**Supplementary Table1: Genotype of our 118 PMM patients.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Mutation category*** | ***Gene*** | ***Gene name*** | ***No.*** | ***Genome*** |
| ***Mitochondrial translation machinery*** | ***tRNA*** | ***transfer RNA*** | ***39*** | ***mtDNA*** |
| ***MRPL44*** | ***Mitochondrial large ribosomal unit*** | ***1*** | ***nDNA*** |
| ***MtDNA deletions*** | ***Twinkle*** | ***Mitochondrial helicase*** | ***4*** | ***nDNA*** |
| ***POLG*** | ***Mitochondrial polymerase gamma*** | ***8*** | ***nDNA*** |
| ***mtDNA*** | ***Single deletion: tRNA and RC subunits*** | ***46*** | ***mtDNA*** |
| ***DNA2*** | ***DNA2 mutation (AD)*** | ***1*** | ***nDNA*** |
| ***OPA1*** | ***OPA1*** | ***2*** | ***nDNA*** |
| ***ANT1*** | ***ANT1*** | ***1*** | ***nDNA*** |
| ***DGUOK*** | ***DGUOK*** | ***1*** | ***nDNA*** |
| ***mtDNA*** | ***Multiple deletions*** | ***12*** | ***mtDNA*** |
| ***RC subunits /******Assembly factors*** | ***ND1*** | ***NADH dehydrogenase subunit 1*** | ***1*** | ***mtDNA*** |
| ***MT-CO3***  | ***Mitochondrially encoded cytochrome C oxidase III*** | ***1*** | ***mtDNA*** |
| ***MT-CYB*** | ***mitochondrially encoded cytochrome b*** | ***1*** | ***mtDNA*** |

**Supplementary Table 2:** Differences among PMM genotypes

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | ***N*** | ***Value*** | ***P value between single deletion and mDNA mutation*** | ***P value between single deletion and nDNA mutation*** | ***P value between mDNA and nDNA mutation*** |
|  |
| ***AGE ONSET*** | mDNA mutation | 44 | 32,8±13,6 | p <0,01 | p <0,05 | ns |
| Single deletion | 41 | 25,6±11,5 |
| nDNA Mutation | 28 | 37,7±17,8 |
| ***6MWT (m)*** | mDNA mutation | 45 | 357,5 (343,5 – 410,5) | p <0,02 | p <0,01 | ns |
| Single deletion | 40 | 428 (378,3 – 472,5) |
| nDNA Mutation | 31 | 372 (243 – 422) |
| ***5XSST (s)*** | mDNA mutation | 46 | 22,6±12,1 | p <0,01 | p <0,01 | ns |
| Single deletion | 40 | 15,7±13,4 |
| nDNA Mutation | 31 | 24±12,3 |
| ***3TUG (s)*** | mDNA mutation | 48 | 33 (23,6 – 39) | p <0,01 | p >0,05 | ns |
| Single deletion | 37 | 24 (13 – 29,7) |
| nDNA Mutation | 29 | 29,3 (12,5 – 40) |
| ***TOMASS*** | mDNA mutation | 25 | 17 (14,5 – 25) | p <0,01 | p <0,01 | ns |
| Single deletion | 9 | 58 (44,5 – 65) |
| nDNA Mutation | 5 | 14 (12 – 20) |
| ***TWST*** | mDNA mutation | 42 | 8 (7-12.1) | ns | ns | ns |
| Single deletion | 37 | 9.4 (6-15.6) |
| nDNA Mutation | 24 | 10 (7-18) |
| ***FEV1*** | mDNA mutation | 26 | 84.1±12  | ns | p=0.053 | ns |
| Single deletion | 15 | 79±17.3 |
| nDNA Mutation | 15 | 92.5±17.3 |
| ***Disease duration*** | mDNA mutation | 34 | 20.9±12 | ns | ns | ns |
| Single deletion | 27 | 25±11.8 |
| nDNA Mutation | 20 | 18±12.7 |
| ***FSS*** | mDNA mutation | 46 | 37.6±12.4 | ns | ns | ns |
| Single deletion | 40 | 39.1±16.3 |
| nDNA Mutation | 30 | 39.9±16.3 |
| ***NMDAS*** | mDNA mutation | 46 | 18±11.3 | ns | ns | ns |
|  | Single deletion | 41 | 19±10.6 |
|   | nDNA Mutation | 31 | 18.5±9.3 |
| ***WHYMPI*** ***(pain severity)*** | mDNA mutation | 44 | 2,7±1 | ns | ns | ns |
|  | Single deletion | 39 | 2,9±1,6 |
|   | nDNA Mutation | 28 | 3±1,5 |
| ***FGF-21*** | mDNA mutation | 33 | 573,5 (403,14 - 825,15) | ns | ns | ns |
|  | Single deletion | 23 | 486,1 (308,6 - 807) |
|   | nDNA Mutation | 24 | 503,5 (239,9 - 699,8) |
| ***GDF-15*** | mDNA mutation | 33 | 2497,4 (1176 - 3923,43) | ns | ns | ns |
|  | Single deletion | 23 | 3049,2 (1964,3 - 4328) |
|   | nDNA Mutation | 24 | 1899 (1139,3 - 3320,5) |
| ***CK*** | mDNA mutation | 33 | Male 171 (135,3 - 446) Female 211 (178 - 420) | ns | ns | ns |
|  | Single deletion | 27 | Male 223,5 (122,5 - 384,3) Female 124 (75 - 254) |
|   | nDNA Mutation | 20 | Male 332,5 (169,5 - 516) Female 138 (63,8 - 271) |
| ***LACTATE*** | mDNA mutation | 33 | 2 (1 - 2,8) | ns | ns | ns |
|  | Single deletion | 26 | 1,7 (1,2 - 2,2) |
|   | nDNA Mutation | 20 | 1,9 (1 - 2,5) |

