Appendix 1

Supplementary data to manuscript "TBX4 mutations (Small Patella Syndrome) are associated with childhood-onset Pulmonary Arterial Hypertension "
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Genetic analysis

aCGH analysis

Array-comparative genomic hybridization (aCGH) analysis was performed using the 180K oligo array from Agilent (custom design ID: 23363; Agilent Technologies Inc., Santa Clara, CA, USA). A mixture of 40 healthy male or 40 female DNA samples was used as reference (sex-matched). Procedures were performed according to the manufacturer's protocol. Data were extracted using Feature Extraction V.9.1 software.

Mutation analysis of TBX2 and TBX4

Mutation analysis of all coding exons and flanking intronic sequences of the *TBX4* gene was carried out using flanking intronic primers (primer sequences are available upon request). The forward primer was designed with a PT1 tail (5'-TGTAAAACGACGGCCAGT-3') and the reverse primer was designed with a PT2 tail (5'-CAGGAAACAGCTATGACC-3'). PCR was performed in a total volume of 15 µl containing 10 µl AmpliTag Gold ®Fast PCR Master Mix (Applied Biosystems), 1.5 pmol/µl of each primer (Eurogentec, Serian, Belgium) and 2 µl genomic DNA. The samples were PCR amplified on a Perkin-Elmer (ABI) Geneamp 9700 using the following PCR program: an initial denaturation at 94°C for 1 minute, followed by 5 cycles of denaturation at 94°C for 5 seconds, annealing starting at 65°C for 30 seconds with a stepdown of 1°C every cycle, and elongation at 72°C for 1 minute, followed by 20 cycles of denaturation at 94°C for 5 seconds, annealing at 60°C for 30 seconds, and elongation at 72°C for 1 minute, followed by another 15 cycles of denaturation at 94°C for 5 seconds, annealing at 55°C for 30 seconds, and elongation at 72°C for 1 minute with a final step at 72°C for 5 minutes, after which the samples were cooled down to 20°C. 5 µl of the PCR products were loaded with 5 µl loading buffer and run on a 2% agarose gel with a FastRuler Low Range DNA Ladder (Fermentas) for comparison. The remainders of all PCR products were purified with ExoSAP-IT (Amersham Pharmacia Biotech, Biscataway, NY, USA) and subjected to direct sequencing on an ABI 3730 automated sequencer, using PT1 and PT2 primers.

MLPA deletion screening of TBX2 and TBX4

For both genes, two uniquely sized probes were developed in accordance with a protocol provided by MRC Holland, The Netherlands). Ten probes were combined in one MLPA assay together with a quantity of DNA and a DNA denaturation control mix (EK-1 kit, MRC Holland). The procedure was further carried out as described by De Vries BB, Pfundt R, Leisink M et al. Diagnostic genome profiling in mental retardation. *Am J Hum Genet* 2005; 77(4):606-616.