**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[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 32 cases and 13 control subjects. We subsequently screened all validated CNVs against an additional 180 control samples from unaffected NYS births using at least one assay targeting the area of interest. Therefore, a total of 193 unaffected controls were screened using at least one assay in the candidate CNV region **(supplemental table 1).**

**References**

1. 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 59:1045-51.

**Supplemental Table 1**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Locus** | **Assay ID** | **Gene** | **Target Coordinates** | **# Cases Tested** | **# Controls Tested** |
| 2p23.1 | Hs04588345\_cn | *LBH* | Chr.2:30459106 | 32 | 193 |
| 2p22.1 | Hs04700397\_cn | *SOS1* | Chr.2:39241498 | 32 | 13 |
| 2p21 | Hs05823760\_cn | *EPAS1* | Chr.2:46547112 | 32 | 13 |
| 2p16.2 | Hs05839009\_cn | *-* | Chr.2:53222335 | 32 | 13 |
| 2q11.2 | Hs01438279\_cn | *COX5B* | Chr.2:98263894 | 32 | 13 |
| Hs00870944\_cn | *ACTR1B* | Chr.2:98272841 | 32 | 193 |
| 3q13.32\* | Hs04261161\_cn | *IGSF11* | Chr.3:118789296 | 32 | 13 |
| Hs03482479\_cn | *IGSF11* | Chr.3:118731106 | 32 | 13 |
| 7p21.1 | Hs04999285\_cn | *-* | Chr.7:20334661 | 32 | 14 |
| Hs04982188\_cn | *-* | Chr.7:20343949 | 32 | 193\*\* |
| 7q11.23 | Hs03621715\_cn | *FKBP6* | Chr.7:72760879 | 32 | 193 |
| Hs01860808\_cn | *ELN* | Chr.7:73466099 | 32 | 13 |
| Hs04941227\_cn | *GTF2I* | Chr.7:74100770 | 32 | 13 |
| 9q34.11\*\*\* | Hs06899673\_cn | *-* | Chr.9:132313069 | 32 | 193# |
| Hs02732589\_cn | *PRRX2* | Chr.9:132481665 | 32 | 13 |
| 9q34.2\* | Hs03719210\_cn | *RXRA* | Chr.9:137284498 | 32 | 13 |
| Hs03710003\_cn | *RXRA* | Chr.9:137323092 | 32 | 13 |
| 10q23.33 | Hs03746767\_cn | *TBC1D12* | Chr.10:96229936 | 32 | 193 |
| Hs05147339\_cn | *HELLS* | Chr.10:96357183 | 32 | 13 |
| 17q24.2\* | Hs05519437\_cn | *-* | Chr.17:66470065 | 32 | 13 |
| Hs05497391\_cn | *-* | Chr.17:66482274 | 32 | 13 |
| 19p13.12 | Hs07154996\_cn | *-* | Chr.19:15860439 | 32 | 13# |
| Hs00963364\_cn | *NOTCH3* | Chr.19:15296321 | 32 | 193 |

\*CNV at this locus failed to validate.

\*\*One low confidence copy-number 1 control for this probe, subject was copy-number 2 for the other probe at this locus

 \*\*\*Validated copy-number 3 for both probes in subject with duplication called from array data. Additional case with high confidence copy-number 3 for Hs06899673\_cn and low confidence copy-number 3 for Hs02732589\_cn.

#One copy-number 1 control for this probe, subject was copy-number 2 for the other probe at this locus