M (%)	+(%)
Controls ($N = 380$)	32.77 ± 9.85*	170 (44.74)*	210 (55.26)	299 (78.7)*
HC ($N = 202$)	31.82 ± 10.2	68 (33.66)	134 (66.33)	169 (83.66)
HHC ($N = 178$)	33.83 ± 9.35	102 (57.30)	76 (42.7)	130 (73.03)
Primary TB ($N = 345$)	25.52 ± 10.51*	204 (59.13)*	141 (40.87)	148 (42.9)*
PTB ($N = 221$)	25.96 ± 11.18	124 (56.11)	97 (43.89)	96 (43.43)
EPTB ($N = 124$)	24.79 ± 9.34	80 (64.52)	44 (35.48)	52 (41.93)
Relapse TB ($N = 100$)	30.36 ± 10.97*	49 (49)	51 (51)	35 (35)

HC healthy unrelated control, *HHC* healthy household controls (apparently healthy, TB-disease status unknown), *PTB* pulmonary TB, *EPTB*- extra-pulmonary TB; Age: homogeneity of variance within the cohort was met. In all categories, age distribution significantly differs from normality

*p value based on χ^2-test, $p < 0.0001$. Patients were younger than controls and there are more female in TB group than controls; BCG status differs in patients and controls; relapse were older compared with primary TB

healthy, TB-status unknown) and healthy controls (HC) are summarized in Table 1. The cohort consisted of 345 primary TB patients (221 pulmonary TB (PTB), 124 extra-pulmonary TB (ETB)) and 100 relapse cases). The control cohort consisted of 202 HC and 178 HHC of the TB patients with similar genetic background and socio-economic status. On average within our cohort patients were significantly younger ($W = 82620$, $p < 2.2e\text{-}16$) than the controls. There were more females within the TB group as compared with the control group (χ^2 (1) = 15.00032, $p < 0.0001$). Thus, whenever appropriate, analyses were adjusted to age and gender. Between controls and patients as expected the difference in BCG vaccination was highly significant (χ^2 (1) = 85.27127, $p < 2.6e\text{-}20$) confirming a protective effect of the vaccination. Compared with primary TB patients relapse cases were significantly older ($W = 10686$, $p < 2.5e\text{-}05$). BCG vaccination status was not different between these two groups (χ^2 (1) = 0.5177839, $p < 0.47$).

Differences in SNP distribution

Allelic frequencies of Indian HC and the German cohort are summarized in Table 2. Differences in allele distribution match HapMap-project data, where they were available except for rs311686. Here, we found a higher frequency of the A-Allele than reported for Central Europe in the German Cohort as well as in the Indian cohort as compared with a Gujarati community in the United States. We have observed significantly different distributions of rs311686, STING 230, and STING 293 allele frequency among Indian and German healthy cohorts, which may point to an importance of these genes and a shift over time due to selective pressure. Hardy–Weinberg equilibrium was confirmed for all SNPs except for cGAS rs311686 ($p < 0.0001$), which was owing to higher frequency of heterozygotes. cGAS SNPs were in moderate Linkage Disequilibrium ($D' = 0.234$), whereas STING SNPs were strongly linked ($D' = 0.997\text{-}1$) (Table 3).

Higher frequency of rs311686G in PTB as compared to ETB, relevance of BCG vaccination status

Distribution of rs311686 SNP frequencies comparing different clinical presentations of TB and healthy controls of the Indian cohort are summarized in Table 4. Comparing both control groups (HHC & HC), HHC showed a higher frequency of the G-allele as compared with the HC group ($p < 0.01$, OR = 0.56 (0.359−0.873)). Similarly, HC and TB patients exhibited a significantly different distribution pattern ($p < 0.003$, OR = 0.56 (0.36−0.813)). HHC and TB patients failed to show any difference ($p < 0.87$, OR = 0.96). Comparing pulmonary TB (PTB) and extra-pulmonary TB (EPTB) cases the frequency of the G-Allele was significantly higher in PTB cases ($p < 0.04$, OR = 0.491), however, after adjustment for gender and age significance was weaker ($p < 0.05$, OR = 0.62 (0.37−1.01)). As mentioned earlier, in our Indian cohort the BCG-status was significantly different between patients and controls ($p < 0.0001$, OR = 0.202) and also between patients and their HHCs ($p < 0.0002$, OR = 0.44). Logistic regression analysis revealed a significant correlation of BCG status and SNP frequency comparing HHC and TB patients ($p < 0.05$, OR = 2.64). Hence, we subdivided the HHC and primary TB patients into BCG-positive and -negative groups. In BCG-negative individuals, the frequency of the G-Allele was significantly higher in HHC than in TB patients (80.49% vs 63.77 %, $p < 0.04$, OR = 1.97). After adjusting for gender and age, however, significance was lost ($p < 0.09$, OR = 2.223). With regard to the BCG-positive individuals, no significant differences were observed between HHC and TB patients (as well as

Table 2 Allele frequencies of the cGAS and STING SNPs investigated here

Allele frequencies	Variation (aa)	Function	German cohort (%)	Indian cohort[a] (%)	Hap-MAP CEU	Hap-Map GIH	HWE in Indian cohort*	German vs Indian cohort**
rs311686_G		8.98 kb upstream might influence the promoter region of cGAS	34.94	44.57	58.8	59.6	<0.0001	< 0.00035
rs610913_C	C->A ([Pro]->[His])	Missense	37.45	33.56	30.8	32.95	0.548	0.261
STING230_C	G->C ([Gly]->[Ala])	Missense	11.98	26.63	NA	NA	0.346	< 0.0001
STING232_A	A->G ([Hist]->[Arg])	Missense	14.06	9.54	12.93	9.52	0.3837	0.085
STING293_T	C->T ([Arg]->[Gln])	Missense	13.08	23.68	10	NA	0.2701	< 0.024

CEU European population, *GIH* Gujarati Indians in Houston population, *NA* not available

[a]Cohort consists of only healthy unrelated controls (HC)

*p value based on χ^2 test, $p < 0.05$ indicates significant difference from assumed HWE

**p value based on χ^2 test

Table 3 Linkage disequilibrium

Linkage disequilibrium[a]	D'
CGAS_3116_6109	0.226
Sting_230_293	1
Sting_230_232	0.998
Sting_232_293	0.997

[a]In healthy unrelated Indian cohort

whole cohorts). Taken together the results indicate protection from TB when the G-Allele is present, but between HHC and TB patients it does not have a direct impact. When comparing related individuals not having received BCG vaccination a significant difference was found. Successful vaccination thus may overcome the increased risk observed for the A-allele. Furthermore, the different distribution between PTB and EPTB points towards a protective effect of the G-Allele against dissemination of the disease (Tables 5 and 6).

Significant difference of rs311686 distribution in TB relapse cases as compared with TB cases

There are strikingly less G-Allele carriers (SNP rs311686) in TB relapse cases as compared with the primary TB group and controls ($p < 0.0001$, OR = 2.702 (1.639 −4.54)), indicating a protective effect of the G-genotype for becoming a relapse case (Table 5). The interaction with gender is also significant ($p < 0.02$, OR = 3.33), so further subgroup analysis was performed: Here, within the male cohort only the difference remained significant ($p < 0.0001$, OR = 4.119), whereas within females no significant difference could be observed ($p < 0.182$, OR = 1.334) (not shown in the Table). No significant interaction with BCG was found ($p = 0.78$, OR = 1.17) between primary and relapse TB cases.

