THE LANCET Neurology

## Supplementary webappendix

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Supplement to: Suomalainen A, Elo JM, Pietiläinen KH, et al. FGF-21 as a biomarker for muscle-manifesting mitochondrial respiratory chain deficiencies: a diagnostic study. *Lancet Neurol* 2011; published online Aug 4. DOI:10.1016/S1474-4422(11)70155-7.

# WEBAPPENDIX

| | Adults | | | Children | | |
|---|---|---|---|---|---|---|
| | **Control subjects** | **Mitochondrial patients** | **Non-mitochondrial neurological patients** | **Control subjects** | **Mitochondrial patients** | **Non-mitochondrial neurological patients** |
| N | 49 | 41 | 22 | 25 | 26 | 12 |
| Age (years) | 44·8±12·0 | 41·5±15·1<br>ns | 56·5±14·9<br>p<0·001 | 8·2±3·7 | 8·0±3·8<br>ns | 10·0±4·9<br>ns |
| BMI | 26·2±4·7 | 22·8±6·7<br>p=0·009 | na | na | na | na |
| % males (n) | 40·8 (20) | 63·4 (26) | 27·2 (6) | 52·0 (13) | 46·2 (12) | 50·0 (6) |
| % plasma (vs serum) | 0 | 0 | 0 | 12·0 (3) | 30·8 (8) | 0 |

**Table 1. General characteristics of the study participants.** Data are presented as mean values and SD. ns, not significant; na, not available.

