



# MST1R mutation as a genetic cause of Lady Windermere syndrome

To the Editor:

The prevalence of pulmonary nontuberculous mycobacterial (pNTM) disease is increasing [1]. The most commonly isolated disease-causing NTMs belong to the *Mycobacterium avium* complex [1]. Susceptibility to and clinical manifestation of NTM disease are largely governed by the immune status of a person. Disseminated or extrapulmonary NTM infections are strongly associated with severe immunosuppression, such as those with frank defects in the interferon (IFN)- $\gamma$ -interleukin (IL)-12 axis [2]. Isolated pNTM is strongly associated with certain underlying conditions, such as cystic fibrosis, chronic obstructive pulmonary disease and primary ciliary dyskinesia [3, 4]. However, substantial numbers of pNTM patients have no apparent risk factors, and a significant proportion of them exhibit a body morphotype characterised by lifelong slender body habitus, pectus excavatum, scoliosis and mitral valve prolapse [5, 6], also called the Lady Windermere syndrome. A modest reduction in IFN- $\gamma$  production and an increase in transforming growth factor (TGF)- $\beta$  levels have been described [7–10]. FOWLER *et al.* [11] quantified ciliary beat frequency of 58 pNTM patients and 40 controls and found reduced ciliary beat frequency in the pNTM patients. SZYMANSKI *et al.* [12] performed whole-exome sequencing on patients with pNTM, their unaffected family members and a control group and concluded that pNTM is a multigenic disease, encompassing potential defects in proteins encoded by cilia genes, the cystic fibrosis transmembrane conductance regulator gene, connective tissue genes and certain immune-related genes.

Since pectus excavatum and scoliosis have been described in several genetic disorders that are not necessarily notable for increased susceptibility to lung infections, this body phenotype in pNTM patients may have a more specific genetic basis, that could account not only for their body phenotype, but also their increased vulnerability to pulmonary infections [13]. Our goal was to determine the genetic basis of Lady Windermere syndrome, namely the pNTM with pectus excavatum and scoliosis (pNTM<sup>PEX/scoliosis</sup>) by performing whole-exome sequencing in 11 individuals with this phenotype and functional validation of the genetic findings.

11 individuals with pNTM<sup>PEX/scoliosis</sup> (two sisters and nine sporadic cases) were recruited from National Jewish Health and the University of Colorado Anschutz Medical Campus (Denver, CO, USA) as part of a prior study [5]. In whole-exome sequencing *MST1R* was the only gene with a previously described role in innate immunity or cilia function that harboured rare nonsynonymous variants in three families.

The two sisters (patients 1 and 2 in family 1; figure 1a) carried a very rare missense variant (p.V900M) in *MST1R*, having a very low population frequency of 0.0004794 or 28 per 58 406 public exomes based on Exome Aggregation Consortium (ExAC) data (<http://exac.broadinstitute.org>). Cosegregation analysis in their extended family revealed that three siblings and three children are carriers of the same *MST1R* variant (figure 1a). Two of the nine sporadic pNTM<sup>PEX/scoliosis</sup> cases also have missense variants in the *MST1R* gene. Patient 3 had a private missense variant not previously reported (p.M1383T) and patient 4 possessed a rare missense variant (p.D176N) with a very low population frequency of 0.0009082 or 53 per 58 360 public exomes (<http://exac.broadinstitute.org>). Cosegregation analyses within the respective families showed that in patients 3 and 4 of families 2 and 3, the *MST1R* variant was paternally inherited (figure 1a). Sanger sequencing revealed that all 29 pNTM patients without pectus excavatum or scoliosis were negative for rare *MST1R* variants.

We next addressed cytokine production of pNTM<sup>PEX/scoliosis</sup> patients. Whole-blood stimulation experiments revealed significantly lower *M. intracellulare*-induced IFN- $\gamma$  production in all pNTM patients compared to the control group, as previously reported [5] (figure 1b). In addition, IL-10 production in the pNTM<sup>PEX/scoliosis</sup> patients was lower after stimulation with lipopolysaccharide, *Staphylococcus epidermidis* or *M. intracellulare* (data not shown). The mean concentrations of IL-6 were not significantly different



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Susceptibility to NTM infections in patients with Lady Windermere syndrome is associated with *MST1R* variants <http://ow.ly/xKgX304uhse>

