



LETTERS

Hereditary pulmonary alveolar proteinosis caused by recessive *CSF2RB* mutations

To the Editors:

Pulmonary alveolar proteinosis (PAP) is a syndrome characterised by accumulation of surfactant in alveoli resulting in respiratory insufficiency [1]. Surfactant homeostasis is critical for lung function and is tightly regulated, in part, by pulmonary granulocyte-macrophage colony-stimulating factor (GM-CSF), which is required for surfactant clearance by alveolar macrophages [2] and alveolar macrophage maturation [1]. The effects of GM-CSF are mediated by cell-surface receptors composed of GM-CSF-binding α -chains and affinity-enhancing β -chains (encoded by *CSF2RA* and *CSF2RB*, respectively) [3]. Ligand binding activates signalling *via* multiple pathways including the signal transducer and activator of phosphorylation (STAT)5 [4]. Disruption of GM-CSF signalling causes PAP by impairing surfactant catabolism in alveolar macrophages [1]. In 90% of patients, PAP is caused by neutralising GM-CSF auto-antibodies [5, 6]. Through the Rare Lung Diseases Network global PAP detection programme, we identified PAP caused by recessive *CSF2RA* mutations and developed novel diagnostic methods to identify patients with PAP caused by GM-CSF receptor dysfunction [4, 7]. Herein, we report a case of hereditary PAP caused by disruption of GM-CSF receptor β -chain function.

A previously healthy 9-yr-old female presented with bilateral pneumonia, followed 3 months later by progressive dyspnoea of insidious onset. The diagnosis of PAP was suggested by chest radiograph findings, high-resolution computed tomography and bronchoalveolar cytology, and was confirmed by surgical lung biopsy. Pulmonary histopathology was typical of primary PAP (fig. 1) and she was successfully treated by serial whole lung lavage therapy. Details of the case history are included in the online supplement. A GM-CSF auto-antibody test was negative and the serum GM-CSF level was increased ($25.9 \text{ pg}\cdot\text{mL}^{-1}$) suggesting GM-CSF receptor dysfunction as the molecular basis of PAP [4, 7]. A molecular evaluation was undertaken and included GM-CSF receptor detection, STAT-5 phosphorylation, *CSF2RA* and *CSF2RB* nucleotide sequencing, and cloning as reported previously [4, 7]. GM-CSF receptor α - and β -chains were detected on blood leukocytes from the patient and all family members by flow cytometry and Western blotting (data not shown) [4, 7]. Nucleotide sequencing of leukocyte mRNA and genomic DNA revealed a normal *CSF2RA* sequence for the patient and all family members (data not shown). A single *CSF2RB* point mutation (c.812C>T) in exon 7 was identified in both mRNA and DNA from the patient (who was homozygous for the mutation) and from