Importance of the presence of the rs610913 SNP for efficacy of BCG vaccination in females

Next, we analyzed the frequency of SNP cGAS rs610913 within our cohorts. We did not find any significant difference in distribution of the rs610913 SNP comparing controls and TB patients as such. However, when taking BCG vaccination into account, the SNP was differently distributed, just failing to reach statistical significance ($p < 0.06$, OR = 0.654). Comparing a potential gender influence, a significant difference in SNP frequency in the female cohort only could be found ($p < 0.03$, OR = 2.141) (not shown in Table), but the SNP itself in the subgroup of BCG vaccinated or unvaccinated females did not reach significance, maybe owing to low statistical power because of the limited case number in this subgroup (Tables 7–9).

Table 4 rs311686 SNP frequencies in the Indian cohorts

rs311686 SNP	Percentage of allele-carriers *N* (%)				
	Controls *N* (%)		TB *N* (%)		Relapse *N* (%)
	HC	HHC	PTB	EPTB	
AA	104 (52.26)	73 (41.47)	75 (37.31)	56 (49.12)	59 (64.13)
AG	23 (11.56)	43 (24.43)	49 (24.38)	18 (15.79)	0
GG	72 (36.18)	60 (34.09)	77 (38.31)	40 (35.09)	33 (35.87)

HC healthy unrelated control, *HHC* healthy household control (apparently healthy, TB-disease status unknown), *PTB* pulmonary TB, *EPTB* extra-pulmonary TB

Table 5 rs311686 SNP comparison between different groups

Comparison	*N* (%) of GG/AG vs AA		χ^2		Logistic regression[a]			Interaction for gender[b]		Interaction for BCG[b]	
			p value	OR	*p* value	OR	CI	*p* value	OR	*p* value	OR
Controls vs TB	198 (52.8)	184 (58.4)	0.145	0.904	0.07	0.725	0.512–1.027	0.59	0.85	0.256	1.56
HC vs HHC	95 (47.74)	103 (58.52)	**0.03**	**0.649**	**0.01**	**0.56**	**0.359–0.873**	0.03	2.63	0.02	0.2
HC vs TB	95 (47.74)	184 (58.41)	**0.01**	**0.651**	**0.003**	**0.56**	0.36–0.813	0.27	1.538	0.26	1.886
HHC vs TB	103 (58.52)	184 (58.41)	0.98	0.995	0.87	0.96	0.62–1.49	0.18	1.72	**0.05**	**2.64**
EPTB vs PTB	58 (50.88)	126 (62.69)	**0.04**	**0.491**	**0.05**	**0.62**	0.37–1.01	0.91	1.01	0.12	2.223
Controls vs relapse	198 (52.8)	33 (35.87)	**0.003**	**1.997**	**0.001**	**2.203**	**1.369–3.57**	**0.03**	**2.857**	0.292	1.82
TB vs relapse	184 (58.43)	33 (35.87)	**0.0001**	**2.506**	**0.0001**	**2.702**	**1.639–4.54**	**0.02**	**3.33**	0.776	1.17

TB primary TB including both PTB and EPTB

[a]Adjusted for age and gender, based on Wald's statistic

[b]Interaction term of a logistic regression model, based on Wald's statistic meaning whether the analysis has any influence with respect to either gender and/or with BCG

P-values of 0.05 and lower are shown in bold

STING 230C is less frequent in HHC as compared with primary TB patients

Within the closely related STING protein, SNPs have been reported that are in linkage disequilibrium. We investigated one representative SNP and found the C allele frequency of STING 230 to be slightly more frequent in TB patients as compared with HHC in a logistic regression model, which just misses statistical significance ($p < 0.06$, OR = 1.493). No other differences between controls or primary TB patients was observed for this SNP, neither significant interactions with gender nor with BCG, indicating that no genuine impact originates from them (Supplementary Tables 1 and 2).

In summary, statistical analyses from our cohort show three significant findings for the cGAS genotype: (i) A significantly reduced frequency of the G-Allele of rs311686 in patients with EPTB compared to PTB, which just reaches significance after adjustment. (ii) A highly significantly lower frequency of the G-Allele of rs311686 in relapse cases as compared with primary TB. And (iii) a significant interaction with the BCG vaccination for rs311686 and rs610913 in females.

Differences in hypothetical cGAS structure resulting from the SNPs investigated

Statistically, we found the SNP rs311686 to have impact on course and susceptibility of TB. Although this SNP is not in a coding region, it indirectly may lead to conformational changes owing to co-segregating SNPs. Looking into the potential influence on protein structure by modeling, as expected we failed to detect any significant structural conformational changes (data not shown). The wildtype and variant forms of cGAS resulting from the rs610913 SNP, in contrast, led to hypothetical structures with conformational differences. The outcome of *SIFT* predictions specified the amino-acid change from P to H at position 261 to be potentially damaging. Likewise, *Polyphen* analysis revealed that the mutation of P at 261 to H is damaging with a score of 0.988. *I-mutant* indicated a decrease of DDG value (Free energy change), i.e., −1.42 Kcal/mole, specifying a decrease in protein stability in the H variant as compared with the P variant. *MutPred* revealed that this mutation (P261H) resulted in loss of helix ($p < 0.044$) and loss of glycosylation at ($p < 0.0446$). Therefore, we conclude that according to in silico analysis the mutation of P to H may

Table 6 BCG influence on rs311686 SNP

	BCG-status	Comparison	*N* (%)		χ^2		Logistic regression[a]		
					p value	OR	*p* value	OR	CI
BCG vaccination effect on TB	BCG vaccinated[b]	HC vs TB	169 (83.66)	148 (42.9)	$\mathbf{8.83 \times 10^{-8}}$	**0.338**	$\mathbf{1.18 \times 10^{-13}}$	**0.12**	**0.06–0.2**
	BCG vaccinated[b]	HHC vs TB	130 (73.03)	148 (42.9)	$\mathbf{3.521 \times 10^{-21}}$	**0.096**	**0.0002**	**0.44**	**0.29–0.68**
rs311686 GG/AG vs AA	Not BCG vaccinated	HHC vs TB	33 (80.48)	63 (63.77)	**0.04**	**1.97**	0.09	2.223	0.91–6.25
	BCG vaccinated	HHC vs TB	63 (54.31)	79 (57.25)	0.63	0.89	0.676	0.89	0.523–1.515

[a]Adjusted for age and gender, based on Wald's statistic

[b]BCG status in whole cohort

P-values of 0.05 and lower are shown in bold

potentially change cGAS function. However, further experimental analyses will be needed to support this theory. Evaluation of the 3D structures of the P and H variants revealed a RMSD difference of 0.01 and 0.01 Å in C alpha and backbone, respectively (Fig. 1a, b). Both the P and H variant had four functionally important nests. Whereas the P variant has 1 tunnel, 3 functional pores, and 64 pockets; the H variant 261 has 1 tunnel, 2 functional pores and 66 pockets in their structure (Fig. 2a, b). The P variant was slightly more flexible as compared with the H variant. The outcome of the analyses of four major clefts (Supplementary Table 3 and Fig. 1a, b) pointed out a significant difference in the volume of the second largest cleft. The P variant had a volume of 1606.92 Å3, whereas the H variant had a larger volume of 1743.61 Å3. This specified a better capacity for binding interactions in the latter. However, the volumes of three other major clefts were slightly higher in the P variant compared with the H variant. In summary, it is likely that some minor conformational changes at the structural level are taking place owing to the mutation.

