Supplementary Material

# Supplementary Data

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# 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 | maternal  AD | 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 novo  AD | 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/Maternal  AR | Peroxisome biogenesis disorder 1A/1B | LP/VOUS | Live birth |
| 4 | 4.40 | Nomal | Nomal | LZTR1(NM\_006767)c.2306C>T(p.T769M) | Heterozygous | Maternal  AD | Noonan syndrome 10 | VOUS | Live birth |
| 5 | 3.20 | Nomal | Nomal | FGFR3(NM\_000142)c.1138G>A(p.G380R) | Heterozygous | De novo  AD | Achondroplasia | P | Live birth |
| 6 | 3.60 | Nomal | Nomal | XYLT1(NM\_022166)c.2456G>T(p.G819V) | Homozygous | Paternal/Maternal  AR | Desbuquois dysplasia 2 | VOUS | TOP |
| 7 | 3.20 | Nomal | Nomal | SOS1(NM\_005633)c.929G>A(p.R310H) | Heterozygous | Maternal  AD | Noonan syndrome 4 | VOUS | Live birth |
| 8 | 3.10 | Nomal | Nomal | PRRX1(NM\_022716)c.146T>C(p.V49A) | Heterozygous | De novo  AD/AR | Agnathia-otocephaly complex | VOUS | Live birth |
| 9 | 3.40 | Nomal | Nomal | RAI1(NM\_030665)c.3445C>T(p.Arg1149Cys) | Heterozygous | De novo  AD | Smith-Magenis syndrome | VOUS | Live birth |
| 10 | 5.60 | Nomal | Nomal | COL2A1(NM\_001844)c.3599G>T(p.Gly1200Val) | Heterozygous | De novo  AD | 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/Maternal  AR | 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 | Maternal  AD/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 | Maternal  AD | 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 novo  AD | Noonan syndrome 10 | LP | TOP |
| 15 | 3.24 | Nomal | Nomal | LZTR1(NM\_006767)c.1201T>G(p.Y401D) | Heterozygous | Paternal  AD/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 novo  AD | 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 | Paternal  AD | Schuurs-Hoeijmakers syndrome | LP | Live birth |
| 19 | 3.60 | Nomal | Nomal | MYH3(NM\_002470)c.3402\_3403delG(p.K1135Tfs\*65) | Heterozygous | De novo  AR/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 novo  AR/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/Maternal  AR | 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/Maternal  AR | 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 novo  AD | 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 novo  AD/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 | Maternal  AD/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 | Maternal  XLR | Ichthyosis, X-linked | VOUS | Live birth |
| 27 | 6.00 | Nomal | Nomal | LZTR1(NM\_006767)c.2325+1G>A | Heterozygous | Paternal  AD/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 novo  AD | Sotos syndrome 1 | P | Live birth |
| 29 | 6.50 | Nomal | Nomal | FGFR3(NM\_001163213)c.742C>T(p.Arg248Cys) | Heterozygous | De novo  AD | 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/Maternal  AR | Osteopetrosis, autosomal recessive 1 | VOUS | Live birth |
| 31 | 4.40 | Nomal | Nomal | SOS2(NM\_006939.3)c.20C>G(p.Pro7Arg) | Heterozygous | Paternal  AD/AR | Noonan syndrome 9 | VOUS | Live birth |
| 32 | 4.10 | Nomal | Nomal | HRAS(NM\_005343.3)c.351G>T(p.Lys117Asn) | Heterozygous | De novo  AD | Costello syndrome | VOUS | TOP |
| 33 | 11.80 | Nomal | Nomal | SOX9(NM\_000346.4)c.788delG(p.Gly263Alafs\*16) | Heterozygous | De novo  AD | Campomelic Dysplasia | LP | Live birth |
| 34 | 5.10 | Nomal | Nomal | COL2A1(NM\_001844.5)c.4196del(p.Tyr1399Phefs\*36) | Heterozygous | De novo  AD | Achondrogenesis, type II | LP | TOP |
| 35 | 3.20 | Nomal | Nomal | GRIN2B(NM\_000834.4)c.655C>T(p.Gln219\*) | Heterozygous | De novo  AD | 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 | Paternal  AD | 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 novo  AD | Achondroplasia/Thanatophoric dysplasia, type I | P | Live birth |
| 38 | 5.00 | Nomal | Nomal | PTPN11(NM\_002834.4)c.124A>G(p.Thr42Ala) | Heterozygous | De novo  AD | Noonan syndrome 1/LEOPARD syndrome 1/Metachondromatosis | P | TOP |
| 39 | 3.10 | Nomal | Nomal | LZTR1(NM\_006767.4)c.27delG(p.Gln10Argfs\*15) | Heterozygous | Paternal  AD | 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 novo  AD | 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.