Supplementary Material

# Supplementary Data

Supplementary Material should be uploaded separately on submission. Please include any supplementary data, figures and/or tables.

Supplementary material is not typeset so please ensure that all information is clearly presented, the appropriate caption is included in the file and not in the manuscript, and that the style conforms to the rest of the article.

# Supplementary Figures and Tables

## Supplementary Tables

Table S1. Details of copy number variant identified by CMA in 44 euploid fetuses with increased NT

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Case ID** | **NT（mm)** | **QF-PCR result** | **Karoytype result** | **CMA result** | **Size** |  **Related syndrome/gene** | **Inheritance** | **Categorization**  | **outcome** |
| 1 | 3.10  | Nomal | Nomal | arr[hg19] 8q24.13q24.21(127,087,342-128,404,823)x3 | 1.3Mb | No specified | N/S | VOUS | Live birth |
| 2 | 3.00  | Nomal | Nomal | arr[hg19] 16p13.11(15,375,911-16,278,133)x3 | 902kb | 6 OMIM genes | maternal | VOUS | Live birth |
| 3 | 3.10  | Nomal | Nomal | arr[hg19] 7q11.21q11.23(62,569,501-75,233,244)×2 hmz | 12.7Mb | AOH | De novo | Others-AOH | Live birth |
| 4 | 3.60  | Nomal | Nomal | arr[hg19] 22q11.21(18,648,855-20,312,661)x3 | 1.7Mb | 22q11.2 microduplication syndrome | De novo | P | Live birth |
| 5 | 3.30  | Nomal | Nomal | arr[hg19] Xq22.2(102,957,372-103,111,680)x2 | 154kb | 2 OMIM genes （RAB9B , PLP1）；Pelizaeus-Merzbacher disease（PMD）、Spastic paraplegia 2, X-linked（SPG2） | N/S | P | TOP |
| 6 | 3.20  | Nomal | Nomal | arr[hg19] 22q11.21(18,648,855-21,461,017)x3 | 2.8Mb | 22q11.2 duplication syndrome | N/S | P/LP | Live birth |
| 7 | 3.70  | Nomal | Nomal | arr[hg19] 12q11q13.12(38,190,102-49,316,412)×2 hmz； 12q23.3q24.13(105,077,945-113,923,939)×2 hmz | 11.1Mb、8.8Mb | AOH | N/S | Others-AOH | Live birth |
| 8 | 3.00  | Nomal | Nomal | arr[hg19] 1q25.1q25.2(175,146,449-176,164,670)x3；Xq26.2(131,028,807-131,820,272)x1 | 1.0Mb、791kb | 3OMIM genes；4 OMIM genes (eg.FRMD7) | N/S | VOUS/P | Live birth |
| 9 | 4.10  | Nomal | Nomal | arr[hg19] 2q14.2q21.2(121,804,081-133,300,702)×2 hmz | 11.5Mb | AOH | N/S | Others-AOH | Live birth |
| 10 | 2.90  | Nomal | Nomal | arr[hg19] 8p22(15,868,963-17,452,339)x3 | 1.6Mb | 8 OMIM genes | N/S | VOUS | Live birth |
| 11 | 3.60  | Nomal | Nomal | arr[hg19] 8q12.1(59,700,319-61,484,612)x3 | 1.8Mb | 3 OMIM genes | N/S | VOUS | Live birth |
| 12 | 3.70  | Nomal | Nomal | arr[GRCh37] 14q22.1q23.3(53159576\_65724107)x2 hmz | 12.6Mb | ROH | N/S | Others-ROH | Live birth |
| 13 | 3.30  | Nomal | Nomal | arr[hg19] 10q26.11q26.12(120,890,519-122,179,122)x3 | 1.3Mb | 8 OMIM genes (eg.BAG3) | N/S | VOUS | Live birth |
| 14 | 2.40  | Nomal | Nomal | arr[hg19] 4q12q13.1(58,193,591-62,730,657)x3 | 4.5Mb | ADGRL3  | N/S | VOUS | Live birth |
| 15 | 3.10  | Nomal | Nomal | arr[hg19] 4p16.3(68,345-2,181,924)x1 | 2.1Mb | Wolf-Hirschhorn syndrome | N/S | P | TOP |
| 16 | 3.20  | Nomal | Nomal | arr[hg19] 3q11.1q11.2(93,519,464-96,949,316)x1 | 3.43Mb | 5 OMIM genes | N/S | VOUS | Live birth |
| 17 | 3.20  | Nomal | Nomal | arr[hg19] 22q11.21(18,648,855-21,800,471)x1 | 3.2Mb | DiGeorge syndrome | N/S | P | TOP |
| 18 | 2.90  | Nomal | Nomal | arr[hg19] 10q11.22q11.23(48,750,425-52,457,367)x3；20p12.1(13,963,727-17,657,339)x1 | 2.6Mb、3.7Mb | 17 OMIM genes；9 OMIM genes | maternal | VOUS/VOUS | Live birth |