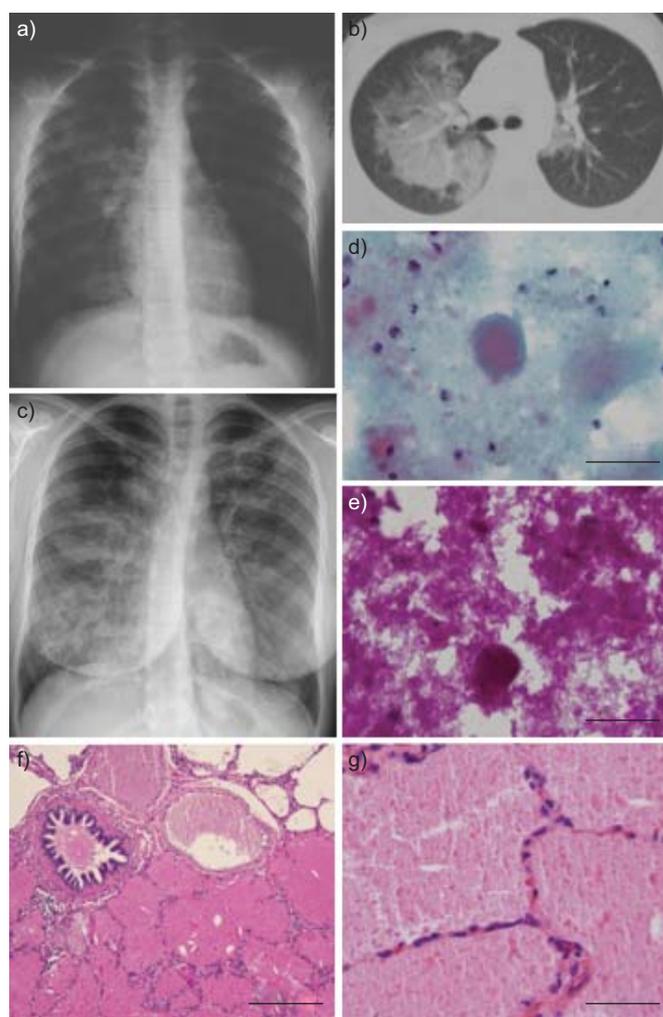


FIGURE 1. Radiographic and histopathological appearance of the lungs of the patients with pulmonary alveolar proteinosis caused by recessive *CSF2RB*^{S271L} mutations. a) Posterior-anterior chest radiograph at the time of diagnosis, 9 yrs of age. Alveolar infiltrates are present throughout both lung fields. b) High-resolution computed tomography of the chest. Ground-glass opacification is superimposed on thickened interlobular and septal lines. c) Chest radiograph prior to an annual whole lung lavage therapy at 17 yrs of age. d) Bronchoalveolar lavage (BAL) fluid cytology at diagnosis (Papanicolaou stain). e) BAL fluid cytology at diagnosis (Periodic acid-Schiff (PAS) stain). f) Surgical lung biopsy obtained at diagnosis. Note the presence of PAS-staining material filling alveoli and also present in terminal airways. g) High-power view showing that alveolar wall architecture is normal (haematoxylin and eosin stain). d, e, g) Scale bars=50 μm . f) Scale bar=200 μm .

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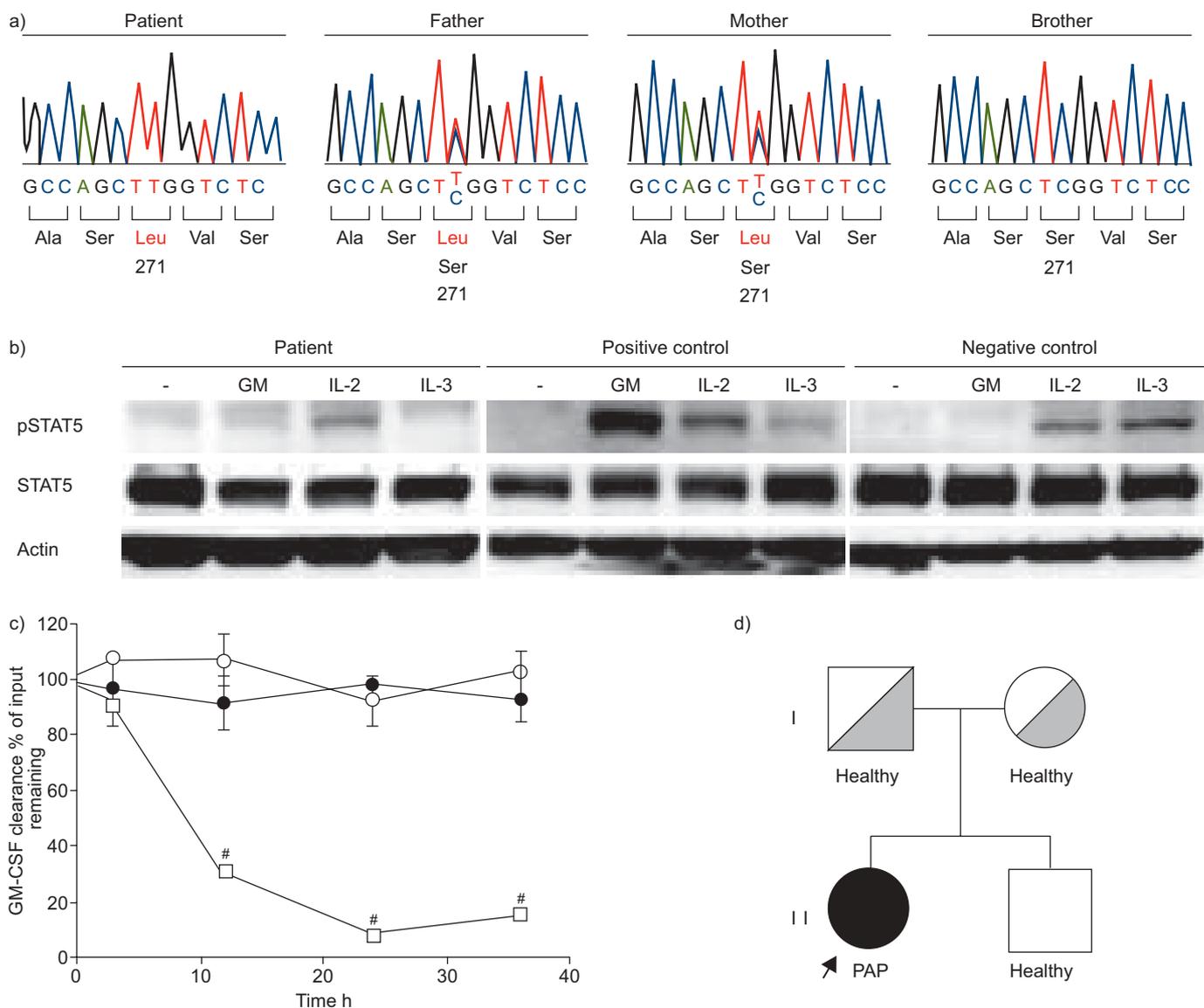


