Supplemental Material

Data S1.

Supplemental Methods

Laboratorial methods

Plasma lyso-Gb3 was quantified by liquid chromatography tandem-mass spectrometry (Agilent, ultra performance liquid chromatography [UPLC] triple quadrupole [QqQ] electrospray ionization [ESI]). The quantification was achieved by multiple reaction monitoring (MRM) of the transitions mass-to-charge ratios (m/z) 786,4→282,3 and, as internal standard 1-β-D-glucosylsphingosine (GSG), 460,5→280,3. The result was extrapolated from the calibration curve obtained from responses of calibrators of known concentrations versus internal standard.

PICP was measured in serum, by an ELISA method (QUIDEL Corporation®, Hannover, Germany; category number 8003), according to manufacturer instructions. The microelisa strip plate is pre-coated with human PICP monoclonal antibody and the kit uses p-nitrophenyl phosphate substrate to measure the immune complex obtained during the reaction; final colorimetric reaction is measured by photometry at 405 nm.

ICTP was measured in serum, by an ECLIA method (Roche® Diagnostics GmBH, Mannheim; Germany; reference 11972308122) in an automated analyser COBAS e411 instrument, according to manufacturer instructions. The detection of the marker in the samples is made using human ICTP monoclonal antibody.

MMP-1 was measured also in serum, in an assay using a double-antibody sandwich ELISA (SunRed® Biotechnology Company; category number 201-12-0917), according to the instructions of the manufacturer. The microelisa strip plate is pre-coated with human MMP-1 monoclonal antibody and the kit uses biotin-streptavidin-HRP technology for measure de immune complex obtained during the reaction; final colorimetric reaction is measured by photometry at 450 nm.

MMP-2 was measured in serum, also by a double-antibody sandwich ELISA assay (SunRed® Biotechnology Company; category number 201-12-0905), according to the instructions of the manufacturer. The microelisa strip plate is pre-coated with human MMP-2 monoclonal antibody and the kit uses biotin-streptavidin-HRP technology for measure de immune complex obtained during the reaction; final colorimetric reaction is measured by photometry at 450 nm.

B-AP was measured in serum, by ELISA methodology (QUIDEL Corporation®, Hannover, Germany; category number 8012), according to the instructions of the manufacturer. The microelisa strip plate is pre-coated with human B-AP monoclonal antibody and thee kit uses p-nitrophenyl

phosphate substrate to measure the immune complex obtained during the reaction; final colorimetric reaction is measured by photometry at 405 nm.

TRAP-5b was measured in serum, by an ELISA assay (ids, Immunodiagnostic Systems®, United Kingdom; category number SB-TR201A), according to the manufacturer instructions. The microelisa strip plate is pre-coated with human TRAP5b monoclonal antibody and the kit uses p-nitrophenyl phosphate substrate to measure the immune complex obtained during the reaction; final colorimetric reaction is measured by photometry at 405 nm.

Table S1. Mutation frequency.

Mutation	n	%
p.N215S	10	16.7
p.F113L	9	15.0
p.G35E	7	11.7
c.700_702del	2	3.3
p.R227X	2	3.3
p.C52G	2	3.3
p.L166P	2	3.3
p.N42V	2	3.3
p.R342Q	2	3.3
unknown	2	3.3
other*	20	33.3

