Clinical and molecular findings in three Japanese patients with
N-acetylneuraminic acid synthetase-congenital
disorder of glycosylation (NANS-CDG)
### Table 1: Rare variants extracted from whole exome sequencing in patient 1, under the assumption of Mendelian inheritance with complete penetrance in the trio analysis.

| Inheritance | Compound heterozygous variants | De novo variants | Hemizygous variant |
|-------------|--------------------------------|----------------|-------------------|
|  | Gene (Chromosome) | | | |
| Maternal | NANS (Chr. 9) | NM_0189464.4 | NM_0189464.4 | NM_0001317446.2 | NM_0001317446.2 | NM_0001251.3 | NM_0001251.3 | NM_001318468.1 | NM_001318468.1 | NM_001101357.3 | NM_001101357.3 | NM_0153844.4 | NM_0153844.4 | NM_015922.3 | NM_015922.3 |
| Paternal | NANS (Chr. 9) | c.207delT | c.979_981dup | c.1854_1862del | c.229G>T | c.1886G>T | c.229G>T | c.229G>T | c.229G>T | c.229G>T | c.229G>T | c.229G>T | c.229G>T | c.229G>T | c.229G>T |
| | KIF21A (Chr. 12) | c.979_981dup | c.979_981dup | c.979_981dup | c.979_981dup | c.979_981dup | c.979_981dup | c.979_981dup | c.979_981dup | c.979_981dup | c.979_981dup | c.979_981dup | c.979_981dup | c.979_981dup | c.979_981dup | c.979_981dup |
| | ZNF624 (Chr. 17) | | | | | | | | | | | | | | | |
| | P2RY10 (Chr. X) | | | | | | | | | | | | | | | |
| | TSC22D3 (Chr. X) | | | | | | | | | | | | | | | |
| | CCDC160 (Chr. X) | | | | | | | | | | | | | | | |
| | ADGRG4 (Chr. X) | | | | | | | | | | | | | | | |
| | NSDHL (Chr. X) | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | <Frequency> | | | | | | | | | | | | | | | |
|  | gnomeAD East Asia | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
|  | HGVD | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
|  | I4KJPN | 0.0000 | 0.0003 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
|  | In-house (n = 218) | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
|  | <Pathogenicity> | | | | | | | | | | | | | | | |
|  | CADD PHRED | Not predicted | Not predicted | Not predicted | 1% most deleterious | Deleterious | 1% most deleterious | Deleterious | 1% most deleterious | Non-deleterious | Deleterious | 1% most deleterious | Non-deleterious | Deleterious | 1% most deleterious | Non-deleterious | Deleterious |
|  | PolyPhen-2 HumVar | Not predicted | Not predicted | Not predicted | Probably damaging | Benign | Probably damaging | Benign | Benign | Possibility damaging | Benign | Benign | Possibility damaging | Benign | Benign | Possibility damaging | Benign | Benign |
|  | SIFT | Not predicted | Not predicted | Not predicted | Damaging | Tolerated | Tolerated | Tolerated | Damaging | Tolerated | Damaging | Tolerated | Damaging | Tolerated | Damaging | Tolerated | Damaging |
|  | MutationTaster | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing |
|  | <Phenotype> | OMIM | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown |

Shown are non-synonymous rare variants with minor allele frequencies of ≤ 0.01 in all the public and in-house databases employed. The URLs utilized are as follows; in silico analyses have been performed using default parameters.

1) GenBank: https://www.ncbi.nlm.nih.gov/genbank.
2) gnomAD (Genome Aggregation Database): http://gnomad.broadinstitute.org/.
3) HGVD (Human Genetic Variation Database): http://www.hgvd.genome.med.kyoto-u.ac.jp/.
4) I4KJPN (Whole-genome sequences of 14,000 healthy Japanese individuals and construction of the highly accurate Japanese population reference panel) https://ijgvd.megabank.tohoku.ac.jp/.
5) CADD (Combined Annotation–Dependent Depletion): http://cadd.gs.washington.edu/ (Current version: 1.6, GRCh37hg19); PHRED scores of > 10–20 are regarded as deleterious, and those of > 20 indicates the 1% most deleterious.
6) Polyphen-2 Hum Var: http://genetics.bwh.harvard.edu/pph2/ (Current version: 2.22, GRCh37/hg19); HumVar scores were evaluated as 0.000 (most probably benign) to 1.000 (most probably damaging).
7) SIFT (Sorting Intolerant From Tolerant): http://sift.jcvi.org/ (Current version: Mar 2013; GRCh37/Ensembl 66); Scores of ≥0.05 and those > 0.05 are assessed as damaging and tolerated, respectively.
8) MutationTaster: http://www.mutationtaster.org/ (MutationTaster2, GRCh37/Ensembl 69); Alterations are classified as disease causing or polymorphisms, and the high scores of ~1.00 indicate the high probability of disease-causing variant or polymorphism.
9) OMIM: https://www.ncbi.nlm.nih.gov/omim.

