Determination of loss of chromosome Y in peripheral blood cells in males with idiopathic pulmonary fibrosis

Received: 5 July 2024
Accepted: 21 Sept 2024

To the Editor:

Idiopathic pulmonary fibrosis (IPF) is a devastating and usually progressive disease associated with ageing [1]. The incidence and prevalence of IPF have been reported to be higher in males than in females, although the reasons are unclear [2–4].

Strong evidence demonstrates that mosaic loss of the Y chromosome (LOY), the male-specific sex chromosome, is a common feature in the blood of elderly men, and that it is associated with short life expectancy, and a wide variety of ageing-related diseases [5, 6]. LOY refers to chromosome Y aneuploidy acquired during lifetime which is the most common post-zygotic variant described in human blood cells [7]. In addition to ageing, LOY is likely enhanced by genetic and environmental factors. Thus, over 150 genetic variants in autosomes associated with LOY have been found, including gene variants involved in the regulation of the cell cycle, chromatin structure during mitosis, and kinetochore structure and function [8]. From environmental factors, tobacco smoking, also associated with IPF, represents the major risk factor for ageing-related LOY, although the effects of smoking on LOY are transient and dose-dependent [9].

In this study, we tested the hypothesis that LOY might be involved in higher frequency of IPF in males.

Blood samples from 57 male patients with IPF from our Interstitial Lung Diseases Clinic, at the National Institute of Respiratory Diseases, diagnosed according to the American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Asociación Latinoamericana de Tórax clinical practice guideline [10], and 57 age-matched (69+7 *versus* 68±6 years old; p=0.4), healthy male controls from our cohort of the Lung Aging Program were collected at the baseline visit. Our Lung Aging Program is an observational, cross-sectional study, enrolling individuals over 60 years of age, without chronic lung diseases and without respiratory symptoms at the time of the study. Patients and controls were of Mexican mestizo ancestry. No differences were found in smoking habits. Thus, 36 IPF patients and 35 controls were ever smokers (89% and 78%, former smokers), without significant differences in the number of years smoked (p=0.3) and in smoking history (18 *versus* 11 pack-years; p=0.07).

Genomic DNA was extracted employing a commercial kit (BDtract Genomic DNA Isolation Kit; Maxim Biotech, San Francisco, CA, USA) following the manufacturer's instructions, and was quantified by ultraviolet spectrophotometry using a NanoDrop 2000 device (Thermo Fisher Scientific, Waltham, MA, USA). The DNA samples were stored at −20°C until use.

Quantification of LOY was conducted by digital PCR (QIAcuity One instrument; Qiagen, Hilden, Germany), a sensitive method for quantifying copy number variation that has been used to measure LOY in blood [11]. The relative amount of Y chromosome was determined as a chromosome Y/chromosome X ratio (Y/X ratio) of fluorescent signal of co-amplified short sequences from the YX homologous amelogenin genes (AMELY and AMELX). These genes are discriminated by a six-base pair deletion in intron 1 of AMELX that is absent in AMELY [11]. As previously described, we evaluated the presence or absence of LOY, coding Y/X ratios <0.9 as LOY and Y/X ratios ⩾0.9 as normal [12].

Using Spearman's correlation coefficients, we did not find a significant negative correlation between age and LOY in either patients or controls, likely because only two decades were virtually included in the

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Idiopathic pulmonary fibrosis prevalence is higher in males. Loss of chromosome Y (LOY) occurs in elderly men, and is associated with ageing-related diseases. This study did not find association between IPF and LOY, likely due to the ethnic background. https://bit.ly/3ZIQHKi

Cite this article as: Espinosa M, Herrera I, Buendía-Roldán I, *et al*. Determination of loss of chromosome Y in peripheral blood cells in males with idiopathic pulmonary fibrosis. *Eur Respir J* 2024; 64: 2401303 [DOI: 10.1183/13993003.01303-2024].

comparison (figure 1a and b). In fact, to support the well-known negative correlation of LOY and age, we included in the control group four young healthy Mexican mestizo individuals (22±2 years, never smokers) which gave a significant negative correlation (figure 1c). These young individuals were not used in the comparison of controls with IPF patients.

The mean±SD of the Y/X ratio was 0.95±0.09 in IPF patients *versus* 0.93±0.10 in controls (p=0.28). There was no significant difference in the frequency of LOY between the IPF and control groups. Thus, 13 of 57 (22.8%) IPF patients and 17 of 57 (29.8%) controls exhibited presence of LOY (Y/X ratio <0.9; p=0.5). Also, among IPF patients we did not find association between LOY values and smoking history, haematic biometry parameters (some correlations have been reported [13]), and lung function tests (forced vital capacity and diffusing capacity of the lung for carbon monoxide).