| 19 | 2.80  | Nomal | Nomal | arr[hg19] 14q22.1q23.3(51,437,619-66,297,540)×2 hmz | 14.9Mb | AOH | De novo | Others-AOH | Live birth |
| 20 | 4.80  | Nomal | Nomal | arr[hg19] 16p13.11(14,910,158-16,458,424)x1 | 1.5Mb | 16p13.11 microdeletion syndrome | N/S | P | TOP |
| 21 | 2.60  | Nomal | Nomal | arr[hg19] 16p13.11p12.3(15,338,152-18,172,468)x3 ma | 2.9Mb | 16p13.11 duplication syndrome | maternal | VOUS | Live birth |
| 22 | 2.70  | Nomal | Nomal | arr[hg19] 1q21.1q21.2(145,895,746-147,830,830)x1 | 1.9Mb | 1q21.1 microdeletion syndrome | maternal? | P | Live birth |
| 23 | 3.60  | Nomal | Nomal | arr[hg19] Xp11.4(40,018,086-40,946,284)x2 | 928kb | 4 OMIM genes | maternal? | VOUS | Live birth |
| 24 | 2.70  | Nomal | Nomal | arr[hg19] Xq22.3(104,675,923-105,815,760)x1 | 1.1Mb | 3 OMIM genes | N/S | VOUS | Live birth |
| 25 | 2.70  | Nomal | Nomal | arr[hg19] 21q11.2q21.1(15,961,155-17,519,310)x1 | 1.6Mb | 3 OMIM genes | N/S | VOUS | Live birth |
| 26 | 3.10  | Nomal | Nomal | Maternal UPD (7) mosaics | - | Russell-Silver syndrome | maternal | Others-mosaics | Live birth |
| 27 | 9.00  | Nomal | Nomal | arr(1-22)×2,(X)×1 | - | Turner syndrome | N/S | P | Live birth |
| 28 | 3.10  | Nomal | Nomal | arr[GRCh37] 1q21.1q21.2(146586250\_147391923)x3 | 806kb | 1q21.1 microdeletion syndrome | De novo | P | Live birth |
| 29 | 2.70  | Nomal | Nomal | arr[GRCh37] 12p13.33p13.32(2024105\_3841392)x1 | 1.8Mb | Timothy syndrome（CACNA1C） | N/S | VOUS | Live birth |
| 30 | 4.00  | Nomal | Nomal | arr[GRCh37] 14q32.31q32.33(101858740\_107279475)x2 hmz | 5.4Mb | ROH | De novo | Others-ROH | Live birth |
| 31 | 2.50  | Nomal | Nomal | arr[GRCh37] 16p11.2(29591327\_30167919)x3 | 577kb | 16p11.2 microduplication syndrome | N/S | P | Live birth |
| 32 | 3.00  | Nomal | Nomal | arr[GRCh37] 16p11.2(29428532\_30190029)x1 | 761kb | 16p11.2 microdeletion syndrome | N/S | P | TOP |
| 33 | 3.11  | Nomal | Nomal | arr[GRCh37] 16p13.11(15058821\_16309046)x3 | 1.25Mb | 10 OMIM genes | maternal? | VOUS | Live birth |
| 34 | 2.90  | Nomal | Nomal | arr[GRCh37] 16p11.2(29581102\_30190029)x1 | 609kb | 16p11.2 microdeletion syndrome | N/S | P | Live birth |
| 35 | 3.00  | Nomal | Nomal | arr[GRCh37] 16p12.2(21841354\_22431031)x1 | 590kb | 16p12.2 microdeletion syndrome | maternal | P | Live birth |
| 36 | 3.60  | Nomal | Nomal | arr[GRCh37] 14q23.2q23.3(64289920\_66098274)x3 | 1.8Mb | 14 OMIM genes | N/S | VOUS | Live birth |
| 37 | 3.50  | Nomal | Nomal | arr[GRCh37] 16p12.2(21946956\_22442007)x3 | 495kb | 4 OMIM genes | N/S | VOUS | Live birth |
| 38 | 3.10  | Nomal | Nomal | arr[hg19] 22q11.21(18,648,855-20,312,661)x3 | 1.7Mb | 22q11.2 duplication syndrome | N/S | P | TOP |
| 39 | 2.80  | Nomal | Nomal | arr[hg19] 16p13.11(15,171,146-16,272,403)x3 | 1.1Mb | 16p13.11 microduplication syndrome | N/S | VOUS | Live birth |
| 40 | 3.20  | Nomal | Nomal | arr[hg19] 22q11.21(18,648,855-21,454,872)x3 | 2.8Mb | 22q11.2 microduplication syndrome | N/S | P | Live birth |
| 41 | 3.00  | Nomal | Nomal | arr(22)×2-3 | - | Mosaics 22 Trisomy（74%） | N/S | Others-mos | TOP |
| 42 | 4.40  | Nomal | Nomal | arr[hg19] 16p13.11(14,892,976-16,538,596)x1 | 1.65Mb | 16p13.11 microdeletion syndrome | N/S | P | Live birth |
| 43 | 3.00  | Nomal | Nomal | arr[hg19] 13q12.12(23,519,917-24,922,373)x3 | 1.4Mb | 8 OMIM genes | N/S | VOUS | Live birth |
| 44 | 2.60  | Nomal | Nomal | arr[hg19] 2q12.3q13(109,373,186-110,442,979)x3 | 1.07Mb | 3 OMIM genes | N/S | VOUS | Live birth |