FIGURE 2. Nucleotide sequence of *CSF2RB*, granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor signalling, GM-CSF clearance and pedigree of the patient's family. a) PCR-based nucleotide sequencing of genomic DNA using *CSF2RB*-specific primers (5'-TCTCGGAGCTGTGGACACA-3', 5'-AAGATGCTCACCCTGCATCTG-3'). Amino acid sequence numbers are based on the human *CSF2RB* cDNA with first base of the ATG codon labelled +1 (Genbank accession number NM_000395). A single C>T substitution at nucleotide 812 results in a single amino acid substitution Ser>Leu at amino acid 271. The patient was homozygous and both parents were heterozygous for this mutation (*CSF2RB*^{S271L}) while the brother did not carry the mutation. b) GM-CSF receptor signalling analysis. Fresh heparinised blood from the patient, a healthy individual (positive control) or a patient with recessive *CSF2RA*^{R217X} mutations (negative control) was incubated alone (-) or with GM-CSF (GM), interleukin (IL)-2 or IL-3 (each at 10 ng·mL⁻¹) for 15 min followed by Western blot analysis to detect phosphorylated signal transducer and activator of transcription (pSTAT5), total STAT5 or actin (to ensure equal loading of cell lysates). The IL-3 control demonstrates that the β -chain can function independently of the GM-CSF receptor α -chain. c) Evaluation of cell/receptor-mediated GM-CSF clearance. Blood leukocytes isolated from the patient (●) or a healthy individual (□) or dishes of culture media without cells (○) were incubated with GM-CSF (1 ng·mL⁻¹) added at time 0. At subsequent times, GM-CSF concentration was measured in culture media by ELISA as described previously [4]. #: significant differences in levels of GM-CSF in media in plates containing control cells compared to plates with no cells. d) Segregation of the *CSF2RB*^{S271L} allele in the patient's family. ●: homozygous individuals; ■: heterozygous individuals; □: non-carriers of the *CSF2RB*^{S271L} allele. The health status of each member is indicated and the arrow indicates the proband.

her parents (both heterozygous) but not from her brother (fig. 2a). This mutation caused a single amino acid change (p.Ser271Leu) in the GM-CSF receptor β -chain that impaired STAT5 phosphorylation in blood leukocytes following stimulation by GM-CSF and interleukin (IL)-3, but not IL-2 (fig. 2b). Since IL-3 stimulates STAT-5 phosphorylation *via* the β -chain

common to GM-CSF, IL-3 and IL-5 receptors [8], we used IL-3 as an alternative means to demonstrate β -chain dysfunction in our patient (fig. 2b). Since IL-2 stimulates STAT5 phosphorylation independent of the β -chain, we used IL-2 as a positive control to demonstrate STAT5 phosphorylation in the patient's cells (fig. 2b). Consistent with disruption of GM-CSF receptor