Discussion

Here we report on the potential importance of cGAS/STING SNPs for TB susceptibility by analyzing patient and control cohorts for SNP distribution. First, we found that genetic variations within cGAS and STING differed significantly when comparing healthy controls from Germany and India. Although this is very hypothetical, this difference could point to a selection of genetic ("potentially beneficial") variants in regions of high prevalence of a disease over long time as it has been shown, i.e., for Malaria [40]. Next, we analyzed the demographic characteristics of our TB cohort and found a disbalance in the male to female ratio in TB patients favoring females, which contrasts with the current WHO report where the male to female ratio is 1.7. We have observed age, gender, and BMI to be strong predictors of primary TB, whereas there was no difference in distribution of these parameters between relapse and primary TB. We have used two different control groups, HC and HHC. HC were individuals living distantly from patients, whereas the HHC group comprised individuals living within the household of patients. Thus, results obtained by comparison of HHC and TB are less vulnerable for confounding environmental factors and put a stronger emphasis on potential genetic links.

cGAS is a recently identified molecule crucial for DNA recognition and control of viral infections and infections caused by intracellular bacterial pathogens [29]. DNA binding to cGAS leads to the induction of type I IFNs through the STING pathway, representing an important innate defense mechanism. Human retroviruses are also able

Table 7 rs610913 SNP frequencies

rs610913 SNP	Percentage of allele carriers *N* (%)				
	Controls *N* (%)		TB *N* (%)		Relapse *N* (%)
	HC	HHC	PTB	EPTB	
AA	87 (45.55)	69 (46)	74 (40.66)	42 (43.75)	37 (41.73)
AC	84 (43.98)	61 (40.66)	82 (45.05)	44 (45.83)	45 (45.32)
CC	20 (10.47)	20 (13.33)	26 (14.29)	10 (10.42)	11 (11.29)

HC healthy unrelated control, *HHC* healthy household control, *PTB* pulmonary TB, *EPTB* extra-pulmonary TB

Table 8 rs610913 SNP Comparison between different groups

comparison	*N* (%) of CC/AC vs AA		χ^2		Logistic regression[a]			Interaction for gender[b]		Interaction for BCG[b]	
			p value	OR	*p* value	OR	C.I	*P* value	OR	*p* value	OR
Controls vs TB	185 (54.25)	162 (58.76)	0.62	0.492	0.454	0.87	0.61–1.25	0.888	0.95	0.193	0.58
HC vs HHC	104 (54.45)	81 (54)	0.934	1.01	0.709	0.92	0.57–1.46	0.422	0.68	0.22	2.28
HC vs TB	104 (54.45)	162 (58.27)	0.411	0.856	0.422	0.84	0.6–1.28	0.629	0.83	0.960	0.97
HHC vs TB	81 (54)	162 (58.27)	0.39	0.841	0.623	0.89	0.56–1.41	0.665	1.21	0.085	0.42
EPTB vs PTB	54 (56.25)	84 (59.34)	0.619	1.13	0.611	1.15	0.67–1.96	0.652	0.79	0.387	0.62
Controls vs relapse	185 (54.25)	56 (60.22)	0.84	0.196	0.340	0.79	0.49–1.27	0.656	0.81	0.726	0.82
TB vs relapse	162 (58.27)	56 (60.22)	0.74	0.992	0.90	0.97	0.58–1.6	0.734	0.85	0.541	1.4

[a]Adjusted for age and gender, based on Wald's statistic

[b]Interaction term of a logistic regression model, based on Wald's statistic meaning whether the analysis has any influence with respect to either gender and/or with BCG

to activate the cGAS pathway through the cytosolic accumulation of reversely transcribed cDNA generated during replication [41]. With respect to TB, cGAS knockout mice have been shown to be more susceptible to *M.tb*, and the mechanism suggested to explain this was the inability to undergo autophagy. Thus, cGAS has an important role in pathogen elimination and it has been reported that there are several checkpoints critical for cGAS activity in order to balance between disease progression and elimination [42].

The role of type I IFN in TB pathogenesis currently is not completely understood. For a long time, it was thought to have just a detrimental effect in chronic disease status [43–46]. Recently, several studies reported a significant enhancing effect of type I IFNs toward innate immunity pathways following mycobacterial infection [47]. For example, in a new vaccination trial using the ESX-1 system extracted from *M. marinum* with BCG yielded a better protection with that of the classical BCG vaccine. This effect correlated with a higher induction of Type I IFNs [48]. Also, there are studies explaining the early production of Type I IFNs to be important to induce pro-inflammatory cytokines like IL-12p70 to develop adaptive immunity for protection against *M.tb* [49]. Support for the importance of Type I IFNs in TB pathogenesis are emerging, i.e., the 2′-5′-oligoadenylate synthetase like (OASL) protein helps mycobacterial survival by inhibiting autophagic mechanisms and antimicrobial peptide expression [50]. OASL is a bi-functional protein, which upon RNA-recognition enhances production of Type I IFNs through RIG-1, whereas upon DNA recognition inhibiting cGAS and type I IFN production. In mycobacterial disease one would assume both DNA and RNA to be potential activators of these pathways, however, little is known about their function in TB yet. Extensive study of this signaling pathway (cGAS/STING/type I IFN/OASL) is needed in order to better understand TB pathogenesis and identify new treatment targets.

Regarding the rs311686 cGAS SNP we found several lines of evidences for a potentially crucial role of the G-Allele within this genetic region. In primary TB patients, the frequency of the SNP was higher as compared with the HC group. An interesting aspect is that in the unvaccinated subcohort primary TB patients displayed lower G-allele frequencies as compared with HHC arguing for a beneficial effect originating from the mutation, which might be masked by (successful) BCG vaccination. Strikingly, comparing PTB and EPTB patients we found the frequency of the G-allele to be lower in EPTB, and even lower in relapse cases. The incidence of TB relapse in those who completed previous treatment can be 30-times higher than the

Table 9 BCG influence on rs610913 SNP

	BCG-status	comparison	*N* (%)		χ^2		Logistic regression[a]			Interaction with gender[b]	
					p value	OR	*p* value	OR	C.I	*P* value	OR
rs610913 CC/AC vs AA	BCG not vaccinated	Controls vs TB	26 (55.32)	63 (52.50)	0.74	1.119	0.08	1.03	0.47–2.27	**0.07**	3.7
	BCG vaccinated	Controls vs TB	140 (54.26)	78 (64.46)	**0.06**	0.654	0.15	0.71	0.5–1.13	0.769	0.877