mm, millimeter; NT, nuchal translucency; CMA, chromosomal microarray analysis; N/S, not specified;P, Pathogenic; VOUS, variant of uncertain significance;TOP, termination of pregnancy ; UPD,uniparental disomy.

Table S2. Clinical and molecular information of 40 fetuses with increased NT identified by WES

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Case ID** | **NT（mm)** | **QF-PCR result** | **CMA result** | **WES result** | **Zygosity** | **Inheritance** | **Disease association(s)** |  **Categorization** | **outcome** |
| 1 | 3.10  | Nomal | Nomal | PTPN11(NM\_002834)c.1124A>G(p.Y375C) | Heterozygous | maternalAD | Noonan syndrome 1/LEOPARD syndrome 1/Metachondromatosis | VOUS | Live birth |
| 2 | 3.40  | Nomal | Nomal | CACNA1E(NM\_000721)c.4615C>T(p.R1539\*)；PITX1(NM\_002653)c.297\_298delinsTT(p.Q99\_Q100delinsH\*) | Heterozygous | De novoAD | Developmental and epileptic encephalopathy 69 / Liebenberg syndrome | LP/LP | Live birth |
| 3 | 4.50  | Nomal | Nomal | PEX1(NM\_000466)c.1583\_1587delTACAA(p.I528Sfs\*13)；PEX1(NM\_000466)c.782\_783delAA(p.Q261Rfs\*8) | Compound Heterozygous | Paternal/MaternalAR | Peroxisome biogenesis disorder 1A/1B | LP/VOUS | Live birth |
| 4 | 4.40  | Nomal | Nomal | LZTR1(NM\_006767)c.2306C>T(p.T769M) | Heterozygous | MaternalAD | Noonan syndrome 10 | VOUS | Live birth |
| 5 | 3.20  | Nomal | Nomal | FGFR3(NM\_000142)c.1138G>A(p.G380R) | Heterozygous | De novoAD | Achondroplasia | P | Live birth |
| 6 | 3.60  | Nomal | Nomal | XYLT1(NM\_022166)c.2456G>T(p.G819V) | Homozygous | Paternal/MaternalAR | Desbuquois dysplasia 2 | VOUS | TOP |
| 7 | 3.20  | Nomal | Nomal | SOS1(NM\_005633)c.929G>A(p.R310H) | Heterozygous | MaternalAD | Noonan syndrome 4 | VOUS | Live birth |
| 8 | 3.10  | Nomal | Nomal | PRRX1(NM\_022716)c.146T>C(p.V49A) | Heterozygous | De novoAD/AR | Agnathia-otocephaly complex | VOUS | Live birth |
| 9 | 3.40  | Nomal | Nomal | RAI1(NM\_030665)c.3445C>T(p.Arg1149Cys) | Heterozygous | De novoAD | Smith-Magenis syndrome | VOUS | Live birth |
| 10 | 5.60  | Nomal | Nomal | COL2A1(NM\_001844)c.3599G>T(p.Gly1200Val) | Heterozygous | De novoAD | Achondrogenesis, type II or hypochondrogenesis | VOUS | TOP |
| 11 | 3.40  | Nomal | Nomal | ETFDH(NM\_004453.4)c.920C>G(p.Ser307Cys)；ETFDH(NM\_004453.4)c.959C>T(p.Ala320Val) | Compound Heterozygous | Paternal/MaternalAR | Multiple Acyl-CoA Dehydrogenase Deficiency(MADD) | LP/VOUS | Live birth |
| 12 | 2.70  | Nomal | Nomal | LZTR1(NM\_006767.4)c.2263C>T(p.Arg755Trp) | Heterozygous | MaternalAD/AR | Noonan syndrome 10/Noonan syndrome 2 | VOUS | Live birth |