**Supplementary Table 3.** Continuous and categorical data were correlated through Pearson correlation test or Spearman rank correlation test, according to distribution of each parameter. To identify possible predictors of NMDAS results and quality of life questionnaires scores, multivariable logistic regression analysis were performed. Before performing partial correlations and regression analysis, variables with a skewed distribution were logarithmically corrected. In all cases, a p value less than 0.05 was considered as significant, a lower value is indicated if it was found.

|  |  |
| --- | --- |
|  | **Relation between functional test, QoL questionnaires, NMDAS** |
| Correlation | Multivariate model (including sex, mutation type and disease duration) |
| Bivariate | Partial (including SEX, MUTATION TYPE and DISEASE DURATION) | B | R | R2 | P value | VIF |
| Coefficient | P value | Coefficient  | P value |
| ***6MWT – FSS*** | -0,399 | P<0,01 | -0,433 | P<0,01 | -22,152±5,34 | 0,482 | 0,232 | P<0,01 | 1,063 |
| ***6MWT – NMDAS*** | -0,51 | P<0,01 | -0,468 | P<0,01 | -14,74±3,2 | 0,613 | 0,376 | P<0,01 | 1,063 |
| ***3TUG – FSS*** | 0,397 | P<0,01 | 0,515 | P<0,01 | 15± 2,92 | 0,554 | 0,307 | P<0,01 | 1,046 |
| ***3TUG – NMDAS*** | 0,325 | P<0,01 | 0,523 | P<0,01 | 9,22± 1,75 | 0,644 | 0,4151 | P<0,01 | 1,047 |
| ***6MWT – WHYMPI pain severity*** | -0,446  | P<0,01 | -0,372 | P<0,05 | -0,75±0,25 | 0,481 | 0,23 | P<0,01 | 1,178 |
| ***3TUG – WHYMPI pain severity*** | 0,339 | P<0,01 | 0,441 | P<0,01 | 0,146±0,039 | 0,512 | 0,262 | P<0,01 | 1,180 |
| ***5XSST – WHYMPI pain severity*** | 0,280 | P<0,05 | 0,122 | P<0,02 | 0,146±0,039 | 0,313 | 0,098 | P>0,05 | 1,178 |
| ***TWST – WHYMPI pain severity*** | 0,215 | P<0,05 | 0,407 | P<0,01 | 0,204±0,062 | 0,498 | 0,248 | P<0,01 | 1,268 |
| ***TWST - FSS*** | 0,366 | P<0,01 | 0,27 | P<0,05 | 5,88±2,51 | 0,373 | 0,139 | P<0,05 | 1,132 |
| ***TWST – NMDAS*** | 0,324 | P<0,01 | 0,297 | P<0,02 | 4,28±1,64 | 0,525 | 0,275 | P<0,02 | 1,132 |

