RTEL1 mutations are associated with heterogeneous pulmonary and extra-pulmonary phenotypes

Supplementary materials

Methods

Whole exome sequencing and bioinformatics analysis

Whole human exome sequencing was performed by Otogenetics (Norcross, Georgia, USA) using the Agilent's V5 and PE Illumina HiSeq2000 sequencing with an estimated 50-fold average coverage. Sequences were aligned to the reference human genome hg19 using the Burrows-Wheeler Aligner. Downstream processing was carried out with the Genome Analysis Toolkit (GATK), SAMtools, and Picard, following documented best practices (http://www.broadinstitute.org/gatk/guide/topic?name=best-practices). Variant calls were made with the GATK Unified Genotyper. All variants were annotated using a software system developed by the Paris Descartes University Bioinformatics platform. All the annotation process was based on the 72 version of ENSEMBL database. Querying and filtering were made by POLYWEB, an in-house bioinformatics tool (Imagine Institute, Université Paris Descartes, Paris, France).

***RTEL1* sequencing**

To confirm the presence of the mutations identified by whole-exome sequencing on the DNA of the probands and to study the cosegregation of the mutation with the phenotype in the families, exons carrying the mutations of *RTEL1* were sequenced by fluorescent bi-directional sequencing (primers sequences are available on request) and compared to the reference sequences of the 2 main *RTEL1* transcripts expressed in human cells (NM_016434.3 and NM_032957.4).

RTEL1 variations were retained to be pathogenic or likely pathogenic as recommended according to several criteria when available: nature of the variant, absence or rare frequency of the variant in the general population; previous report, segregation data, telomere length, computational and predictive in silico data (1).

Telomere length measurement

Telomere length measurement was performed by Telomeric Restriction Fragment assay (TRF). Genomic DNA (800ng) extracted from whole blood cells was digested with HinfI and RsaI enzymes, resolved by a 0.7% agarose gel, and transferred to a nylon membrane. Hybridization was performed using EasyHyb solution (Roche) with **γ**-32P-labeled $(TTAGGG)_4$ probe. After washes, membranes were exposed over a PhosphorImager. PhosphorImager exposures of telomere-probed Southern blots were analyzed with the ImageJ program. The digitalized signal data were then transferred to Microsoft Excel and served as the basis for calculating mean TRF length using the formula L = (ODi)/(ODi/Li), where ODi = integrated signal intensity at position i and Li = length of DNAfragment in position i.

RESULTS

PATIENTS

Eleven patients were previously described [16, 20]. Of note, 4 probands with RA-ILD were evidenced from the cohort of 101 patients with RA-ILD and 13 other probands were evidenced from the cohort of 151 patients with suspected monogenic pulmonary fibrosis. Median age at diagnosis of ILD of the patients from the second generation was 51.0 years vs 53.6 years for the patients from the first generation (p=0.12). Altogether only 6 patients (17%) were neither smoker nor had any known exposure that may be associated with ILD. Ten patients (28%) were considered to have exposure to inhaled fibrogenic particles: birds

(n=4), cocaine (n=1), sanding (n=2), solvent (n=1), hay (n=1), metals dust (n=1). One patient received radiotherapy for breast cancer a few months before the diagnosis of ILD.

CT scan was available for central reading for 31 patients (88%) and classified as typical UIP (n=17, 54.8%), probable UIP (n=8, 25.8%), indeterminate for UIP (n=1, 3.2%), and inconsistent with UIP (n=5, 16.1%) (Figure 2). Among these 5 patients, CT scan was suggestive of non-specific interstitial pneumonia (n=3), sarcoidosis (n=1) or pneumoconiosis (n=1). None was suggestive of pleuro parenchymal fibro-elastosis (PPFE). Six patients (17.1%) had emphysema in addition to ILD on CT scan.

Lung biopsy was available for review for 10 patients (surgical lung biopsy (n=8) and/or lung transplantation (n=4)). The histological pattern was definite UIP for 6 patients (all with possible or typical UIP pattern on CT scan) and probable UIP for 1 patient (Figure 2), non-specific interstitial pneumonia for 2 patients superimposed with desquamative interstitial pneumonia for one (both patients presented inconsistent UIP pattern on CT scan), and pneumoconiosis in one patient (who presented an increased load of mica particles in the lung).

Regarding patients with IPAF, one patient presented Raynaud's phenomenon, auto-immune hepatitis with antinuclear antibodies $\geqslant$1:320, and a typical UIP pattern on CT scan. Two patients presented a Raynaud's phenomenon with an NSIP pattern on CT scan with ANCA without specificity. During follow-up, one patient developed lung cancer.

The pulmonary diagnosis was IPF (n=17, 48%) or unclassifiable diagnosis with a working diagnosis of IPF (n=3, 8%), RA-ILD (n=4, 18%), interstitial pneumonia with auto-immune features (IPAF, n=3, 12%), chronic hypersensitivity pneumonitis (n=2, 7%), sarcoidosis (n=1, 4%), pneumoconiosis (n=1), unclassifiable fibrosis (n=4, 18%).

One patient complained of difficulty to become pregnant, the 34 other patients had one or more children. One patient presented skeletal abnormalities (agenesis of several fingers) as previously described (2), however neither his mother nor his daughters, all carriers of the same mutation, presented skeletal abnormalities. Four patients presented peripheral arthritis and were diagnosed with RA seropositive for rheumatoid factor and/or anti-citrullinated peptide antibodies.

Four patients developed bone marrow disease: transient agranulocytosis post cyclophosphamide (n=2), acute leukemia (n=1), pancytopenia after lung transplantation (Tables 2 and 3). Two patients from the same family presented neutropenia after cyclophosphamide treatment, whereas 4 other patients received cyclophosphamide without cytopenia. No other specific serious adverse events attributable to therapy were reported.

Among those patients, one patient received liver transplantation, developed lung fibrosis 10 years after transplantation and died from lung fibrosis 12 years after liver transplantation. No patient presented an hepato-pulmonary syndrome.

Of note, one female patient developed breast cancer.

Four patients received lung transplantation and were treated with prednisone, mycophenolate mofetil and tacrolimus after transplantation. One patient developed chronic rejection, and received another lung transplantation. He developed again chronic rejection and finally died of respiratory insufficiency 50 months after first transplantation. Two patients experienced hematological complications after transplantation. One presented cytopenia while also receiving cotrimoxazole and ganciclovir and the cytopenia resolved with suppression of the above-cited cytotoxic drugs. The other one presented macrocytosis and thrombocytopenia at ILD diagnosis and developed severe pancytopenia after lung

transplantation. She finally died 52 months after lung transplantation from gastro-intestinal bleeding attributable to thrombocytopenia. Two patients still presented pulmonary improvement 35 and 47 months after transplantation.

Bibliography

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