| 13 | 3.00  | Nomal | Nomal | ZMIZ1(NM\_020338.4)c.1342C>T(p.Gln448\*) | Heterozygous | MaternalAD | Neurodevelopmental disorder with dysmorphic facies and distal skeletal anomalies | LP | Live birth |
| 14 | 4.10  | Nomal | Nomal | LZTR1(NM\_006767)c.740G>T(p.S247I) | Heterozygous | De novoAD | Noonan syndrome 10 | LP | TOP |
| 15 | 3.24  | Nomal | Nomal | LZTR1(NM\_006767)c.1201T>G(p.Y401D) | Heterozygous | PaternalAD/AR | Noonan syndrome 10/Noonan syndrome 2 | VOUS | Live birth |
| 16 | 4.10  | Nomal | Nomal | CHD4(NM\_001273)c.4018C>T(p.R1340C) | Heterozygous | De novoAD | Sifrim-Hitz-Weiss syndrome | LP | Live birth |
| 17 | 3.90  | Nomal | Nomal | VARS1(NM\_006295)c.94G>T(p.Gly32\*)；VARS1(NM\_006295)c939G>C(p.Trp313Cys) | Compound Heterozygous | Maternal/PaternalAR | Neurodevelopmental disorder with microcephaly, seizures, and cortical atrophy | LP/VOUS | Live birth |
| 18 | 3.40  | Nomal | Nomal | PACS1(NM\_018026) heterozygous deletion of exons 12-24 | Heterozygous | PaternalAD | Schuurs-Hoeijmakers syndrome | LP | Live birth |
| 19 | 3.60  | Nomal | Nomal | MYH3(NM\_002470)c.3402\_3403delG(p.K1135Tfs\*65) | Heterozygous | De novoAR/AD | Arthrogryposis, distal, type 2A/type 2B3;Contractures, pterygia, and spondylocarpostarsal fusion syndrome 1A/1B | LP | Live birth |
| 20 | 4.00  | Nomal | Nomal | PTPN11(NM\_002834)c.155C>T(p.T52I) | Heterozygous | De novoAR/AD | Noonan syndrome 1 | LP | Live birth |
| 21 | 3.20  | Nomal | Nomal | TRIP11(NM\_004239)c.2138C>A(p.T713N);c.3705G>C(p.M1235I) | Compound Heterozygous | Paternal/MaternalAR | Achondrogenesis, type IA;Odontochondrodysplasia 1 | VOUS | Live birth |
| 22 | 12.90  | Nomal | Nomal | PHGDH(NM\_006623)c.1015C>G(p.L339V),PHGDH(NM\_006623)c.1037G>A(p.W346\*) | Compound Heterozygous | Paternal/MaternalAR | Neu-Laxova syndrome 1;Phosphoglycerate dehydrogenase deficiency | LP/VOUS | TOP |
| 23 | 3.60  | Nomal | Nomal | SOS1(NM\_005633)c.508A>G(p.K170E) | Heterozygous | De novoAD | Noonan syndrome 4 | P | TOP |
| 24 | 3.90  | Nomal | Nomal | SOS1(NM\_005633)c.587C>T(p.S196L);COL3A1(NM\_000090)c.1682G>A(p.G561D) | Heterozygous | Paternal/De novoAD/AD | Noonan syndrome 4;Ehlers-Danlos syndrome, vascular type | VOUS/VOUS | Live birth |
| 25 | 3.17  | Nomal | Nomal | LZTR1(NM\_006767)c.2075T>C(p.F692S) | Heterozygous | MaternalAD/AR | Noonan syndrome 10/Noonan syndrome 2 | VOUS | Live birth |
| 26 | 4.10  | Nomal | Nomal | STS(NM\_000351.6)c.463G>A(p.G155S) | Hemizygous | MaternalXLR | Ichthyosis, X-linked | VOUS | Live birth |
| 27 | 6.00  | Nomal | Nomal | LZTR1(NM\_006767)c.2325+1G>A | Heterozygous | PaternalAD/AR | Noonan syndrome 10/Noonan syndrome 2 | VOUS | Live birth |