In contrast to our results, in a recent study (currently published as a preprint prior to full peer review) it was found that there was an effect of peripheral blood LOY in patients with pulmonary fibrosis (mostly IPF), but it was minimal, and was likely driven primarily by age and telomere length [14]. Moreover, bidirectional Mendelian randomisation did not find any causal effects, but gave indicative evidence that shorter telomere length was on the causal pathway for LOY [14]. Interestingly, although the mechanisms by which cells lose Y chromosome are unclear, it has been suggested that one of them is the influence of telomere shortening that occurs during ageing, leading to greater instability and degradation of Y chromosome [15].

In the aforementioned study by WANG *et al.* [14], a genetic approach was used, but it has been demonstrated that there is a high concordance between estimates of LOY in samples pairwise evaluated with whole genome sequencing and digital PCR, method that we used in our study [11]. With this method, our results suggest that LOY is not involved in the higher frequency of IPF in males.

In part, the reason for the different result may be related with ancestry. In this context, a recent multi-ancestry study, involving 25 517 ancestrally diverse male participants, showed that the age-adjusted frequency of LOY is higher in European American individuals compared to East Asian, African American and Hispanic American individuals, the latter most closely related to the Mexican mestizo population that

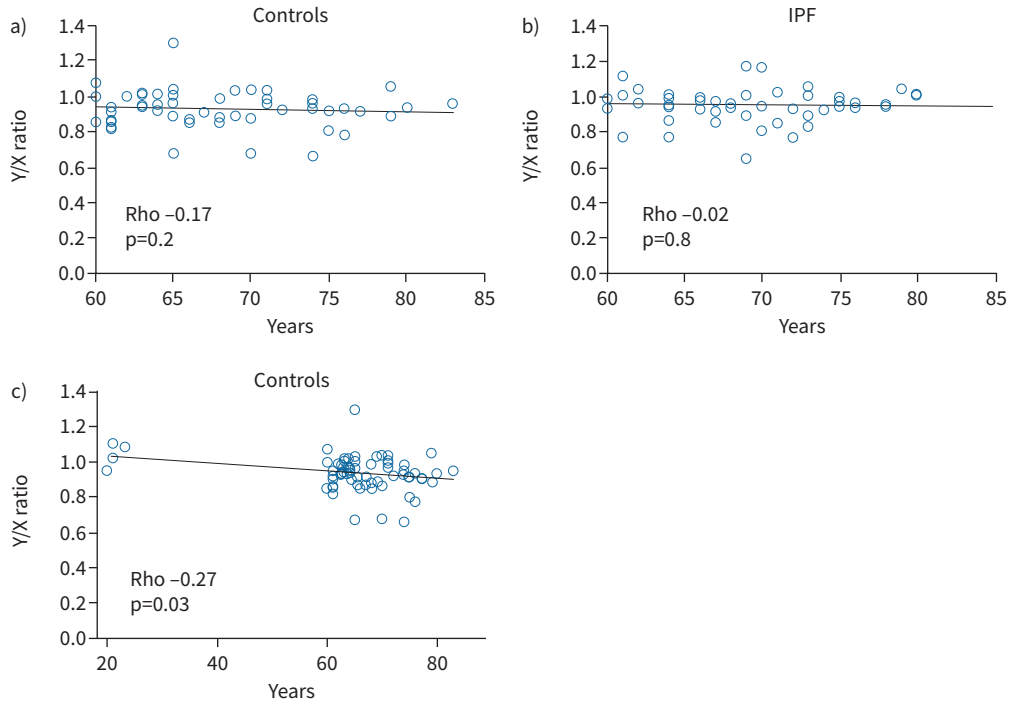

FIGURE 1 Relationship between age and Y/X ratio in blood samples from a) controls, b) patients with idiopathic pulmonary fibrosis (IPF) and c) controls including four young healthy Mexican mestizo individuals. All p-values and rho values were calculated using Spearman's test.

was evaluated in our study [15]. These differences appeared to be driven at least partially by common protective variants at *TCL1A* (T cell leukaemia/lymphoma protein 1A), an oncogene, and *BCL2L1* (Bcl2-like protein 1), a key antiapoptotic gene, which are more common in African American and Hispanic American individuals.

On the other hand, it is important to emphasise that serial analysis of LOY in healthy individuals has revealed a deep inter-individual variation over time. In some subjects, the level of LOY increased with age, while in others, the level did not change substantially during a long follow-up time. Moreover, in a few subjects there is an initial increase followed by a decrease, or *vice versa* [11].

Our study has some limitations that necessitate cautious interpretation. These are primarily related to the small sample size, since the limited statistical power may make it difficult to detect a weak association such as that found by Wang *et al*. [14]. Conversely, the strengths of the study include the similar age and smoking habits between IPF patients and controls, since strong evidence supports the association between these factors and LOY.

In conclusion, our results suggest that, at least in a Mexican population, LOY does not contribute to the higher frequency of IPF in males, and indicate that future studies approaching LOY in this disease should carefully identify the ethnic background of the patients and controls.

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Ethics statement: The study protocol was approved by the scientific and ethics committee, and informed consent was obtained from patients and controls.

Conflict of interest: The authors have no potential conflicts of interest to disclose.

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