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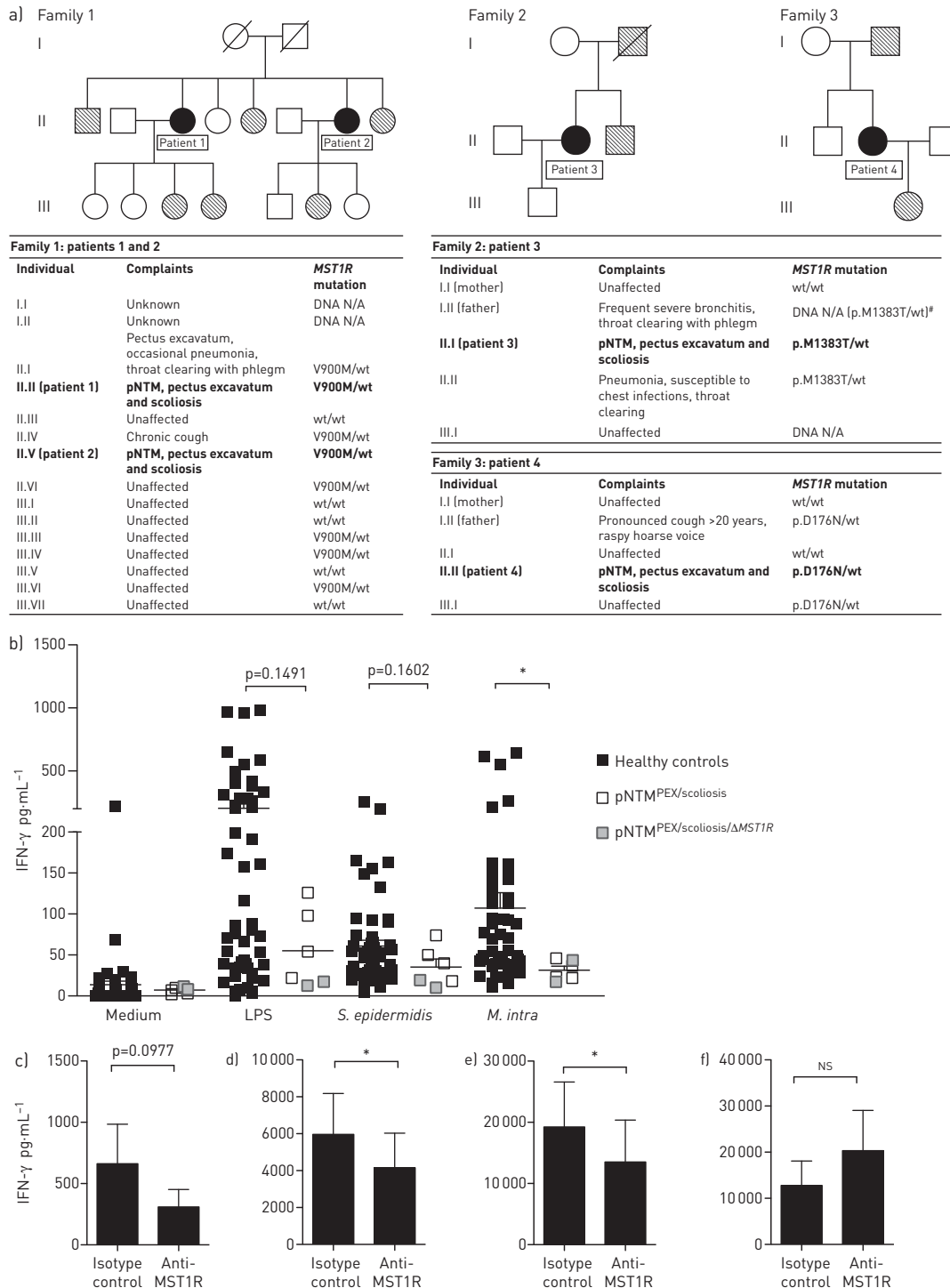


FIGURE 1 a) Family 1: the two sisters with pulmonary nontuberculous mycobacterial disease with pectus excavatum and scoliosis (pNTM<sup>PEX/scoliosis</sup>) carry a mutation ( $\Delta$ ) in *MST1R*. Three of their siblings also have  $\Delta$ *MST1R* and two of them have chronic respiratory symptoms. Family 2: the index patient with pNTM<sup>PEX/scoliosis</sup> has  $\Delta$ *MST1R*. One brother of the index patient has the same *MST1R* mutation and has frequent chest infections. <sup>#</sup>: because two of his children carry the *MST1R* mutation, the deceased father of the family is the expected carrier of the mutation, although his DNA was not available. Family 3: the index patient with pNTM<sup>PEX/scoliosis</sup> has  $\Delta$ *MST1R*. Both the father and daughter of the index patient also carry the *MST1R* mutation; the father has a chronic cough, whereas the daughter has no health issues or complaints. b) Whole blood of healthy controls (n=52), patients with pNTM<sup>PEX/scoliosis</sup> (n=4) and pNTM<sup>PEX/scoliosis</sup> patients carrying the *MST1R* mutation (pNTM<sup>PEX/scoliosis</sup>/ $\Delta$ *MST1R*) (n=2) was stimulated with a medium control, lipopolysaccharide (LPS), heat-killed *Staphylococcus epidermidis* and live *Mycobacterium intracellulare*. Interferon (IFN)- $\gamma$  was measured in the supernatant using ELISA. The Mann-Whitney U-test was used to determine whether the means were significantly different. c-f) Peripheral blood mononuclear cells of healthy donors were stimulated for 48 h with c) heat-killed *M. avium* (n=6); d) live *M. avium* (n=6); e) heat-killed *M. avium* plus interleukin (IL)-12 (n=6) or f) IL-12/IL-18 (n=7) in the presence of a *MST1R*-neutralising antibody (anti-*MST1R*) or its isotype control antibody. IFN- $\gamma$  was measured in the cell culture supernatant using ELISA. The Wilcoxon signed rank test was used to determine whether the mean values were significantly different. N/A: not available; wt: wild-type; \*: p<0.05; NS: nonsignificant.

