**Supplementary Material 1**

**CNV Validation**

CNVs were validated in the laboratory using two to three quantitative real-time PCR (qPCR) TaqMan assays (Applied Biosystems, Carlsbad, CA) per region. Genomic DNA was extracted from one 3-mm DBS [[Saavedra-Matiz et al., 2013](#_ENREF_1)], diluted 1:10 in water, and amplified using TaqMan Environmental Master Mix (ABI) in 5µl reaction volumes. A fragment of the RNaseP H1 RNA gene was co-amplified and used as an internal control (TaqMan Copy Number Reference Assay, ABI). Assays were run in quadruplicate on either an ABI 7900HT or an ABI QuantStudio. CopyCaller software v2.0 (ABI) was used to analyze the real-time data using relative quantitation (2-ΔΔCt method). The manual Ct threshold was set to 0.2 with the automatic baseline on. CopyCaller software parameters were as follows: the median ΔCt for each experiment was used as the calibrator, wells with an RNaseP Ct > 38 were excluded and the zero copy ΔCt threshold was set to 6. The average copy number and a software-generated confidence value were calculated for each subject. Samples with confidence values ≥ 0.95 were considered valid; samples with confidence values <0.95 were rerun in quadruplicate. All assays were tested in each of the 17 cases and 13 control subjects. We subsequently screened all validated CNVs against an additional 188 control samples from unaffected NYS births using at least one assay targeting the area of interest. Therefore, a total of 201 unaffected controls were screened using at least one assay in the candidate CNV region **(supplementary table 1).**

**Reference**

Saavedra-Matiz CA, Isabelle JT, Biski CK, Duva SJ, Sweeney ML, Parker AL, Young AJ, DiAntonio LL, Krein LM, Nichols MJ, Caggana M. 2013. Cost-Effective and Scalable DNA Extraction Method from Dried Blood Spots. *Clin Chem* **59**: 1045-1051.

**Supplementary Table I.** CNVs present in cases and absent in controls.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Locus** | **Assay ID** | **Gene** | **Target Coordinates** | **# Cases Tested** | **# Controls Tested** |
| 1p13.2 | Hs05760729\_cn | - | Chr.1:113,599,852 | 17 | 201 |
| Hs01528582\_cn | LRIG2 | Chr.1:113,616,080 | 17 | 13 |
| 1q42.3 | Hs06575556\_cn | - | Chr.1:235,177,040 | 17 | 201 |
| Hs06567844\_cn | - | Chr.1:235,183,842 | 17 | 13 |
| 2q37.3\*\* | KT\_1343\_CXVI3RU | ING5 | Chr.2:242,659,600 | 17 | 201 |
| Hs01490888\_cn | GAL3ST2 | Chr.2:242,741,278 | 17 | 14 |
| 4q23 | Hs04834234\_cn | - | Chr.4:101,051,884 | 17 | 201 |
| Hs04831759\_cn | - | Chr.4:101,063,569 | 17 | 13 |
| 5q11.2 | c3\_start\_CX1RUMN | - | Chr5:56,350,589 | 17 | 13 |
| c3\_stop\_CX0IWGF | - | Chr5:56,366,155 | 17 | 201 |
| 6q12 | Hs03089526\_cn | - | Chr.6:63,687,776 | 17 | 201 |
| Hs03260635\_cn | - | Chr.6:63,708,948 | 17 | 13 |
| 6q16.1 | Hs06739017\_cn | - | Chr.6:93,750,741 | 17 | 201 |
| Hs06750296\_cn | - | Chr.6:93,851,942 | 17 | 13 |
| 7p14.3 | Hs04988480\_cn | PDE1C | Chr.7:32,041,346 | 17 | 201 |
| Hs04960929\_cn | Chr.7:32,077,956 | 17 | 15 |
| 7p13\* | Hs04952152\_cn | CAMK2B | Chr.7:44,267,651 | 17 | 13 |
| Hs04941806\_cn | Chr.7:44,289,134 | 17 | 13 |
| 7p21.1 | Hs03646420\_cn | HDAC9 | Chr.7:18,267,017 | 17 | 201 |
| Hs03638416\_cn | Chr.7:18,331,281 | 17 | 13 |
| 8p12 | Hs06173267\_cn | - | Chr.8:34,907,726 | 17 | 13 |
| Hs05052219\_cn | - | Chr.8:34,941,912 | 17 | 201 |
| 10q24.1\* | Hs02010805\_cn | SLIT1 | Chr.10:98,762,731 | 17 | 13 |
| Hs00409538\_cn | Chr.10:98,773,543 | 17 | 13 |
| 18q23 | Hs06465115\_cn | - | Chr.18:75,552,966 | 17 | 201 |
| Hs03317078\_cn | - | Chr.18:76,028,173 | 17 | 13 |
| Hs03318976\_cn | - | Chr.18:76,150,822 | 17 | 13 |
| 19p13.3\* | Hs04016793\_cn | AP3D1 | Chr.19:2,144,267 | 17 | 13 |
| Hs04013238\_cn | DOT1L | Chr.19:2,167,559 | 17 | 13 |
| 20q11.23 | Hs07197533\_cn | DLGAP4 | Chr.20:35,019,755 | 17 | 201 |
| Hs07174315\_cn | Chr.20:35,040,587 | 17 | 13 |

\*CNV at this locus failed to validate.