[a]Adjusted for age and gender, based on Wald's statistic

[b]Interaction term of a logistic regression model, based on Wald's statistic meaning whether the analysis has any influence with respect to gender

P-values of 0.05 and lower are shown in bold

incidence of active TB in the general population [51]. Based on the data analyzed over a period of 19 years at Bhagwan Mahavir Medical Research Centre (BMMRC) the recurrent TB rates were 10–12% within 6 months to 2 years of primary TB attack. Globally, they vary from 4.9 to 47% depending on several factors including drug resistance of the bacterial strain. Studying relapse cases is particularly important as multi-drug resistant (MDR) TB is significantly rising currently [52]. According to a recent WHO report an estimated rate of 19% of recurrent TB cases had MDR TB, compared with only 4.1% of the newly diagnosed cases. Little is known about individual genetic risk factors that could influence recurrent TB and to our knowledge no clear correlation with a SNP of PRRs has been found. From our results, we might hypothesize that cGAS has an impact on containing the disease, which is modulated by the G-Allele region of the cGAS gene. cGAS-induced immunity may help against dissemination of TB as well as against developing a relapse, in which dissemination of APCs, at least to the lymph node, would be necessary to activate central memory T cells, but also supports spreading of *M.tb*.

The rs311686 SNP is located upstream of the cGAS gene and most likely affects the transcription rates of the gene. cGAS is known to be highly regulated and cGAS levels are crucial for the threshold of DNA sensing and cellular activation [53]. cGAS is known to be induced by Interferons [54], and further in vitro studies have to be carried in order to check whether the rs311686 variation leads to a different activation pattern of the cGAS promoter. Different levels of expression of cGAS and eventual IFN production might beneficially shift the balance of recognition of *M.tb* and thus antigen-presentation to evoke Th1-response, on the other hand also dissemination, which needs to be further addressed by future studies.

As BCG does not have the ESX-1 locus helping in translocation of bacteria into the cytosol, activation of the cGAS/STING pathway seems unlikely to play a direct role during vaccination. A recent study, however, revealed that activation and maturation of bone marrow derived dendritic cells after *M. bovis* infection is regulated by cGAS/STING/TBK1/IRF3 signaling pathway [55]. We have seen an influence of the rs311686 SNP within the unvaccinated group, thus hinting that it rather acts upon re-infection, when a memory has already been built, and might regulate the efficacy of reactivation. However, further investigation of SNPs associated with cGAS/STING and/or type I IFN production may help us to better understand this pathway and its interaction with BCG vaccination. Uptake and recognition of *M. tuberculosis* by cGAS/Sting and how the genetic variation rs311686 analyzed here potentially may interfer with this pathway is summarized in Fig. 3.

It has been shown that STING has anti-proliferation effects by inducing memory T cells, and the mutations in

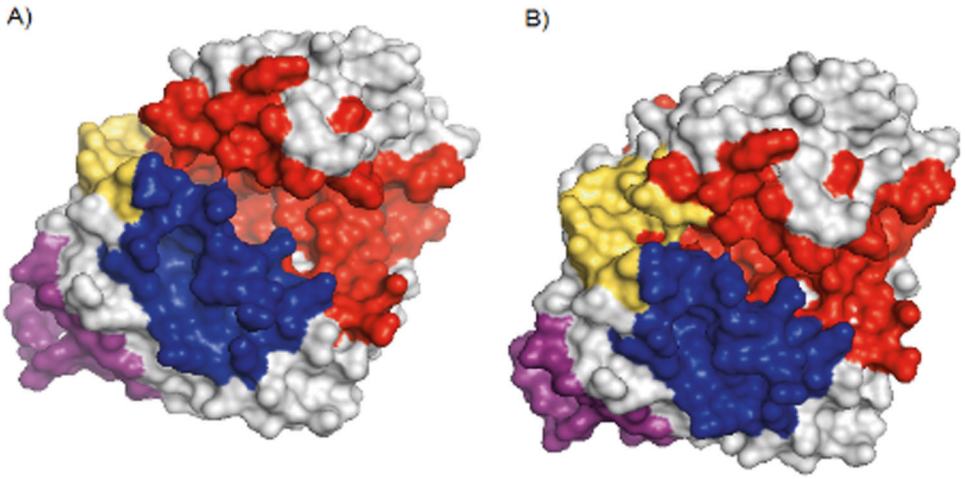

Fig. 1 Structure prediction models of cGAS rs610913 C and A Distribution of functional clefts in **a** P261 variant **b** H261 variant of cyclic GMP-AMP synthase protein. Red, purple, yellow, and blue indicate color of clefts

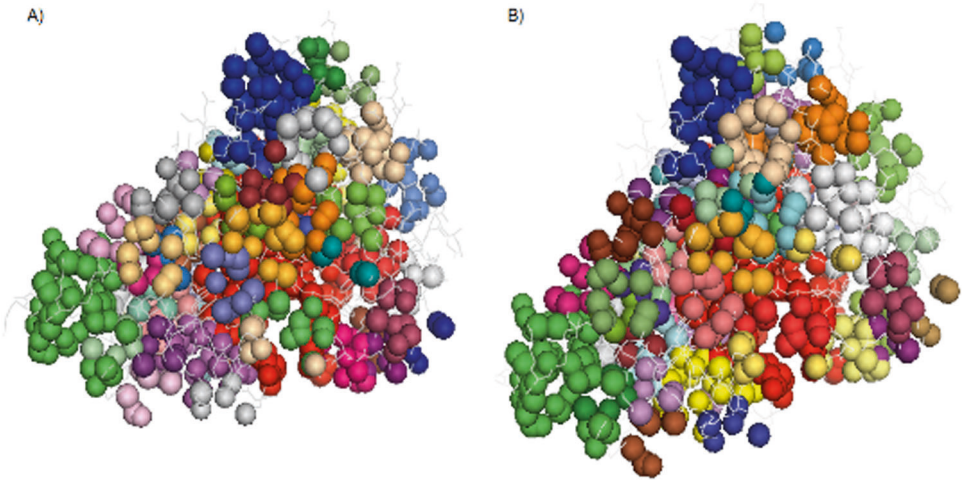

Fig. 2 Functional pockets of cyclic GMP-AMP synthase in different colors representing **a** P261 variant (64 pockets) and **b** H261 variant (66 pockets)

the c-terminal domain of STING might modulate this effect [56]. This also has been discussed to have a role in different types of diseases such as autoimmune diseases, cancer, and infections caused by intracellular pathogens. Hence, functional analysis of this SNP might be beneficial to explain the potential mechanism in disease progression. We failed to find any effects of genetic variations of STING for susceptibility and course of TB.

Structure analysis showed interesting results with respect to the rs610913 sequence of cGAS. At position 261 Proline changes to Histidine upon C to A conversion of the DNA sequence. *MutPred* analysis revealed that this mutation results in loss of Helix ($p < 0.04$) as well as loss of glycosylation ($p < 0.04$), which might cause changes during DNA-induced oligomerization of cGAS. The polymorphism investigated seems to affect the active site of cGAS. The H variant is predicted to have 66 pockets, whereas the P variant would have only 64 pockets making the P variant slightly more flexible. Also there would be a larger volume in the second cleft for the H variant. It has been suggested that larger clefts in proteins correlate with larger binding sites and better chances for increased bonding interactions [57]. Thus, occurrence of a larger cleft in the H variant could hint towards an increased binding interaction. Functional analyses with recombinantly expressed variants of cGAS will be needed to clearly understand the role of the genetic variations for protein function, however.

