**SUPPLEMENTARY**

## Table S1: Primers used for Sanger sequencing and real-time qPCR

### A. Primers for *CLPP* Sanger sequencing

| Covered exon | Forward primer (M13 tailed) | Reverse primer (M13 tailed) |
|---|---|---|
| **CLPP exon 1 + 2** | 5'-*TGTAAAACGACGGCCAGT*GAAGCTGTAGTTCCGCCATC-3' | 5'-*CAGGAAACAGCTATGACC*GAGTCAGAAGGGGAGGGAAG-3' |
| **CLPP exon 3** | 5'-*TGTAAAACGACGGCCAGT*CCCAGCCTCCCTTAAAACTC-3' | 5'-*CAGGAAACAGCTATGACC*TCTGGTCCCCTGAGTCACC-3' |
| **CLPP exon 4** | 5'-*TGTAAAACGACGGCCAGT*CCTCCCCAGGTTTAGGAGAT-3' | 5'-*CAGGAAACAGCTATGACC*GCCCAGTCAGTCCTTCTGTC-3' |
| **CLPP exon 5** | 5'-*TGTAAAACGACGGCCAGT*CCCTGTGAGGGGCTGAC-3' | 5'-*CAGGAAACAGCTATGACC*GGAGAAGCCCTGGCTTAGAG-3' |
| **CLPP exon 6** | 5'-*TGTAAAACGACGGCCAGT*AACATTCAGGCCAAAACAGG-3' | 5'-*CAGGAAACAGCTATGACC*GTCTGGCAGGCCTCTGG-3' |

### B. Primers for quantifying *CLPP* mRNA expression by real-time qPCR

| Gene/exon | Forward primer | Reverse primer |
|---|---|---|
| **CLPP exon 2 + 3 (5' side)** | 5'-GCGCGCCTATGACATCTACT-3' | 5'- AGAGGAGCTGTGCGATAACAA-3' |
| **CLPP exon 5 + 6 (3' side)** | 5'-CCATCCAGGCAGAGGAGA-3' | 5'-AGGGTGGACCAGAACCTTG-3' |
| **TBP exon 5 + 6** | 5'- CACGAACCACGGCACTGATT-3' | 5'-TTTTCTTGCTGCCAGTCTGGAC-3' |

### C. Primers for mtDNA and *CLPP* copy-number determination by qPCR

| Gene/exon | Forward primer | Reverse primer |
|---|---|---|
| **ND5 (mtDNA)** | 5'-GCCTTCTCCACTTCAAGTCAA-3' | 5'-TGGGTACAGATGTGCAGGAAT-3' |
| **B2M (nDNA)** | 5'-TGCTGTCTCCATGTTTGATGTATCT-3' | 5'-TCTCTGCTCCCCACCTCTAAGT-3' |
| **CLPP exon 3** | 5'-GCCTAAATTCTTCCCCATCC-3' | 5'-AGAGGAGCTGTGCGATAACAA-3' |
| **CLPP exon 4A** | 5'-CCTCCCCAGGTTTAGGAGAT-3' | 5'-TTGAGGATGTACTGCATCGTG-3' |
| **CLPP exon 4B** | 5'-ATGGGCTCCCTGCTTCTC-3' | 5'-GCCCAGTCAGTCCTTCTGTC-3' |
| **CLPP exon 6** | 5'-AACATTCAGGCCAAAACAGA-3' | 5'-AGGGTGGACCAGAACCTTG-3' |

**Table S2: Clinical findings**

| **Patient** | **Patient 1.1** | **Patient 1.2** | **Patient 2** | **Patient 3.1** | **Patient 3.2** |
|---|---|---|---|---|---|
| **Year of birth** | **1995** | **1993** | **1994** | **1989** | **1994** |
| **Patient and family characteristics** | | | | | |
| Gender | Male | Male | Male | Male | Male |
| Siblings (affected/unaffected) | 1 (pat.1.2) / 1 | 1 (pat.1.1) / 1 | 0/1 | 1 (pat.3.2) / 1 | 1 (pat.3.1) / 1 |
| Consanguinity of the parents | Yes, full cousins | Yes, full cousins | Not known | Not known | Not known |
| **History** | | | | | |
| Pregnancy/delivery | Premature | Nl/breech delivery | Nl | Premature | Nl |
| Neonatal period | Feeding problems | Feeding problems | Irritable | No information | Nl |
| Initial psychomotor development | Delayed | Delayed | Delayed | Delayed | Nl |
| Unsupported walking (years) | 14 months | 14 months | 15 months | Unknown | 16 months |
| Highest motor milestone | Walking without support | Walking without support | Walking without support, riding bike with training wheels | Walking without support | Walking without support |
| **Clinical picture** | | | | | |
| Age at presentation | 1 year | 16 mths | 1 year | 4 years | 3 years |
| Clinical signs | Congenital deafness, psychomotor retardation, autism, behavioral problems, epilepsy, height and weight < 2SD hernia inguinalis | Deafness from 16 months, psychomotor retardation, autism, behavioral problems, stunted growth, height/ weight <p3, | Deafness noted at age 1 year; mild retardation of initial psychomotor development; stunted growth; developed epilepsy and ataxia at age 3; sensorimotor neuropathy noted at age 16 | Deafness without speech development; psychomotor retardation; spastic diplegia; microcephaly, stunted growth; developed epilepsy (photosensitive myoclonic absences) at 11 years; diabetes mellitus at 20 years | Deafness without speech development; psychomotor retardation; spastic diplegia; microcephaly, stunted growth; no epilepsy and EEG Nl; at 20 years hypothyreosis |
| Disease course | Slow decline | Slow decline | Slow decline, additional episodes of steep decline following illness or stress | Slow decline | Slow decline |
| Episodes of regression / number | Yes / multiple | Yes / multiple | Yes, more than ten | Not observed | One |
| Provoking factors | Febrile illness | Viral infections | Viral infection, puberty | - | None identified |
| Improvement after regression | Yes | Yes, but never back to baseline | Yes, but never back to baseline | - | Yes |
| Present status | Wheelchair bound, loss of cognitive skills, uses sign language, swallowing problems | Wheelchair bound, loss of cognitive skills, uses sign language, swallowing problems | Wheelchair bound, also loss of fine motor and cognitive skills; uses sign language; GMFCS level V | Wheelchair bound GMFCS level V | Wheelchair bound GMFCS level IV |