* Mutations presented by only one patient within the study

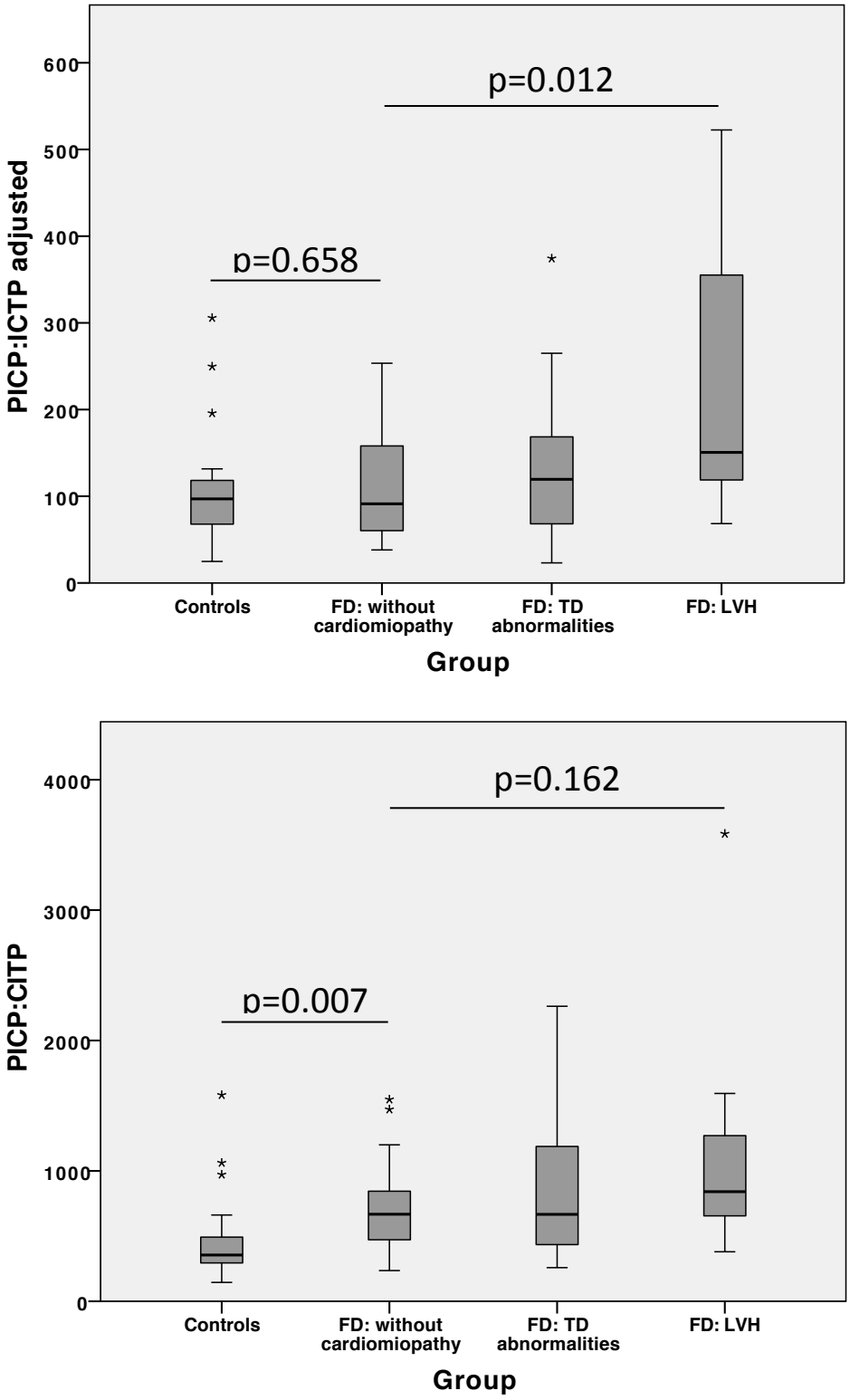

Figure S1. Boxplot of PICP to ICTP ratio (before and after adjustment for bone collagen turnover) in controls and FD subgroups.

PICP: carboxy-terminal propeptide of procollagen type I; ICTP: carboxy-terminal telopeptide of type I collagen.

