Supplemental Material S1. Cytogenetic and molecular analyses.

Karyotype analysis

Peripheral venous blood lymphocytes were grown following standard protocols and collected after 72 hours. A moderate resolution G-banding (550 bands) karyotyping by trypsin (Gibco 1x trypsin® and Leishmann stain) was subsequently performed. Microscopic analysis was conducted with a Nikon® eclipse 50i optical microscope and the IKAROS Karyotyping System (MetaSystem® software). DNA from the patient and her parents was extracted from 100 µl of EDTA-anticoagulated whole blood using MagNA Pure (Roche Diagnostics, West Sussex, UK) and used for subsequent analyses.

Multiplex ligation-dependent probe amplification (MLPA)

MLPA was conducted to detect abnormal copy-number variations (CNVs) in subtelomeric regions of the chromosomes, as well as frequent interstitial CNVs. MLPA consists on the amplification of different probes using a single PCR primer pair. Each probe detects a specific subtelomeric DNA sequence. One kit from MRC-Holland was used: SALSA® MLPA® probemix P245 Microdeletion Syndromes-1A. This kit contains 50 MLPA probes with amplification products between 130 and 499 nt. These probes detect sequences involved in diverse microdeletion and microduplication disorders: 1p36 deletion syndrome, 2p16.1-p15 microdeletion syndrome, 2q23.1 microdeletion/microduplication syndrome, Glass syndrome, 3q29 microdeletion syndrome, 3q29 microduplication syndrome, Wolf-Hirschhorn syndrome, Cri-du-Chat syndrome, Sotos syndrome, Williams-Beuren syndrome, Williams-Beuren duplication syndrome, Langer-Giedion syndrome, 9q22.3 microdeletion syndrome, DiGeorge syndrome-2, Prader-Willi syndrome, Angelman syndrome, Witteveen-Kolk/15q24 microdeletion syndrome, Rubinstein-Taybi syndrome, Miller-Dieker syndrome,

Lissencephaly-1, Smith-Magenis syndrome, Potocki-Lupski syndrome, NF1 microdeletion syndrome, Koolen-de Vries syndrome, 17q21.31 microduplication syndrome, DiGeorge syndrome, 22q11.2 microduplication syndrome, Distal 22q11.2 deletion syndrome, Phelan-McDermid syndrome, Rett syndrome, and MECP2 duplication syndrome. The PCR products were analyzed by capillary electrophoresis in an automatic sequencer Hitachi 3500 and further analyzed with the Coffalyser V 1.0 software from MRC-Holland.

Microarrays for whole-genome CNVs search and chromosome aberrations analysis

The DNA from the patient and his parents was hybridized on a CGH platform (Agilent Technologies). The derivative log ratio spread (DLRS) value was 0.172204. The platform included 60.000 probes. Data were analyzed with AgilentCytoGenomics3.0.5.1 and qGenViewer, and the ADM-2 algorithm (threshold = 6.0; aberrant regions had more than 4 consecutive probes).