Natural history and modifiers of respiratory and cardiac function in the Italian Duchenne muscular dystrophy Network

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While the natural history of Duchenne has been well described in relation to progressive muscle weakness, respiratory and cardiac parameters have not been as extensively characterized, despite their impact on life quality and expectancy. We aimed to collect retrospective data regarding cardio-respiratory function, i.e. standard spirometry and echocardiography, in a large DMD cohort followed at Centers in the Italian DMD Network. Besides describing longitudinal trajectories of these measures in Italian DMD patients, we focused on potential modifiers: glucocorticoid corticosteroid (GC) treatment, *DMD* mutations amenable to different molecular treatments, and known genetic modifiers, i.e. single nucleotide polymorphisms (SNPs) in genes different from *DMD*.

We collected Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 second (FEV1), and Peak Expiratory Flow (PEF) from longitudinal spirometries carried out in 327 patients over a mean follow-up of 4.5±3.9 years; and Ejection Fraction (EF), Shortening Fraction (SF), and Left Ventricular End Diastolic Volume (LV-EDV) from longitudinal echocardiograms carried out in 372 patients over 2.6±3.7 years. We used Generalized Estimating Equation models to evaluate the yearly change in these measures, as well as the effects of specific *DMD* mutations (e.g. involving vs. spare=ing the short dystrophin isoforms Dp140/Dp71; amenable to skipping of exons 8, 44, 45, 51, and 53; nonsense) and of four SNP modifiers: rs28357094 in the promoter of the osteopontin-coding *SPP1* gene, rs10880 within the IAAM haplotype of Latent Transforming growth factor-β Binding Protein 4 (*LTBP4*), rs1883832 in the 5'UTR of *CD40*, and the nonsense *ACTN3* rs1815739 variant.

We observed an annual decrease of FVC of -4.2%, FEV1 -5.1%, and PEF -2.9%. EF and SF also decreased steadily with age (-0.7% and -0.4% per year, respectively), while LV-VTD did not show a clear linear increase. GC treatment strongly predicted improved respiratory function (FVC higher by 14.6%), but not as much of improved cardiac function. *DMD* mutations involving Dp140 showed a clear negative effect on respiratory function (-6.8% for FVC, p=0.002), but not on cardiac parameters. Patients amenable to

skipping of exon 8 had a dramatically higher PEF (+23.0%). We observed an association of *SPP1* rs28357094 (dominant model) with reduced FVC (-7.5%, p=0.025), to be validated in independent cohorts, and a strong association of the LTBP4 IAAM haplotype with preserved left ventricular function and size (EF +4.5%, SF +4.4%, VTD -10.6 mL/m^2, $p < 0.01$).

These findings strengthen natural history trajectories of cardio-respiratory measures employed in the clinical monitoring of DMD; highlight the potential of continued GC treatment in the non-ambulatory phase for preserving respiratory function; and identify genetic features (both *DMD* mutations and trans-active SNP modifiers) that will be relevant for prognosis, counseling, clinical management, and stratification in clinical trials.