\*\*CNV at this locus was found in a control.

**Supplementary Table II.** Selected phenotypes from CNV reports in ISCA and DECIPHER overlapping study case CNVs.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Locus** | **Genomic Coordinates** | **Size (bp)** | **Type** | **Genes/ Transcripts** | **ISCA** | **DECIPHER** |
| 1p13.2 | 113,595,229 -113,617,151 | 21,923 | Dupl | *LRIG2; RP11-31F15.2* | NA | NA |
| 1q42.3 | 235,167,295 - 235,191,818 | 24,524 | Het Del | *Intergenic* | NA | 265891-Hemifacial hypoplasia |
| 2q37.3 | 242,646,350 - 242,744,703 | 98,354 | Dupl | *ING5; D2HGDH; GAL3ST2; NEU4;* | nssv577770-Abnormality of limb bone morphology Abnormality of the skeletal system | 252280-Cavernous hemangioma |
| 4q23 | 101,044,442 - 101,065,531 | 21,090 | Het Del | *AC121157.1; RP11-15B17.1* | NA | 285906-Hemiatrophy |
| 5q11.2 | 56,345,093 - 56,382,661 | 37,569 | Het Del | *Intergenic* | NA | 249915-Postaxial polydactyly |
| 6q16.1 | 93,747,735-93,855,183 | 107,449 | Dupl | *RP1-23E21.2* | NA | NA |
| 7p21.1 | 18,263,964 -  18,336,338 | 72,375 | Het Del | *HDAC9* (uc011jya.2, uc003sua.1) | NA | 255320- Abnormal facial shape 259698- Cutaneous finger syndactyly |
| 7p14.3 | 32,040,979 -  32,082,768 | 41,790 | Het Del | *PDE1C* | NA | 314309-Hemifacial hypoplasia |
| 8p12 | 34,902,928 - 34,946,452 | 43,525 | Het Del | *Intergenic* | NA | NA |
| 18q23 | 75,522,402 -  76,163,540 | 641,139 | Dupl | *RNA5SP461; RP11-100K18.1; RP11-671C19.1; RP11-671C19.2, U6* | nssv577662-Abnormal facial shape Abnormality of limb bone morphology | NA |
| 20q11.23 | 35,015,926 - 35,043,790 | 27,865 | Dupl | *DLGAP4* | NA | 263052-Polydactyly |

**Supplementary Table III.** Case Phenotypes.

|  |  |  |
| --- | --- | --- |
| **Case ID** | **Diagnosis** | **Additional Phenotypic Information** |
| 1 | KLIPPEL-TRENAUNAY SYNDROME | AFFECTING LEFT ARM |
| 2 | KLIPPEL-TRENAUNAY SYNDROME | NO ADDITIONAL DATA |
| 3 | KLIPPEL-TRENAUNAY SYNDROME | THYROGLOSSAL CYST |
| 4 | KLIPPEL-TRENAUNAY SYNDROME | NO ADDITIONAL DATA |
| 5 | KLIPPEL-TRENAUNAY SYNDROME | LEFT HYDRONEPHROSIS  VENTRICULAR SEPTAL DEFECT  VATER ASSOCIATION  PATENT DUCTUS ARTERIOSUS |
| 6 | KLIPPEL-TRENAUNAY SYNDROME | VARICOSE VEINS ON LEFT LEG |
| 7 | KLIPPEL-TRENAUNAY SYNDROME | AGENESIS OF SACRUM |
| 8 | KLIPPEL-TRENAUNAY SYNDROME | UNSPECIFIED SKULL AND FACIAL BONE ANOMALY |
| 9 | KLIPPEL-TRENAUNAY SYNDROME | NO ADDITIONAL DATA |
| 10 | KLIPPEL-TRENAUNAY SYNDROME | VENOUS MALFORMATIONS |
| 11 | KLIPPEL-TRENAUNAY SYNDROME | PORT WINE NEVUS ON RIGHT LOWER LEG |
| 12 | KLIPPEL-TRENAUNAY SYNDROME | LEG HEMANGIOMAS |
| 13 | KLIPPEL-TRENAUNAY SYNDROME | UPPER EXTREMITY VESSEL ANOMALY |
| 14 | KLIPPEL-TRENAUNAY SYNDROME | NO ADDITIONAL DATA |
| 15 | KLIPPEL-TRENAUNAY SYNDROME | NO ADDITIONAL DATA |
| 16 | KLIPPEL-TRENAUNAY SYNDROME | LYMPHANGIOMA, HEMANGIOMA |
| 17 | KLIPPEL-TRENAUNAY SYNDROME | CONGENITAL HAMARTOSES  TALIPES EQUINOVARUS  PATENT DUCTUS ARTERIOSUS |