Previous studies indicate that the residues involved in minor groove binding, arginine fingers and the Zn-thumb were targets to bind dsDNA. Changes in these active sites affect the catalytic rate of cGAS [58]. Others have shown that cGAS interacts with two dsDNA through two binding sites and site B has an important role in the cooperative DNA binding [59]. Another study focused on the necessity of two sites with site A playing a major role in conformational change and site B being involved in DNA binding [60].

We did not observe any significant trend in the frequency of the STING 230 C allele in TB patients as compared to HHC. A previous study on HPV infection also failed to find any significance for the distribution of cGAS 610913 and STING 230 SNPs [61]. Previous studies on structural

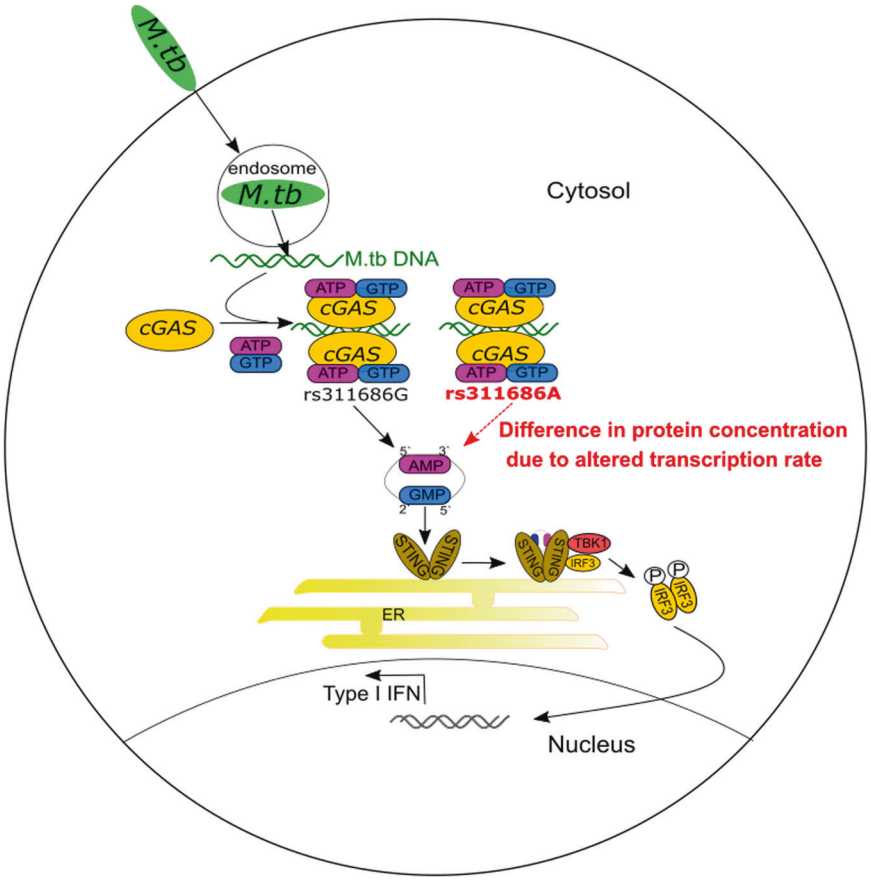

Fig. 3 Schematic overview on *M.tb* DNA recognition by the cGAS/STING system and a potential influence of the rs311686 SNP. After entry of the mycobacteria into the cell via the endosome mycobacterial DNA is released in to the cytosol. This is recognized by cGAS dimers leading to the synthesis of 2'3'-cGAMP from ATP and GTP, which is a ligand for STING. STING together with cGAMP translocates to the endoplasmic reticulum to recruit TBK1 and IRF3. IRF3 subsequently dimerizes and translocates into the nucleus to induce the production of type I IFNs. SNP rs311686G may lead to different concentrations of cGAS followed by a change in dimerization of cGAS finally leading to an altered induction of type I IFN

analyses of STING SNPs revealed that the G230A SNP present in the C-terminal domain has slightly better binding affinity with c-di-GMP as compared with that of wildtype or R232H. The G230A variant is more flexible as compared with the WT variant and can bind more efficiently c-di-GMP [62]. There were several other reports of STING germline missense mutations causing infectious diseases owing to gain-of-function mutations. However, the mechanism of STING SNPs in TB needs to be investigated in further studies. A recent study shows that STING pathway is crucial for DC activation during *M.tb* infection but not involved in host protection in vivo [63]. Although, we did not find any differences in distribution of SNPs in German and Indian healthy cohort, it is important to study these SNPs in larger sample sizes and in different ethnic groups.

In summary, our results obtained from genotyping patient and control cohorts support the hypothesis that the cGAS/STING-induced type I IFN might be important in susceptibility and course of TB. As we have seen differences in the frequency of the rs311686 SNP comparing EPTB and relapse cases, these pathways may be particularly important during the long-term interaction of the host with *M.tb*. cGAS might influence dissemination and memory building in mycobacterial disease, hence, it is important to completely understand the cGAS/STING triggered immunological mechanism in order to potentially develop intervention strategies based on these results. Confirmatory trials including even larger sample size and functional studies of the proteins involved and their genetic variations are needed, which may help us to better stratify risk groups in order to improve prevention and therapy of TB.

Materials and methods

Cohort description

In brief, 725 subjects were studied consisting of 345 TB patients with PTB or EPTB disease, as well as relapse cases, and 380 Controls (HC) including household contacts (HHC). Patients, who attended Free Chest TB Clinic PPM DOTS at Mahavir Hospital and Research Centre, Hyderabad, were confirmed with the sputum microscopy for AFB, culture and chest X-ray or histopathology as per the guidelines of Revised National Tuberculosis Control Program (RNTCP). Patients with diabetes, hypertension, HIV and other comorbid conditions were excluded from the study. Informed consent was obtained from all subjects. The study was approved by the institutional ethics committee of

Bhagwan Mahavir Medical Research Centre (BMMRC), Hyderabad and Charité Medical University Berlin as described earlier [39]. The German cohort consisted of 309 volunteers, all details about this cohort have been described earlier [64]. Buccal swabs were collected to test the volunteer genotypes. Genomic DNA was extracted using the Flexi gene DNA kit from Qiagen, Hilden, Germany, according to the manufacturer's protocol. Quantity of DNA was confirmed by Nano Drop and DNA was stored at − 20 °C.

SNP selection

After analyzing the relevant literature SNPs described in the cGAS/Sting pathway were selected on minor allele frequency and likely functional relevance. This included rs610913 and rs311686 (8.98 kb upstream in the cis-regulatory region) of cGAS. This genetic region was termed c6orf150 and includes the cGAS gene as well as a gene termed DDX43 [65]. Although rs311686 is in the coding region of DDX43 (and consequently now is listed is DDX43 SNP in the databases) it also was suggested to influence the promoter region of cGAS, which is located just downstream of the DDX43 gene [66]. Co-segregation of other genetic variations may also play a role and examination of haplotypes in the future may be needed. The following SNPs for STING were included rs78233829 G/C (aa code G230A), rs1131769 A/G (aa code H232R) and rs7380824 C/T (aa code R293Q). The selected SNPs were non-synonymous, and if in a coding region led to an amino-acid change potentially affecting function of the protein.