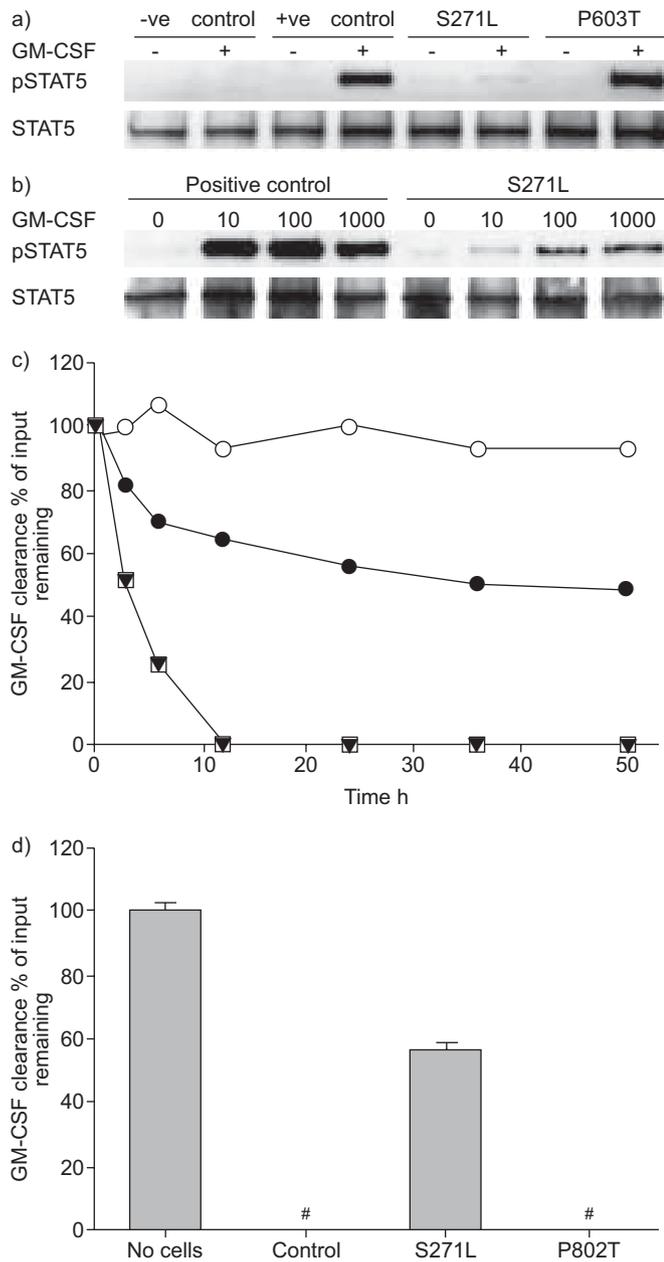


FIGURE 3. Reproduction and function of granulocyte-macrophage colony-stimulating factor (GM-CSF) receptors harbouring the *CSF2RB*^{S271L} mutation. Human embryonic kidney epithelial cells (293 cells) were evaluated alone (negative (-ve) control) or after transfection-mediated expression of a normal *CSF2RA* allele together with either a normal *CSF2RB* allele (positive (+ve) control), the patient's allele (*CSF2RB*^{S271L}) or a previously proposed mutant *CSF2RB* allele (*CSF2RB*^{P603T}; previously named P602T using a different naming convention [9]). a) Cells were incubated with (+) or without (-) GM-CSF (10 ng·mL⁻¹, 15 min) and evaluated by immunoprecipitation and Western blotting as described previously [4] to detect phosphorylated signal transducer and activator of transcription (pSTAT)5 or total STAT5. Note, the *CSF2RB*^{P603T}-derived receptors resulted in signalling equal to the normal GM-CSF receptors. b) The same experiment was performed as in a) but increased GM-CSF concentrations were used for stimulation. Note, the partial signalling of *CSF2RB*^{S271L}-derived GM-CSF receptors. c) Evaluation of the effects of the *CSF2RB*^{S271L} (●) or *CSF2RB*^{P603T} (▼) alleles on cell-mediated GM-CSF clearance. GM-CSF (1 ng·mL⁻¹) was added at time 0 and subsequently measured in culture media at the indicated times by ELISA. Note, the *CSF2RB*^{S271L}-derived receptors cleared GM-CSF at an intermediate rate compared to healthy control (*CSF2RB*^{Normal}; □) and that *CSF2RB*^{P603T}-derived receptors had normal GM-CSF clearance. ○: no cells. d) Evaluation of the effects of *CSF2RB*^{S271L} and *CSF2RB*^{P603T} mutations on cell-mediated GM-CSF clearance. Results are similar to those in c) except that measurements were made 24 h after initiating GM-CSF exposure (n=3 independent determinations in each group). p<0.01 for comparison to cell-free control. #: GM-CSF was undetectable.