| Patient | Sex | Age serum sample | Key clinical features | ASAT U/l | ALAT U/l | GT U/l | P-AFOS U/l | Bilirubin µmol/l | CK max U/l | FGF21 pg/ml | Liver disease |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Adults with mitochondrial disease** | | | | | | | | | | | |
| 1 | F | 55 | CMP, DM, HI | 36 | 18 | 55 | 65 | 9 | 140 | **1762** | NO |
| 6 | M | 53 | CMP, myopathy, HI, DM, RR | 34 | 34 | 22 | 75 | 13 | 143 | **562** | NO |
| 7 | F | 67 | DM, HI | 21 | 18 | 16 | 47 | 20 | 62 | **395** | NO |
| 9 | M | 52 | DM, HI, ataxia, ptosis | 19 | 18 | 19 | 45 | 5 | 65 | **289** | NO |
| 11 | M | 39 | headaches | 34 | 34 | 23 | 71 | 15 | 120 | **88** | NO |
| 13 | F | 65 | ptosis and restricted eye movements | nd | 33 | 26 | 92 | 6 | 106 | **1283** | NO |
| 14 | F | 50 | PEO, myopathy | **50** | 38 | 14 | **26** | 14 | **471** | **659** | NO |
| 15 | M | 49 | PEO, myopathy, fatty liver | **78** | **133** | **131** | 40 | 13 | **715** | **586** | YES, fatty liver |
| 16 | M | 51 | PEO, myopathy | **64** | 61 | 30 | **25** | **23** | 130 | **570** | Possibly |
| 17 | M | 60 | PEO, proximal muscle weakness in lower extremities, sensoral polyneuropathy | **48** | 46 | 26 | 65 | nd | 234 | **347** | NO |
| 18 | M | 56 | PEO, myopathy | **48** | 36 | 88 | **111** | **53** | 147 | **338** | YES, chirrosis |
| 19 | M | 38 | PEO, myopathy | 34 | 56 | 24 | 44 | **22** | 196 | **237** | NO |
| 20 | M | 50 | PEO, neuropathy, myopathy, parkinsonism | **54** | 14 | 24 | 75 | 6 | **1012** | **177** | NO |
| 31 | M | 51 | MIRAS | 27 | 37 | 29 | 66 | 12 | 89 | **537** | NO |
| 33 | M | 43 | MIRAS | 25 | 62 | 35 | 56 | 11 | 96 | **291** | NO |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 34 | M | 51 | MIRAS, HI | 34 | 56 | 40 | 51 | **22** | 188 | **246** | NO |
| 35 | F | 22 | MIRAS, epilepsy, migraine, liver transplant | 19 | 17 | 37 | 47 | 3 | 38 | **173** | NO |
| 36 | M | 38 | MIRAS | 23 | 36 | **202** | 81 | 4 | 172 | **149** | NO |
| 37 | M | 30 | MIRAS | 32 | 32 | 32 | **154** | 17 | 263 | **131** | NO |
| 38 | M | 52 | MIRAS | 31 | 43 | 87 | 93 | 11 | 103 | **116** | NO |
| 39 | M | 49 | MIRAS | 31 | 10 | 16 | 50 | 15 | 318 | **54** | NO |
| 40 | F | 25 | MIRAS | 23 | 17 | 12 | 52 | 6 | 102 | **50** | NO |
| 41 | M | 41 | MIRAS | 42 | 59 | 107 | 77 | 8 | **571** | **25** | NO |
| **Adults with neurological non-mitochondrial disease** | | | | | | | | | | | |
| 42 | F | 57 | Dystrofia myotonica type-2 | nd | **61** | 45 | 80 | nd | **1095** | **701** | NO |
| 43 | M | 40 | Spinal muscular atrophy type-2 | 25 | 10 | 19 | 96 | nd | 36 | **436** | NO |
| 44 | F | 71 | Inclusion body myositis | **62** | **64** | 24 | **243** | 10 | **555** | **392** | NO, muscle-origin |
| 45 | F | 76 | Inclusion body myositis | 31 | **80** | 11 | 51 | 18 | **310** | **253** | NO |
| 46 | F | 77 | Oculopharyngeal muscular dystrophy | **61** | **73** | **178** | **396** | **29** | 38 | **251** | YES |
| 47 | M | 65 | Motoneuron disease | **65** | **106** | 52 | 68 | 13 | **1760** | **190** | NO, muscle-origin |
| 48 | F | 54 | Dystrofia myotonica type-1 | 32 | 21 | 42 | **32** | 8 | **330** | **162** | NO |
| 49 | F | 40 | Spinal muscular atrophy III | **48** | **113** | 39 | 64 | 7 | 137 | **135** | NO, muscle-origin |
| 50 | F | 51 | Dystrofia myotonica type-2 | **60** | **82** | 18 | 42 | nd | **7794** | **131** | NO, muscle-origin |
| 51 | M | 39 | Becker's muscle dystrophy | **104** | **129** | 34 | 88 | **21** | **3725** | **114** | NO, muscle-origin |
| 52 | F | 68 | CMP, muscle weakness of lower limbs | **136** | **68** | 36 | **475** | **112** | 42 | **98** | YES |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 54 | M | 85 | Inclusion body myositis | **47** | **82** | **475** | **169** | nd | **650** | **68** | YES |
| 55 | F | 55 | Dystrofia myotonica type-2 | 33 | **58** | 25 | 73 | 11 | **310** | **56** | NO |
| 56 | M | 65 | Inclusion body myositis | **159** | 32 | **154** | 104 | **115** | **321** | **46** | YES |
| 57 | F | 48 | Dystrofia myotonica type-2 | 22 | 24 | 15 | **124** | nd | 175 | **42** | NO |
| 58 | F | 71 | Inclusion body myositis | 24 | 36 | 23 | 40 | nd | **283** | **40** | NO |
| 59 | F | 57 | Welander's muscular dystrophy | 23 | 38 | 20 | **170** | 14 | **254** | **24** | NO |
| 60 | F | 48 | Dystrofia myotonica type-1 | **71** | **50** | 17 | **32** | nd | 155 | **24** | NO, muscle-origin |
| 61 | F | 23 | Pompe's disease, late-onset | **300** | **306** | 22 | 71 | 18 | **1807** | **18** | YES |
| 62 | F | 41 | Dystrofia myotonica type-1 | nd | 31 | 59 | 65 | 10 | **740** | **17** | NO |
| 63 | M | 58 | Inclusion body myositis | **93** | **149** | 47 | nd | nd | **2114** | **17** | NO, muscle-origin |
| **Children with mitochondrial disease** | | | | | | | | | | | |
| 64 | M | 1.3 | Alpers-Huttenlocher | **168** | **168** | nd | **600** | 6 | 163 | **4358** | YES |
| 65 | M | 0.3 | Reversible COX-deficiency | **120** | 35 | nd | nd | 6 | **662** | **4248** | NO |
| 66 | F | 10 | Enceph.myopathy, cardiomyopathy | **414** | **126** | nd | 108 | 2 | **2288** | **4232** | NO, muscle-origin |
| 67 | M | 0.1 | Reversible COX-deficiency | 44 | 35 | nd | 372 | 19 | 193 | **4161** | NO |
| 70 | M | 0.2 | LCHAD crisis, delayed growth, decreased liver function | **2247** | **104** | **297** | 445 | **27** | **294** | **3314** | YES |
| 72 | M | 13.5 | Retinitis pigmentosa, short stature | **53** | 25 | nd | 180 | 4 | **325** | **2134** | NO |
| 75 | F | 0.3 | Reversible COX-deficiency | 50 | 25 | nd | nd | 4 | 53 | **1465** | NO |
| 77 | F | 9.9 | MELAS | **66** | **47** | nd | nd | 10 | 205 | **1090** | NO |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 78 | F | 3.5 | Alpers/ refractory epilepsy+psychomotor retardation | 49 | 62 | nd | 318 | 3 | nd | **1062** | NO |
| 79 | F | 17 | Mitochondrial myopathy | 23 | 16 | nd | 52 | 8 | **247** | **728** | NO |
| 85 | M | 3.3 | Leigh syndrome | **60** | 52 | nd | nd | 2 | **620** | **335** | NO |
| **Children with non-mitochondrial disease** | | | | | | | | | | | |
| 87 | M | 2.8 | progeria; CMP, Raynaud, growth retardation | **214** | 157 | nd | 119 | 10 | 70 | **556** | NO |
| **LCHAD after diet treatment** | | | | | | | | | | | |
| 99 | F | 9.2 | LCHAD in diet | nd | 57 | nd | 561 | nd | **437** | **185** | YES |