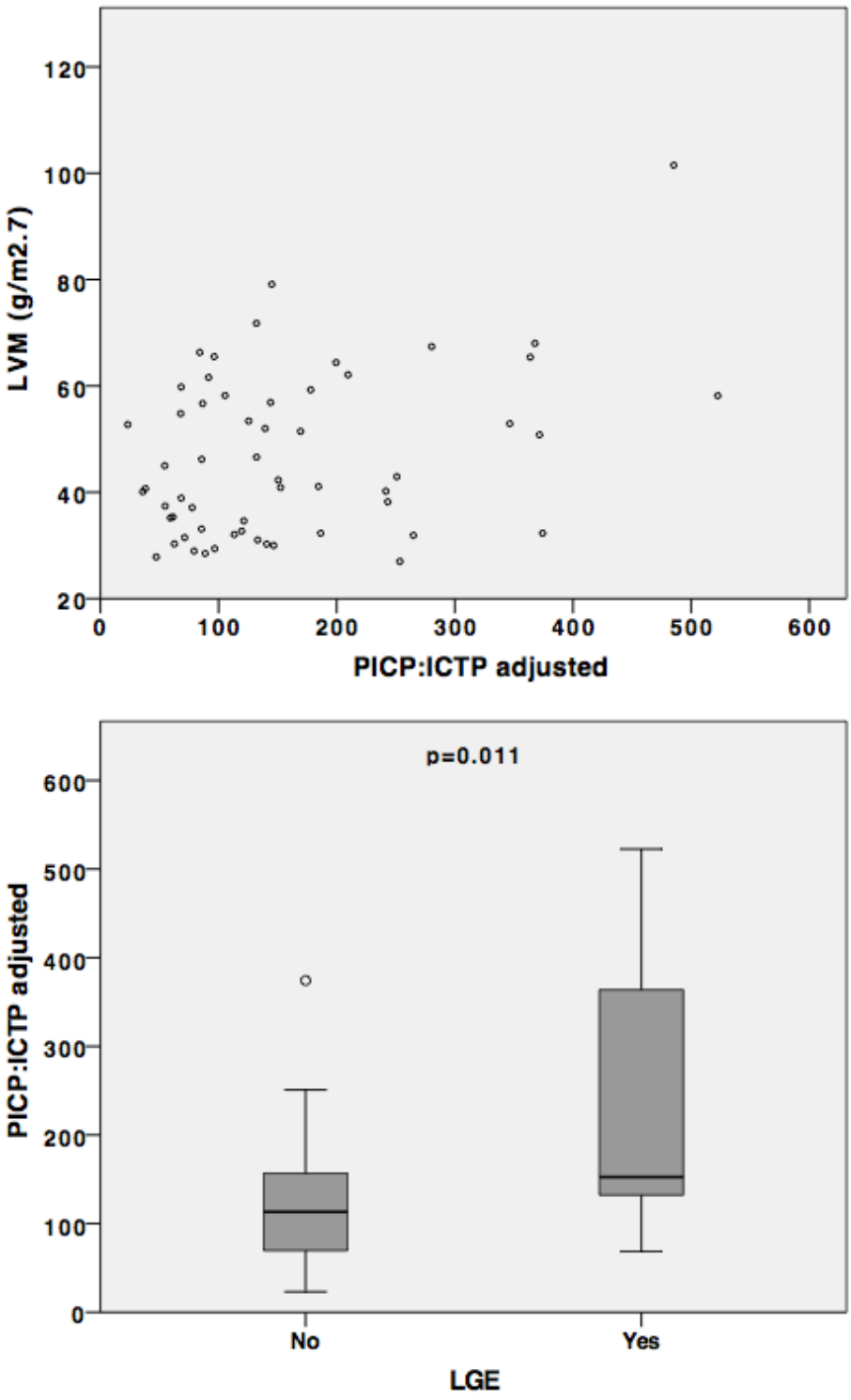

Figure S2. Correlation between PICP to ICTP ratio (after adjustment for bone collagen turnover) and left ventricular mass (upper image); boxplot of PICP to ICTP ratio (after adjustment for bone collagen turnover) in LGE negative and positive patients (lower image).

PICP: carboxy-terminal propeptide of procollagen type I; ICTP: carboxy-terminal telopeptide of type I collagen; LVM: left ventricular mass; LGE: late gadolinium enhancement.