Previously, a *CSF2RB*-point mutation causing a substitution at amino acid 603 (P603T) was proposed as the molecular basis of PAP [10]. We evaluated this mutation using the gene cloning approach described previously. The P603T mutation did not affect GM-CSF receptor function (fig. 3a) or cell-mediated GM-CSF clearance (fig. 3c and d). Furthermore, population studies showed it is present in ~6% of the general population (NCPI single nucleotide polymorphism Database ID rs1801122; www.genecards.org). Based on these results, *CSF2RB*^{P603T} represents a sequence polymorphism rather than a genetic cause of PAP as proposed [10]. Thus, this is the first reported case of hereditary PAP due to *CSF2RB* mutations.

Our findings demonstrate that *CSF2RB* is critical for surfactant homeostasis in humans and that homozygous but not heterozygous *CSF2RB* mutations impairing GM-CSF receptor function cause a hereditary form of primary PAP. They extend prior findings that PAP is caused by the absence of GM-CSF receptor β in mice [10]. The age at onset, presentation, biomarkers, pulmonary histopathology, natural history and response to whole lung lavage therapy are similar to those of hereditary PAP caused by *CSF2RA* mutations [4, 7]. Except for the earlier age at onset and some biomarkers (increased serum GM-CSF and absence of GM-CSF auto-antibodies), many features are similar to those of patients with autoimmune PAP [1]. The remarkable similarities in pathogenic, radiographic, histopathological and clinical findings in PAP caused by *CSF2RA* [4, 7], *CSF2RB* mutations or high levels of neutralising GM-CSF auto-antibodies in humans [1], by injection of patient-derived human GM-CSF auto-antibodies in healthy non-human primates [6] or by *CSF2RB* mutations or GM-CSF deficiency in mice [2], suggest the pathogenesis of each is similar and support the usefulness of grouping them together as primary PAP. The partial functioning of *CSF2RB*^{S271L}-derived receptors suggests that aerosolised GM-CSF therapy may be of clinical benefit for this patient.

function, cell-mediated clearance of GM-CSF was impaired in the patient's leukocytes compared to those from a healthy control (fig. 2c). These results demonstrate that homozygous but not heterozygous *CSF2RB*^{S271L} mutations impair GM-CSF receptor function in parallel with the occurrence of PAP (fig. 2d).

Gene cloning and expression of GM-CSF receptors in human 293 cells [4, 7] reproduced the signalling defect caused by the *CSF2RB*^{S271L} mutation (fig. 3a). Interestingly, use of increased concentrations of GM-CSF for stimulation demonstrated partial functioning GM-CSF receptors derived from the *CSF2RB*^{S271L} allele (fig. 3b). Consistent with this, cell-mediated clearance of GM-CSF by receptors harbouring this mutation was reduced but not absent (fig. 3c and d).

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The CIBERES Pulmonary Biobank Consortium: an opportunity for cooperative international respiratory research

To the Editors:

Research into the pathogenesis of diseases often requires access to appropriate tissue specimens [1]. The lung is not an easily accessible organ. Hence, respiratory research is often hampered by the lack of a large number of adequately preserved lung samples harvested from patients whose phenotype had been carefully and consistently characterised [2].

Since 2006, the Spanish government has funded a national network for respiratory research (CIBERES), which is currently formed of 34 research groups working cooperatively on the investigation of basic, clinical and epidemiological aspects of the main respiratory diseases (www.ciberes.org) [3]. To facilitate translational respiratory research, CIBERES has taken advantage of its multicentric nature and has designed, organised and established a nonprofit CIBERES Pulmonary Biobank Consortium (CPBC) that follows the recommendations of the International Society for Biological and Environmental

Repositories [4] and Organisation for Economic Co-operation and Development [5]. The CPBC initiative is fully funded by public, competitive research funds, and is similar to the Lung Tissue Research Consortium (LTRC) sponsored by the National Institutes of Health in the USA. Its goal is to coordinate and manage the common collection of lung tissue samples and other related samples (whole blood, plasma and serum) from well-characterised patients in order to support studies on chronic obstructive pulmonary disease, asthma, lung cancer and other respiratory pathologies, as well as smoking effects. The text that follows describes briefly the main characteristics of the CPBC to the European community of respiratory researchers. Those interested in knowing more about the CPBC or contacting it are encouraged to visit its website (<http://biobancopulmonar.ciberes.org>).

The CPBC is a network currently formed by ten tertiary Spanish hospitals (see Acknowledgements section) that voluntarily joined the initiative and agreed to provide: 1) lung tissue