Spondyloepimetaphyseal dysplasia, Camera-Genevieve type
Fibrosis of extraocular muscles
Unknown
Unknown
Unknown
Unknown
Unknown
Unknown
Unknown
CHILD syndrome
**Supplementary Table 2.** Rare variants extracted from whole exome sequencing in patient 2, under the assumption of Mendelian inheritance with complete penetrance in the trio analysis.

| Gene (Chromosome) | Homozygous variant | De novo variants | Compound heterozygous variants |
|-------------------|--------------------|-----------------|-------------------------------|
| NANS (Chr. 9)     | NM_018946.4        | NM_01170820.4   | NM_001277115.2                |
| CD274 (Chr. 9)    | NM_014143.4        | NM_001170820.4   | NM_001277115.2                |
| PRK1 (Chr. 9)     | NM_000507.4        | c.791-4C>G      |                  |
| OR13C8 (Chr. 9)   | NM_001004483.1     | c.274G>C        |                  |
| IFITM10 (Chr. 11)| NM_001170820.4     | c.514A>G        |                  |
| DNAH11 (Chr. 7)   | NM_001277115.2     | c.7012C>T       |                  |
| DNAH11 (Chr. 7)   | NM_001277115.2     | c.5491T>A       |                  |

| Variant (GenBank) | Inheritance | Frequency |
|-------------------|-------------|-----------|
| NM_018946.4       | Maternal    | gnomeAD East Asia 0.0000, HGVD 0.0000, 14KJPN 0.0003, In-house (n = 218) 0.0000 |
| NM_014143.4       | Maternal    | gnomeAD East Asia 0.0000, HGVD 0.0000, 14KJPN 0.0003, In-house (n = 218) 0.0000 |
| NM_000507.4       | Maternal    | gnomeAD East Asia 0.0000, HGVD 0.0000, 14KJPN 0.0003, In-house (n = 218) 0.0000 |
| NM_001004483.1    | Maternal    | gnomeAD East Asia 0.0000, HGVD 0.0000, 14KJPN 0.0003, In-house (n = 218) 0.0000 |
| NM_001170820.4    | Maternal    | gnomeAD East Asia 0.0000, HGVD 0.0000, 14KJPN 0.0003, In-house (n = 218) 0.0000 |
| c.791-4C>G        | Maternal    | gnomeAD East Asia 0.0000, HGVD 0.0000, 14KJPN 0.0003, In-house (n = 218) 0.0000 |
| c.274G>C          | Maternal    | gnomeAD East Asia 0.0000, HGVD 0.0000, 14KJPN 0.0003, In-house (n = 218) 0.0000 |
| p.(Val92Leu)      | Maternal    | gnomeAD East Asia 0.0000, HGVD 0.0000, 14KJPN 0.0003, In-house (n = 218) 0.0000 |
| p.(Ile372dup)     | Maternal    | gnomeAD East Asia 0.0000, HGVD 0.0000, 14KJPN 0.0003, In-house (n = 218) 0.0000 |
| p.(Ser1831Thr)    | De novo     | gnomeAD East Asia 0.0000, HGVD 0.0000, 14KJPN 0.0003, In-house (n = 218) 0.0000 |