Genotyping

The cGAS/STING SNPs were analyzed with the Light Cycler® 480 (Roche). The technique has been previously described [64]. The following primers and probes were designed and synthesized by TIBmolbiol, Berlin, Germany.

rs610913 F: 5′-AATCAAAAGGCAAAAGTCTTACC-3′, R: 5′-GCACCTAATGAATTTGATGTCATG-3′,

Sensor AGCATCTTAGAAGCTGATAATATTTCACCT-FL, Anchor LC640-AACTGACTCAGATGATTTTCT-PH

rs311686 F: 5′-TGTGCGATTATCTGAAATGATTTG-3′, R: 5′-CAAAGCCTAGTTTAAGTAAGCAGG-3′,

Sensor GTTGATGTGCCTCAAACCTCTC-FL, Anchor LC640-GCCATTGATACAAGCTCCTCTGGAATACTC-PH

STING G230A F: 5′-GGGTCTCACTCCTGAATCAGGT-3′, R: 5′-CCGATCCTTGATGCAAGCA-3′,

Sensor GGTCAGCGGTCTGCTGG-FL, Anchor LC640-CAGTTTATCCAGGAAGCGAATGTTGGG-PH

STING H232R F: 5′-CCCACTCCCCTGCACACTT-3′, R: 5′-TGACCCCAACATTCGCTTCC-3′,

Sensor: GGTGACCATGCTGGCATC-FL, Anchor AGGATCGGGTTTACAGCAACAGCA-PH

STING R293Q F: 5′-ACCCTGGTAGGCAATGA-3′, R: 5′-GCTTAGTCTGGTCTTCCTCTTAC-3′,

Sensor: CCTCAAGTGTCCGGCAGAAGAGTT-FL, Anchor: LC640-GGCCTGCTCAAGCCTATCCTCCCGG-PH

In silico analysis

The amino-acid sequence of human cGAS (Accession number: NP_612450.2) was taken from NCBI. *SIFT (v5.1)* [67] and *Polyphen* (http://genetics.bwh.harvard.edu/pph2/) software was used to predict damaging impact of amino-acid substitutions. *SIFT* prediction relied upon nature of conserved amino-acid residues in sequence alignments from PSI-BLAST, whereas *Polyphen* investigated the role of substitution based on physical and evolutionary characteristics. A support vector machine (SVM)-based methodology *SNPs & GO* [68] was implemented to detect mutation related to disease using the protein sequence as input. It uses *Gene Ontology* annotation data to predict disease affinity of a mutation. *Mutpred* [69] was utilized to predict whether the amino-acid substitution was pathogenic or benign. *I-Mutant suite* [70] was instrumental to predict stability changes owing to the mutation from amino-acid sequence. rs610913 was analyzed by taking the X-ray crystal structure of human cGAS (P variant, i.e, P261) in PDB (4KM5) as template, the model of H variant (H261) of the protein was generated using *Swiss Mode* [71]. We analyzed refinement and quality assessments of the model with *Swiss PDB viewer* [72], *ProSA* [73] and *SAVES* server (https://servicesn.mbi.ucla.edu/SAVES/). A comparison of P and H variants with regards to surface topography, nests, tunnels, pores, clefts, cavities, and pockets lodging functional residues were carried out using *ProFunc* [74] and *CASTp* [75]. The structures were visualized with *PyMOL v1.6.0.0*. (www.pymol.org). Figure 3 was drawn using Inkscape version 0.92.3.

Statistical analysis

R (version 3.3.2) was used for all statistical analyses and a p value of < 0.05 was considered significant for all tests except for Hardy–Weinberg equilibrium. The assumptions of homogeneity of variance within the cohorts were met. Ethnical differences in the proposed SNPs of the German and Indian cohort were analyzed by frequency distribution and compared with Hapmap data available at NCBI, which they resembled except for the rs311686 SNP. Baseline characteristics were analyzed by χ^2 tests or Student's t test as appropriate. Comparison of genotype frequencies between patients, HHC, and HC was carried out using a generalized linear model adjusted for age and gender

summarizing for minor genotypes if not otherwise specified. Confidence intervals were calculated for these adjusted p values.

Acknowledgements We thank the staff of the free chest clinic Mahavir PPM DOTS, Tuberculosis Unit (T.U), Bhagwan Mahavir Trust and all patients and volunteers participated in this study. We furthermore thank Dr. Lutz Hamann of the Institute for Microbiology, Charité, Berlin for his help in SNP selection and genotyping. We are grateful to Juan Ruiz Moreno, Department of Infectious Diseases, Charité, Berlin for important discussions of STING SNPs, and we thank Dr. Surabhi Goyal, Institute for Microbiology, Charité, Berlin, and ZIK Septomics, Jena, Germany for her co-operation during cohort collection in India. We finally thank Frauke Schreiber of the Institute for Microbiology, Charité, Berlin for her excellent technical assistance.

Funding Special thanks to our financial supporters, the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) International Research Training Group (IRTG), GRK 1673 "Functional molecular infection epidemiology" project A5 (to RRS) and project A4 (to HS) and to the Sonnenfeld Stiftung, Berlin, Germany (to ST).

Compliance with ethical standards

Conflict of interest All authors have no conflict of interest.