Supplementary **Table 4:** Differences among PMM genotypes (Lethonen subgroups).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | ***N*** | ***Value*** | ***P value between Mitochondrial translation machinery and mDNA deletions*** | ***P value between mitochondrial translation machinery deletion and Rc subunit/ assembly factors*** | ***P value between mtDna deletions and Rc subunits/assembly factors***  |
|  |
| ***AGE ONSET*** | Mitochondrial translation machinary | 38 | 35±13 | ns | p <0,01 | p < 0,01 |
| MtDNA deletions | 72 | 30±15 |
| Rc subunits /Assembly factors | 3 | 16±2 |
| ***6MWT (m)*** | Mitochondrial translation machinary | 39 | 389 (346 – 423) | ns | ns | ns |
| MtDNA deletions | 74 | 409 (336 – 449,25) |
| Rc subunits /Assembly factors | 3 | 313,3±145,7 |
| ***5XSST (s)*** | Mitochondrial translation machinary | 40 | 20,98±12,1 | ns | ns | ns |
| MtDNA deletions | 74 | 20,4±13,7 |
| Rc subunits /Assembly factors | 3 | 21,4±7,7 |
| ***3TUG (s)*** | Mitochondrial translation machinary | 39 | 31,6±15 | ns | ns | ns |
| MtDNA deletions | 72 | 29±19,8 |
| Rc subunits /Assembly factors | 3 | 39,6±6,4 |
| ***TOMASS*** | Mitochondrial translation machinary | 20 | 24,25±13,8 | ns | ns | ns |
| MtDNA deletions | 19 | 34,5±21,4 |
| Rc subunits /Assembly factors | 0 |  |
| ***TWST*** | Mitochondrial translation machinary | 37 | 8 (7 – 12,6) | ns | ns | ns |
| MtDNA deletions | 64 | 10 (6,8 – 15) |
| Rc subunits /Assembly factors | 2 | 6,31±0,44 |
| ***FEV1*** | Mitochondrial translation machinary | 21 | 88,67±17,8 | ns | ns | ns |
| MtDNA deletions | 33 | 82,9±14 |
| Rc subunits /Assembly factors | 2 | 80±18,3 |
| ***Disease duration*** | Mitochondrial translation machinary | 28 | 18,75±11,54 | ns | ns | ns |
| MtDNA deletions | 50 | 22,6±12,6 |
| Rc subunits /Assembly factors | 3 | 29±10,8 |
| ***FSS*** | Mitochondrial translation machinary | 40 | 39,38±12,3 | ns | ns | ns |
| MtDNA deletions | 73 | 39,2±16 |
| Rc subunits /Assembly factors | 3 | 47±13 |
| ***NMDAS*** | Mitochondrial translation machinary | 40 | 16,75±10,97 | ns | ns | ns |
|  | MtDNA deletions | 75 | 19,3±9,7 |
|   | Rc subunits /Assembly factors | 3 | 21,7±22,14 |
| ***WHYMPI*** ***(pain severity)*** | Mitochondrial translation machinary | 38 | 2,52±1 | ns | p < 0,05 | ns |
|  | MtDNA deletions | 70 | 2,2±1,87 |
|   | Rc subunits /Assembly factors | 3 | 4,1±1 |
| ***FGF-21*** | Mitochondrial translation machinary | 28 | 688,4±476,2 | ns | ns | ns |
|  | MtDNA deletions | 51 | 648,3±564,3 |
|   | Rc subunits /Assembly factors | 1 | 117,9 |
| ***GDF-15*** | Mitochondrial translation machinary | 28 | 3009,8±1702 | ns | ns | ns |
|  | MtDNA deletions | 51 | 3111,2±2228 |
|   | Rc subunits /Assembly factors | 1 | 795,8 |
| ***CK*** | Mitochondrial translation machinary | 1118 | Male 172 (166 – 704)Female 201 (118 – 421) | ns | ns | ns |
|  | MtDNA deletions | 2029 | Male 208 (122,5 – 399)Female 150 (69,5 – 258) |
|   | Rc subunits /Assembly factors | 11 | Male 262 Female 213  |
| ***LACTATE*** | Mitochondrial translation machinary | 28 | 2,32±1,5 | ns | ns | ns |
|  | MtDNA deletions | 49 | 1,87±1,2 |
|   | Rc subunits /Assembly factors | 2 | 1,57±0,32 |

**Supplementary Table 5:** Bivariate correlation among mitochondrial biomarkers, QoL questionnaires, functional tests, NMDAS and FEV1 were conducted separately in the subgroups according to Lethonen (not in RC mutated patients because it is poorly represented). In all cases, a p value less than 0.05 was considered as significant, a lower value is indicated if it was found. Only statistical significant data are showed.

**Mitochondrial translation machinery**

|  |  |
| --- | --- |
|  | **Bivariate correlation between demographic features and functional test, QoL questionnaires and NMDAS** |
| *Coefficient* | *P value* |
| **FGF-21 and GDF-15**  | 0,606 | P<0,01 |
| **FGF-21 and Heteroplasmy levels**  | 0,889 | P<0,05 |
| **GDF-15 and Heteroplasmy levels**  | 0,889 | P<0,05 |
| **GDF-15 and NMDAS**  | 0,668 | P<0,01 |
| **Lactate and NMDAS** | 0,374 | P<0,05 |
| **CK and TOMASS** | 0,570 | P<0,05 |

**MtDNA deletions**

|  |  |
| --- | --- |
|  | **Bivariate correlation between demographic features and functional test, QoL questionnaires and NMDAS** |
| *Coefficient* | *P value* |
| **FGF-21 and GDF-15** | 0,676 | P<0,01 |
| **GDF-15 and Lactate** | 0,404 | P<0,05 |
| **CK and Lactate** | 0,389 | P<0,01 |
| **FGF-21 and TWST** | 0,340 | P<0,05 |