**Table 2. Liver function tests of the patients.** ASAT (aspartyl aminotransferase): children <1 month, <80U/l; children 1 month-nd16 years, <50U/l; men, 15-45 U/l; women, 15-35 U/l.ALAT (alanyl aminotransferase): children 0-16 years, <40 U/l; men 10-70 U/l; women 10-45 U/l. GT (glutamyl transferase): children <1 month, <300 U/l; children 1 month-16 years, <50 U/l; men 18-39 years, 10-80 U/l; women 18-39 years, 10-45 U/l; men >40 years, 15-115 U/l; women >40 years, 10-75 U/l. AFOS (alkaline phosphatase): children 0-14 days, 60-275 U/l; children 15 days-1 year, 115-460 U/l; children 2-5 years, 115-390 U/l; children 6-7 years, 115-460 U/l; children 8-9 years, 115-345 U/l; boys 10-11 years, 115-335 U/l; girls 10-11 years, 115-435 U/l; boys 12-13 years, 125-405 U/l; girls 12-13 years, 90-335 U/l; boys 14-15 years, 80-445 U/l; girls 14-15 years, 80-210 U/l; boys 16-18 years, 55-330 U/l; girls 16-18 years, 35-125 U/l, adults, 35-105 U/l. Bilirubin: children 0-1 day, <100 µmol/l; children 2-6 days, <200 µmol/l; children 7-20 days, <100 µmol/l; children 21-30 days, <50 µmol/l; >1 month, 4-20 µmol/l. Creatine kinase (CK): children <5 days, <800 U/l; children 5 days-2 years, 50-270 U/l; children 3-15 years, 20-220 U/l; boys 16-17 years, <270 U/l; girls 16-17 years, <150 U/l; men 18-49 years, 50-400 U/l; women 35-210 U/l; men 50+ 40-280 U/l. Abbreviations: CMP, cardiomyopathy cardiomyopathy; DM, diabetes mellitus; HI, hearing impairment; LVH, left ventricular hypertrophy; PEO, progressive external ophthalmoplegia; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; mtDNA, mitochondrial DNA; IFG, impaired glucose tolerance; RR, increased blood pressure; PMR, psychomotor retardation; LCHAD, Long-chain 3 hydroxyacyl CoA dehydrogenase deficiency; MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; MIRAS, mitochondrial recessive ataxia syndrome; MERRF, myoclonic epilepsy with ragged red fibers; RRF, ragged red fiber; COX-neg, cytochrome-c-oxidase negative muscle fibers; CI, CIV, respiratory chain complex I and/or IV deficiency; nd, not determined; na, not available. Interpretation: if ALAT and/or ASAT is increased, combined with increased CK, the source of these is likely the muscle, not liver ("no, muscle origin". A muscle disease was interpreted in the case of increased ALAT/ASAT in the absence of CK increase, and if other markers such as bilirubin was increased. The source of AFOS may be liver in adults, but most often bone in children.