| Patient number | Patient 1.1 | Patient 1.2 | Patient 2 | Patient 3.1 | Patient 3.2 |
|---|---|---|---|---|---|
| **Year of birth** | **1995** | **1993** | **1994** | **1989** | **1994** |
| **Physical examination** | | | | | |
| Age at latest exam (years) | 20 years | 22 years | 20 years | 25 years | 21 years |
| Dysmorphic features | Midface hypo-plasia, thick lips | Coarse facies, hirsutism, diastasis recti, broad short hands | No | Broad face, thick lips, acanthosis nigricans | Broad face, thick lips, acanthosis nigricans |
| Head circumference | < P3 | P 10 | Nl, 58cm | P10 | P10 |
| Height / weight | <P3 (1.59m) / <P3 50.5 kg) | <p3 (1.60m), <P3 58 kg) | <P3 / <P3 | <P3 / P25 | <P3 / P50 |
| Internal organs | Nl | Nl | Nl | Nl | Nl |
| Intelligence | Impaired | Impaired (IQ 50-55) | Impaired | Impaired | Impaired |
| Vision | 0.1 | 0.5 ri , 0.6 left | Nl | Myopia | Nl |
| Fundoscopy | Nl | Nl | Nl | Nl | Nl |
| Eye movements | Nl | Nl | Nl | Strabism | Nl |
| Nystagmus | No | Nl | Nl | No | No |
| Ptosis | No | Nl | Nl | No | No |
| Hearing | Deaf ri 97 and le 90db) | Deaf 108db ri and 103 db le | Deaf | Deaf | Deaf |
| Dysarthria | Severe | Severe | Severe | Severe | Severe |
| Dysphagia, tube feeding | Yes/ no | Yes, no | Occasional, no tube | No | No |
| Arms | | | | | |
| Tone | Increased | Increased | Nl | Increased | Increased |
| Reflexes | Brisk | Brisk | Nl | Brisk | Brisk |
| Muscle strength | Wheelchair bound | Generalized paresis | Nl | Nl | Nl |
| Spasticity | Yes | Yes | Nl | Yes, severe | Yes, severe |
| Ataxia | Yes, intention tremor, tremor head and neck | Yes, intention tremor, tremor head and neck | No | Yes, dysmetria | Yes, dysmetria |
| Sensory function | Nl | Nl | Nl | Not tested | Not tested |
| Extrapyramidal signs | Mild dystonic | Mild dystonic | No | Mildly dystonic | Mildly dystonic |
| Legs | | | | | |
| Tone | Increased | Increased | Increased | Markedly increased | Markedly increased |
| Reflexes | Brisk - clonus | Brisk | Brisk | Brisk | Brisk |
| Babinski signs | +/+ | +/+ | Not sure | +/+ | +/+ |
| Muscle weakness | No | No | No | No | No |
| Spasticity | + | + | Yes | Yes, more severe than arms | Yes, more severe than arms |
| Ataxia | + | + | Gait ataxia, when still walking | No | No |
| Sensory function | Nl | Nl | Decreased, consistent with peripheral neuropathy | Not tested | Not tested |
| Extrapyramidal signs | Mild dystonia | Mild dystonia | Tremor in legs | Mild dystonia | Mild dystonia |
| Gait | Spastic-ataxic, uses walker and wheelchair | Spatic-ataxic | Gait ataxia, when still walking | Wheelchair dependent, but at transfers evident spasticity | Wheelchair dependent, but at transfers spastic-ataxic |

Nl, normal; -, absent, +, present