between pNTM<sup>PEX/scoliosis</sup> patients and controls (data not shown). In contrast, the anti-inflammatory cytokine TGF- $\beta$  was significantly increased in pNTM<sup>PEX/scoliosis</sup> patients compared to controls (data not shown), also shown in a prior study [7]. Comparing pNTM<sup>PEX/scoliosis</sup> patients with and without the variant in *MST1R* revealed equally low or even lower levels of IFN- $\gamma$  in whole-blood stimulation (figure 1b).

To investigate the immunological relevance of *MST1R*, we stimulated peripheral blood monocyte cells (PBMCs) from healthy individuals with heat-killed *M. avium*, live *M. avium*, heat-killed *M. avium*+IL-12 or IL-12+IL-18 in the absence or presence of a *MST1R*-neutralising antibody, and measured IFN- $\gamma$  levels (figure 1d–f). Blocking *MST1R* resulted in a significantly lower level of IFN- $\gamma$  in response to live *M. avium*  $\pm$ IL-12 and a trend toward reduced IFN- $\gamma$  production with heat-killed *M. avium* (figure 1c–e). However, with IL-12+IL-18 stimulation of the PBMCs, there was no difference in IFN- $\gamma$  production with or without anti-*MST1R* antibody (figure 1f). Furthermore, blocking *MST1R* had no effect on *M. avium* (heat-killed or live) stimulation of IL-10, IL-6 or TGF- $\beta$  by the PBMCs (data not shown). Taken together, the increased TGF- $\beta$  response was independent of *MST1R*, since it was observed in all pNTM patients, while the *M. avium*-specific IFN- $\gamma$  response was specifically dependent on *MST1R*. This reveals a previously undescribed potential mechanism by which *MST1R* plays a host defence role against NTM.

Interestingly, *MST1R* variants were recently reported in three pNTM patients in a cohort of 77 NTM patients [12]. While this is consistent with our findings, it is interesting to speculate that the higher frequency of *MST1R* variants in our smaller cohort may be due to more stringent selection of patients with both pectus excavatum and scoliosis. To evaluate this possibility, we performed Sanger sequencing in 29 patients without these physical characteristics and found no rare genetic variation in *MST1R*. How *MST1R* variants might be related to the presence of pectus excavatum/scoliosis is unknown; furthermore, because pectus excavatum/scoliosis are also present in some patients without *MST1R* mutation there is a strong possibility that pectus excavatum/scoliosis are associated with anomalies of more than one gene. Indeed, this notion is supported by the finding that pectus excavatum/scoliosis is seen in Marfan syndrome and in several other connective tissue disorders, such as the Loeys–Dietz and Shprintzen–Goldberg syndromes [13]. Interestingly, all these connective tissue disorders are caused by increased TGF- $\beta$  signalling, albeit through different mechanisms.

*MST1R* is highly expressed on airway epithelial cells and increases mucociliary function, *i.e.* binding and activation of *MST1R* by its ligand macrophage-stimulated protein leads to a significant increase of the ciliary beat frequency [14]. Thus, a defect in the mucociliary transport due to a *MST1R* mutation could lead to impaired clearance of NTM, resulting in a vicious cycle of airway inflammation and infection leading to the bronchiectasis typically seen in patients with pNTM. These genetic findings are consistent with the study showing that adult patients with pNTM and without known predisposing factors have reduced ciliary beat frequency [11]. Thus, defects in *MST1R* function can specifically attenuate IFN- $\gamma$  production as well as decrease airway ciliary function, with both defects increasing susceptibility to pNTM. The decrease in IFN- $\gamma$  production was specific for the stimulation with live *M. avium*, since blocking *MST1R* did not decrease IL-12+IL-18-induced IFN- $\gamma$  production. The reason for this phenomenon is not known; it might be speculated that *M. avium* has a direct ligand for *MST1R*.

In conclusion, we have identified rare variants in *MST1R* in four out of 11 pNTM<sup>PEX/scoliosis</sup> patients, and suggest that these genetic variants contribute to Lady Windermere syndrome by decreasing airway ciliary function and reducing IFN- $\gamma$  production in response to NTM.

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