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References

1. Gringhuis SI, den Dunnen J, Litjens M, van Het Hof B, van Kooyk Y, Geijtenbeek TB. C-type lectin DC-SIGN modulates Toll-like receptor signaling via Raf-1 kinase-dependent acetylation of transcription factor NF-kappaB. Immunity. 2007;26:605–16.
2. Yonekawa A, Saijo S, Hoshino Y, Miyake Y, Ishikawa E, Suzukawa M, et al. Dectin-2 is a direct receptor for mannose-capped lipoarabinomannan of mycobacteria. Immunity. 2014;41:402–13.
3. Ishikawa E, Ishikawa T, Morita YS, Toyonaga K, Yamada H, Takeuchi O, et al. Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. J Exp Med. 2009;206:2879–88.
4. Jo EK. Mycobacterial interaction with innate receptors: TLRs, C-type lectins, and NLRs. Curr Opin Infect Dis. 2008;21:279–86.
5. Stamm LM, Morisaki JH, Gao LY, Jeng RL, McDonald KL, Roth R, et al. Mycobacterium marinum escapes from phagosomes and is propelled by actin-based motility. J Exp Med. 2003;198:1361–8.
6. Hagedorn M, Rohde KH, Russell DG, Soldati T. Infection by tubercular mycobacteria is spread by nonlytic ejection from their amoeba hosts. Science. 2009;323:1729–33.
7. Mostowy S, Bonazzi M, Hamon MA, Tham TN, Mallet A, Lelek M, et al. Entrapment of intracytosolic bacteria by septin cage-like structures. Cell Host Microbe. 2010;8:433–44.
8. van der Wel N, Hava D, Houben D, Fluitsma D, van Zon M, Pierson J, et al. M. tuberculosis and M. leprae translocate from the phagolysosome to the cytosol in myeloid cells. Cell. 2007;129:1287–98.
9. Smith J, Manoranjan J, Pan M, Bohsali A, Xu J, Liu J, et al. Evidence for pore formation in host cell membranes by ESX-1-secreted ESAT-6 and its role in Mycobacterium marinum escape from the vacuole. Infect Immun. 2008;76:5478–87.
10. Stanley SA, Johndrow JE, Manzanillo P, Cox JS. The Type I IFN response to infection with Mycobacterium tuberculosis requires ESX-1-mediated secretion and contributes to pathogenesis. J Immunol. 2007;178:3143–52.
11. Sun L, Wu J, Du F, Chen X, Chen ZJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science. 2013;339:786–91.
12. Dorhoi A, Nouailles G, Jorg S, Hagens K, Heinemann E, Pradl L, et al. Activation of the NLRP3 inflammasome by Mycobacterium tuberculosis is uncoupled from susceptibility to active tuberculosis. Eur J Immunol. 2012;42:374–84.
13. Kupz A, Zedler U, Staber M, Perdomo C, Dorhoi A, Brosch R, et al. ESAT-6-dependent cytosolic pattern recognition drives noncognate tuberculosis control in vivo. J Clin Invest. 2016;126:2109–22.
14. Labzin LI, Lauterbach MA, Latz E. Interferons and inflammasomes: cooperation and counterregulation in disease. J Allergy Clin Immunol. 2016;138:37–46.
15. Wu J, Sun L, Chen X, Du F, Shi H, Chen C, et al. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science. 2013;339:826–30.
16. Ishikawa H, Barber GN. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature. 2008;455:674–8.
17. Saitoh T, Fujita N, Hayashi T, Takahara K, Satoh T, Lee H, et al. Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response. Proc Natl Acad Sci USA. 2009;106:20842–6.
18. Tanaka Y, Chen ZJ. STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway. Sci Signal. 2012;5:ra20.
19. Liu S, Cai X, Wu J, Cong Q, Chen X, Li T, et al. Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation. Science. 2015;347:aaa2630.
20. Zhang Y, Yang J, Bai G. Cyclic di-AMP-mediated interaction between Mycobacterium tuberculosis DeltacnpB and macrophages implicates a novel strategy for improving BCG vaccination. Pathog Dis. 2018;76:2.
21. Burdette DL, Vance RE. STING and the innate immune response to nucleic acids in the cytosol. Nat Immunol. 2013;14:19–26.
22. Watson RO, Manzanillo PS, Cox JS. Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. Cell. 2012;150:803–15.
23. Collins AC, Cai H, Li T, Franco LH, Li XD, Nair VR, et al. Cyclic GMP-AMP synthase Is an Innate Immune DNA Sensor for Mycobacterium tuberculosis. Cell Host Microbe. 2015;17:820–8.
24. Wassermann R, Gulen MF, Sala C, Perin SG, Lou Y, Rybniker J, et al. Mycobacterium tuberculosis differentially activates cGAS- and inflammasome-dependent intracellular immune responses through ESX-1. Cell Host Microbe. 2015;17:799–810.
25. Watson RO, Bell SL, MacDuff DA, Kimmey JM, Diner EJ, Olivas J, et al. The cytosolic sensor cGAS detects mycobacterium tuberculosis DNA to induce type I interferons and activate autophagy. Cell Host Microbe. 2015;17:811–9.
26. Tamayo R, Pratt JT, Camilli A. Roles of cyclic diguanylate in the regulation of bacterial pathogenesis. Annu Rev Microbiol. 2007;61:131–48.
27. Nandakumar R, Tschismarov R, Meissner F, Prabakaran T, Krissanaprasit A, Farahani E, et al. Intracellular bacteria engage a STING-TBK1-MVB12b pathway to enable paracrine cGAS-STING signalling. Nat Microbiol. 2019;4:701–13.
28. Van Dis E, Sogi KM, Rae CS, Sivick KE, Surh NH, Leong ML, et al. STING-activating adjuvants elicit a Th17 immune response