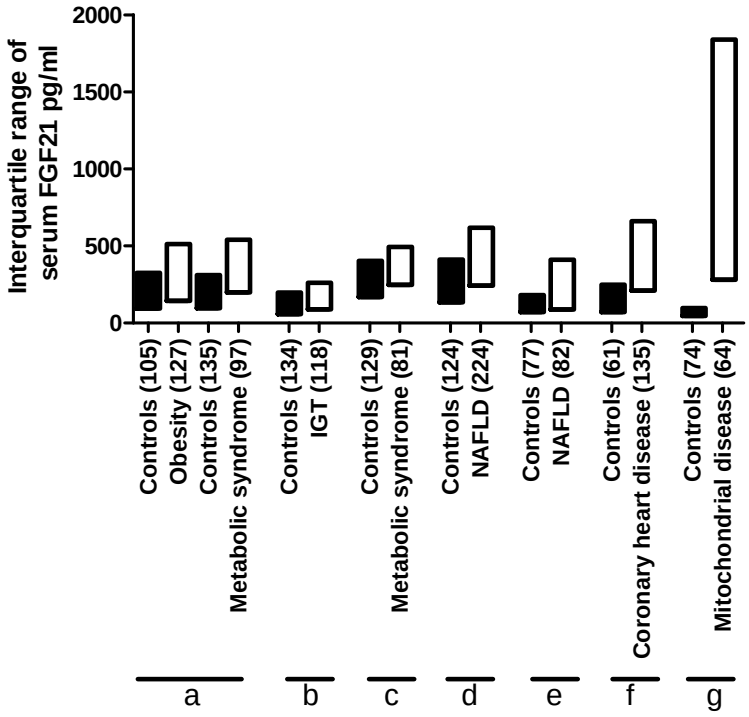


**Figure 1. Summary of literature; clinical conditions in which [S-FGF21] has been reported increased.** Included in the figure are previously published data sets measuring [S-FGF21] with sample size >20, with a significant difference identified between control subjects and patients, and the values of interquartile range available. We included those studies, which used the same Biovendor ELISA kit for [S-FGF21] measurement. The interquartile ranges are shown for the control and the patient groups for each study, number of studied samples in parenthesis. References **a**, [1] **b**, [2] **c**, [3] **d**, [4] **e**, [5] **f**, [6] **g**, this study. IGT, Impaired glucose tolerance; NAFLD, non-alcoholic fatty liver disease.

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