| 28 | 5.70  | Nomal | Nomal | NSD1(NM\_022455)c.1525\_1540del(p.Thr509Ala fs\*22) | Heterozygous | De novoAD | Sotos syndrome 1 | P | Live birth |
| 29 | 6.50  | Nomal | Nomal | FGFR3(NM\_001163213)c.742C>T(p.Arg248Cys) | Heterozygous | De novoAD | Achondroplasia/Thanatophoric dysplasia, type I | P | Live birth |
| 30 | 3.72  | Nomal | Nomal | TCIRG1(NM\_006019)c.1037\_1040dupGTGC(p.Val348Cysfs\*143),(NM\_006019)c.2218\_2219delCT(p.Leu740Glufs\*90) | Compound Heterozygous | Paternal/MaternalAR | Osteopetrosis, autosomal recessive 1 | VOUS | Live birth |
| 31 | 4.40  | Nomal | Nomal | SOS2(NM\_006939.3)c.20C>G(p.Pro7Arg) | Heterozygous | PaternalAD/AR | Noonan syndrome 9 | VOUS | Live birth |
| 32 | 4.10  | Nomal | Nomal | HRAS(NM\_005343.3)c.351G>T(p.Lys117Asn) | Heterozygous | De novoAD | Costello syndrome | VOUS | TOP |
| 33 | 11.80  | Nomal | Nomal | SOX9(NM\_000346.4)c.788delG(p.Gly263Alafs\*16) | Heterozygous | De novoAD | Campomelic Dysplasia | LP | Live birth |
| 34 | 5.10  | Nomal | Nomal | COL2A1(NM\_001844.5)c.4196del(p.Tyr1399Phefs\*36) | Heterozygous | De novoAD | Achondrogenesis, type II | LP | TOP |
| 35 | 3.20  | Nomal | Nomal | GRIN2B(NM\_000834.4)c.655C>T(p.Gln219\*) | Heterozygous | De novoAD | Developmental and epileptic encephalopathy 27;Intellectual developmental disorder, autosomal dominant 6, with or without seizures | LP | Live birth |
| 36 | 4.20  | Nomal | Nomal | EPHB4(NM\_004444.5)c.805C>T(p.Arg269\*) | Heterozygous | PaternalAD | Capillary malformation-arteriovenous malformation 2;Lymphatic malformation 7 | VOUS | Live birth |
| 37 | 3.00  | Nomal | Nomal | FGFR3(NM\_000142.4)c.742C>T(p.Arg248Cys) | Heterozygous | De novoAD | Achondroplasia/Thanatophoric dysplasia, type I | P | Live birth |
| 38 | 5.00  | Nomal | Nomal | PTPN11(NM\_002834.4)c.124A>G(p.Thr42Ala) | Heterozygous | De novoAD | Noonan syndrome 1/LEOPARD syndrome 1/Metachondromatosis | P | TOP |
| 39 | 3.10  | Nomal | Nomal | LZTR1(NM\_006767.4)c.27delG(p.Gln10Argfs\*15) | Heterozygous | PaternalAD | Noonan syndrome 10/Noonan syndrome 2/Schwannomatosis-2 | P | Live birth |
| 40 | 5.00  | Nomal | Nomal | MAP2K1(NM\_002755.4)c.608A>G(p.Glu203Gly) | Heterozygous | De novoAD | Cardiofaciocutaneous syndrome 3 | P | Live birth |

mm, millimeter; NT, nuchal translucency; TOP, termination of pregnancy ; QF-PCR,quantitative fluorescent polymerase chain reaction;WES, Whole Exome Sequencing; CMA, chromosomal microarray analysis; P, Pathogenic;LP,Likely Pathogenic; VOUS, variant of uncertain significance;AD, autosomal dominant; AR, autosomal recessive.