| Pathogenicity     | CADD PHRED  | PolyPhen-2 HumVar | SIFT | MutationTaster |
|-------------------|------------|-------------------|------|----------------|
| Not predicted     | 1.6        | Not predicted     | 0.003| Disease causing|
| Not predicted     | 12.8       | Not predicted     | 0.115| Disease causing|
| Not predicted     | 12.4       | Not predicted     | 0.001| Disease causing|
| Not predicted     | 1% most deleterious | Not predicted | 27.4| Disease causing|
| Non-deleterious   | 12.4       | Not predicted     | 0.001| Disease causing|
| Deleterious       | 0.003      | Not predicted     | 1% most deleterious | Disease causing|
| 1% most deleterious| 24.0       | Not predicted     | Disease causing |
| 1% most deleterious| 34.0       | Not predicted     | Disease causing |
| 1% most deleterious| 27.4       | Not predicted     | Disease causing |

| Phenotype         | OMIM        | MutationTaster |
|-------------------|-------------|----------------|
| Spondyloepimetaphyseal dysplasia, Camera-Genevieve type | Unknown | Disease causing |
| Fructose-1,6-bisphosphatase deficiency | Unknown | Disease causing |
| Unknown | Unknown | Ciliary dyskinesia, primary |

Shown are non-synonymous rare variants with minor allele frequencies of ≤0.01 in all the public and in-house databases employed.

The URLs utilized are shown in the footnotes of Supplementary Table 1; *in silico* analyses have been performed using default parameters.
**Supplementary Table 3.** Rare variants extracted from whole exome sequencing in patient 3, under the assumption of Mendelian inheritance with complete penetrance in the trio analysis.

| Gene (Chromosome) | Compound heterozygous variants | De novo variants | Hemizygous variant |
|-------------------|--------------------------------|-----------------|-------------------|
| **Variant (GenBank)** | **NM_018946.4** | **NM_018946.4** | **NM_001304360.2** | **NM_001324140.2** |
| Inheritance | Maternal | Paternal | Maternal | Paternal |
| **Frequency** | gnomeAD East Asia | HGVD | 14KJPN | In-house (n = 218) |
| Score | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| PolyPhen-2 HumVar | 24.2 | 13.9 | 16.7 | 13.5 |
| SIFT | 0.950 | 0.000 | 0.804 | 0.918 |
| MutationTaster | 0.000 | 0.000 | 0.003 | 0.000 |
| OMIM | 1.000 | 1.000 | 0.999 | 1.000 |
| **Pathogenicity** | CADD PHRED | PolyPhen-2 HumVar | SIFT | MutationTaster |
| Score | 1% most deleterious | 1% most deleterious | 1% most deleterious | 1% most deleterious |
| PolyPhen-2 HumVar | Deleterious | Deleterious | Deleterious | Deleterious |
| SIFT | Benign | Possibility damaging | Not predicted | Not predicted |
| MutationTaster | Disease causing | Not predicted | Not predicted | Not predicted |
| OMIM | Spondyloepimetaphyseal dysplasia, Camera-Genevieve type | Unknown | Unknown | Unknown |
| **Phenotype** | OMIM | | | |
| Score | Neurodegeneration with brain iron accumulation 5 | Unknown | Unknown | Unknown |

Shown are non-synonymous rare variants with minor allele frequencies of ≤ 0.01 in all the public and in-house databases employed.

The URLs utilized are shown in the footnotes of Supplementary Table 1; *in silico* analyses have been performed using default parameters.
**Supplemental Table 4.** Primers used in this study.

| Primers used in this study | Sequence analysis using gDNA | Forward | Reverse |
|---------------------------|-----------------------------|---------|---------|
| c.133-12T>A & c.207delG   | TGGGTGAAAGAGCGAAACTC        | GGGAACAGAAAGGATGAGCA |
| c.607T>C                 | GGCTCAACCAGATACGCTTC        | CTTCTCATTGCAGCCATCT |
| c.979_981dup            | AGCCCTTTCCCTTTGCACTTT       | ACAGCATCAGCAAGGGAACT |

| Sequence and qPCR analyses using cDNA | Forward | Reverse |
|-------------------------------------|---------|---------|
| c.133-12T>A                        | CCGGACCAGACTGGTAGT (P1)   | ACTGGTCATGGCTGAACCTCC (P2) |
| c.607T>C                           | TCCAGTGGGATGCAATCAAT       | CTTCTCATTGCAGCCATCT |

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Supplementary Figure 1. Roentgenograms showing longitudinal striations. Epiphyseal findings of patient 3 appear sclerotic and irregular (flocky).