and protect against mycobacterium tuberculosis infection. Cell Rep. 2018;23:1435–47.
29. Chen Q, Sun L, Chen ZJ. Regulation and function of the cGAS-STING pathway of cytosolic DNA sensing. Nat Immunol. 2016;17:1142–9.
30. Gao D, Li T, Li XD, Chen X, Li QZ, Wight-Carter M, et al. Activation of cyclic GMP-AMP synthase by self-DNA causes autoimmune diseases. Proc Natl Acad Sci USA. 2015;112:E5699–705.
31. Gall A, Treuting P, Elkon KB, Loo YM, Gale M Jr., et al. Autoimmunity initiates in nonhematopoietic cells and progresses via lymphocytes in an interferon-dependent autoimmune disease. Immunity. 2012;36:120–31.
32. Lio CW, McDonald B, Takahashi M, Dhanwani R, Sharma N, Huang J, et al. cGAS-STING signaling regulates initial innate control of cytomegalovirus infection. J Virol. 2016;90:7789–97.
33. Shu C, Yi G, Watts T, Kao CC, Li P. Structure of STING bound to cyclic di-GMP reveals the mechanism of cyclic dinucleotide recognition by the immune system. Nat Struct Mol Biol. 2012;19:722–4.
34. Yin Q, Tian Y, Kabaleeswaran V, Jiang X, Tu D, Eck MJ, et al. Cyclic di-GMP sensing via the innate immune signaling protein STING. Mol Cell. 2012;46:735–45.
35. Huang YH, Liu XY, Du XX, Jiang ZF, Su XD. The structural basis for the sensing and binding of cyclic di-GMP by STING. Nat Struct Mol Biol. 2012;19:728–30.
36. Abel L, Fellay J, Haas DW, Schurr E, Srikrishna G, Urbanowski M, et al. Genetics of human susceptibility to active and latent tuberculosis: present knowledge and future perspectives. Lancet Infect Dis. 2018;18:e64–e75.
37. Schroder NW, Schumann RR. Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious disease. Lancet Infect Dis. 2005;5:156–64.
38. Ugolini M, Gerhard J, Burkert S, Jensen KJ, Georg P, Ebner F, et al. Recognition of microbial viability via TLR8 drives TFH cell differentiation and vaccine responses. Nat Immunol. 2018;19:386–96.
39. Dittrich N, Berrocal-Almanza LC, Thada S, Goyal S, Slevogt H, Sumanlatha G, et al. Toll-like receptor 1 variations influence susceptibility and immune response to Mycobacterium tuberculosis. Tuberculosis (Edinb). 2015;95:328–35.
40. Brinkworth JF. Infectious disease and the diversification of the human genome. Hum Biol. 2017;89:47–65.
41. Rasaiyaah J, Tan CP, Fletcher AJ, Price AJ, Blondeau C, Hilditch L, et al. HIV-1 evades innate immune recognition through specific cofactor recruitment. Nature. 2013;503:402–5.
42. Ablasser A, Gulen MF. The role of cGAS in innate immunity and beyond. J Mol Med (Berl). 2016;94:1085–93.
43. Dorhoi A, Yeremeev V, Nouailles G, Weiner J 3rd, Jorg S, Heinemann E, et al. Type I IFN signaling triggers immunopathology in tuberculosis-susceptible mice by modulating lung phagocyte dynamics. Eur J Immunol. 2014;44:2380–93.
44. Antonelli LR, Gigliotti Rothfuchs A, Goncalves R, Roffe E, Cheever AW, Bafica A, et al. Intranasal Poly-IC treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen-permissive monocyte/macrophage population. J Clin Invest. 2010;120:1674–82.
45. Manca C, Tsenova L, Freeman S, Barczak AK, Tovey M, Murray PJ, et al. Hypervirulent M. tuberculosis W/Beijing strains upregulate type I IFNs and increase expression of negative regulators of the Jak-Stat pathway. J Interferon Cytokine Res. 2005;25:694–701.
46. Manca C, Tsenova L, Bergtold A, Freeman S, Tovey M, Musser JM, et al. Virulence of a Mycobacterium tuberculosis clinical isolate in mice is determined by failure to induce Th1 type immunity and is associated with induction of IFN-alpha /beta. Proc Natl Acad Sci USA. 2001;98:5752–7.
47. Desvignes L, Wolf AJ, Ernst JD. Dynamic roles of type I and type II IFNs in early infection with Mycobacterium tuberculosis. J Immunol. 2012;188:6205–15.
48. Groschel MI, Sayes F, Shin SJ, Frigui W, Pawlik A, Orgeur M, et al. Recombinant BCG expressing ESX-1 of Mycobacterium marinum combines low virulence with cytosolic immune signaling and improved TB protection. Cell Rep. 2017;18:2752–65.
49. Keegan C, Krutzik S, Schenk M, Scumpia PO, Lu J, Pang YLJ, et al. Mycobacterium tuberculosis transfer RNA induces IL-12p70 via synergistic activation of pattern recognition receptors within a cell network. J Immunol. 2018;200:3244–58.
50. de Toledo-Pinto TG, Ferreira AB, Ribeiro-Alves M, Rodrigues LS, Batista-Silva LR, Silva BJ, et al. STING-dependent 2'-5' oligoadenylate synthetase-like production is required for intracellular mycobacterium leprae survival. J Infect Dis. 2016;214:311–20.
51. Crofts JP, Andrews NJ, Barker RD, Delpech V, Abubakar I. Risk factors for recurrent tuberculosis in England and Wales, 1998-2005. Thorax. 2010;65:310–4.
52. van der Werf MJ, Langendam MW, Huitric E, Manissero D. Multidrug resistance after inappropriate tuberculosis treatment: a meta-analysis. Eur Respir J. 2012;39:1511–9.
53. Ablasser A, Chen ZJ. cGAS in action: expanding roles in immunity and inflammation. Science. 2019;363:6431.
54. Schoggins JW, MacDuff DA, Imanaka N, Gainey MD, Shrestha B, Eitson JL, et al. Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. Nature. 2014;505:691–5.
55. Li Q, Liu C, Yue R, El-Ashram S, Wang J, He X, et al. cGAS/STING/TBK1/IRF3 signaling pathway activates BMDCs maturation following Mycobacterium bovis Infection. Int J Mol Sci. 2019;20:E895.
56. Cerboni S, Jeremiah N, Gentili M, Gehrmann U, Conrad C, Stolzenberg MC, et al. Intrinsic antiproliferative activity of the innate sensor STING in T lymphocytes. J Exp Med. 2017;214:1769–85.
57. Laskowski RA, Luscombe NM, Swindells MB, Thornton JM. Protein clefts in molecular recognition and function. Protein Sci. 1996;5:2438–52.
58. Civril F, Deimling T, de Oliveira Mann CC, Ablasser A, Moldt M, Witte G, et al. Structural mechanism of cytosolic DNA sensing by cGAS. Nature. 2013;498:332–7.
59. Li X, Shu C, Yi G, Chaton CT, Shelton CL, Diao J, et al. Cyclic GMP-AMP synthase is activated by double-stranded DNA-induced oligomerization. Immunity. 2013;39:1019–31.
60. Shu C, Li X, Li P. The mechanism of double-stranded DNA sensing through the cGAS-STING pathway. Cytokine Growth Factor Rev. 2014;25:641–8.
61. Xiao D, Huang WH, Ou ML, Guo CC, Ye XG, Liu Y, et al. Interaction between susceptibility loci in cGAS-STING pathway, MHC gene and HPV infection on the risk of cervical precancerous lesions in Chinese population. Oncotarget. 2016;7:84228–38.
62. Yi G, Brendel VP, Shu C, Li P, Palanathan S, Cheng Kao C. Single nucleotide polymorphisms of human STING can affect innate immune response to cyclic dinucleotides. PLoS ONE. 2013;8:e77846.
63. Marinho FV, Benmerzoug S, Rose S, Campos PC, Marques JT, Bafica A, et al. The cGAS/STING pathway is important for dendritic cell activation but is not essential to induce protective immunity against mycobacterium tuberculosis infection. J Innate Immun. 2018;10:239–52.
64. Oh DY, Taube S, Hamouda O, Kuecherer C, Poggensee G, Jessen H, et al. A functional toll-like receptor 8 variant is associated with HIV disease restriction. J Infect Dis. 2008;198:701–9.
65. Perry JR, Corre T, Esko T, Chasman DI, Fischer K, Franceschini N, et al. A genome-wide association study of early menopause and

the combined impact of identified variants. Hum Mol Genet. 2013;22:1465–72.
66. Serre D, Gurd S, Ge B, Sladek R, Sinnett D, Harmsen E, et al. Differential allelic expression in the human genome: a robust approach to identify genetic and epigenetic cis-acting mechanisms regulating gene expression. PLoS Genet. 2008;4:e1000006.
67. Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003;31:3812–4.
68. Calabrese R, Capriotti E, Fariselli P, Martelli PL, Casadio R. Functional annotations improve the predictive score of human disease-related mutations in proteins. Hum Mutat. 2009;30:1237–44.
69. Li B, Krishnan VG, Mort ME, Xin F, Kamati KK, Cooper DN, et al. Automated inference of molecular mechanisms of disease from amino acid substitutions. Bioinformatics. 2009;25:2744–50.
70. Capriotti E, Calabrese R, Casadio R. Predicting the insurgence of human genetic diseases associated to single point protein mutations with support vector machines and evolutionary information. Bioinformatics. 2006;22:2729–34.
71. Guex N, Peitsch MC, Schwede T. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: a historical perspective. Electrophoresis. 2009;30:S162–73.
72. Kaplan W, Littlejohn TG. Swiss-PDB viewer (Deep View). Brief Bioinform. 2001;2:195–7.
73. Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res. 2007;35:W407–10.
74. Laskowski RA, Watson JD, Thornton JM. ProFunc: a server for predicting protein function from 3D structure. Nucleic Acids Res. 2005;33:W89–93.
75. Dundas J, Ouyang Z, Tseng J, Binkowski A, Turpaz Y, Liang J. CASTp: computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues. Nucleic Acids Res. 2006;34:W116–8.