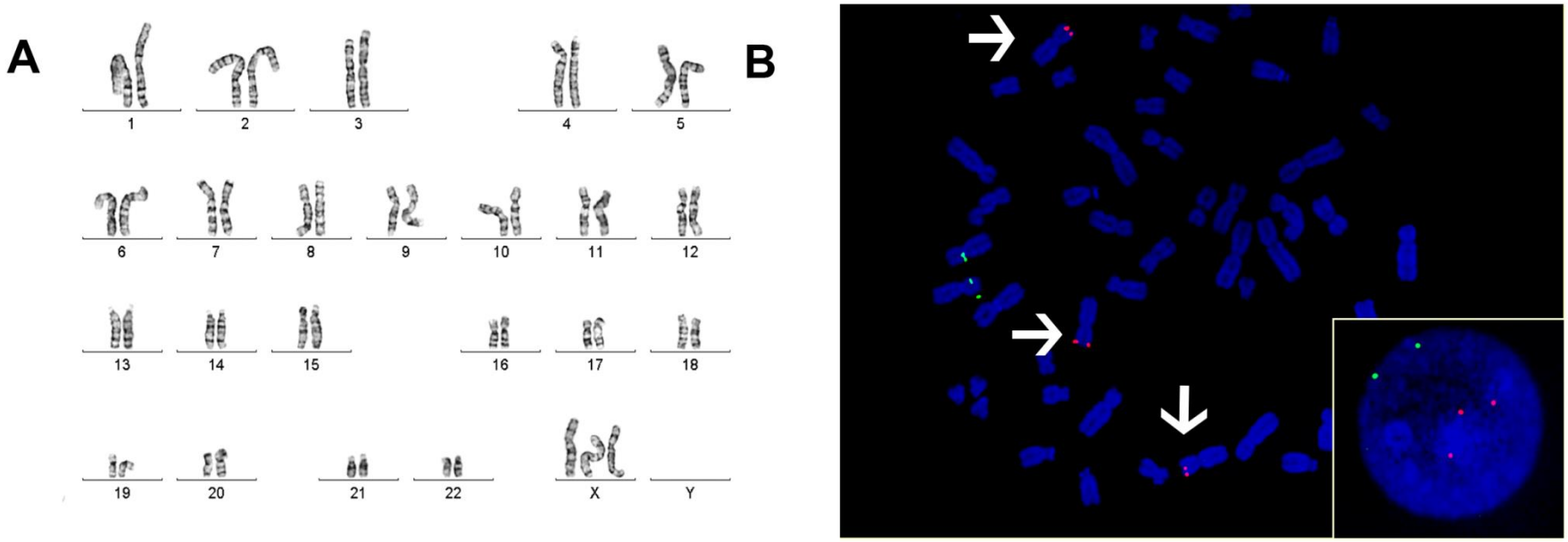
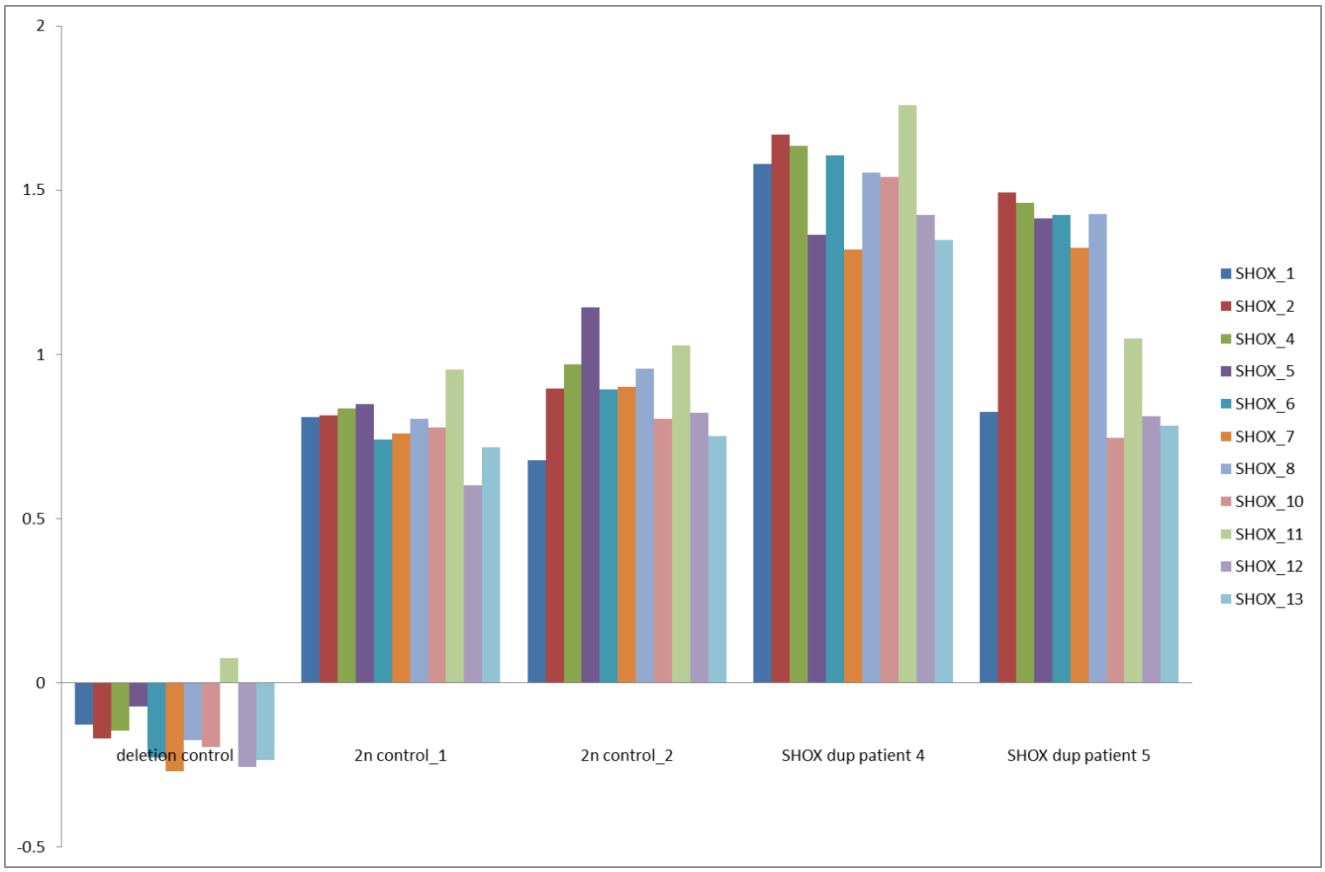
Supplementary figure 1 **Triple X syndrome karyotyping and FISH**

GTG-Giemsa banded karyotype of triple X patient 1 (A), confirmed with DNA FISH (B). X chromosomes are indicated with an arrow. BAC probe on Xp22 (RP11-800K15; red) and a control probe on 8p12 (RP11-489E7; green).



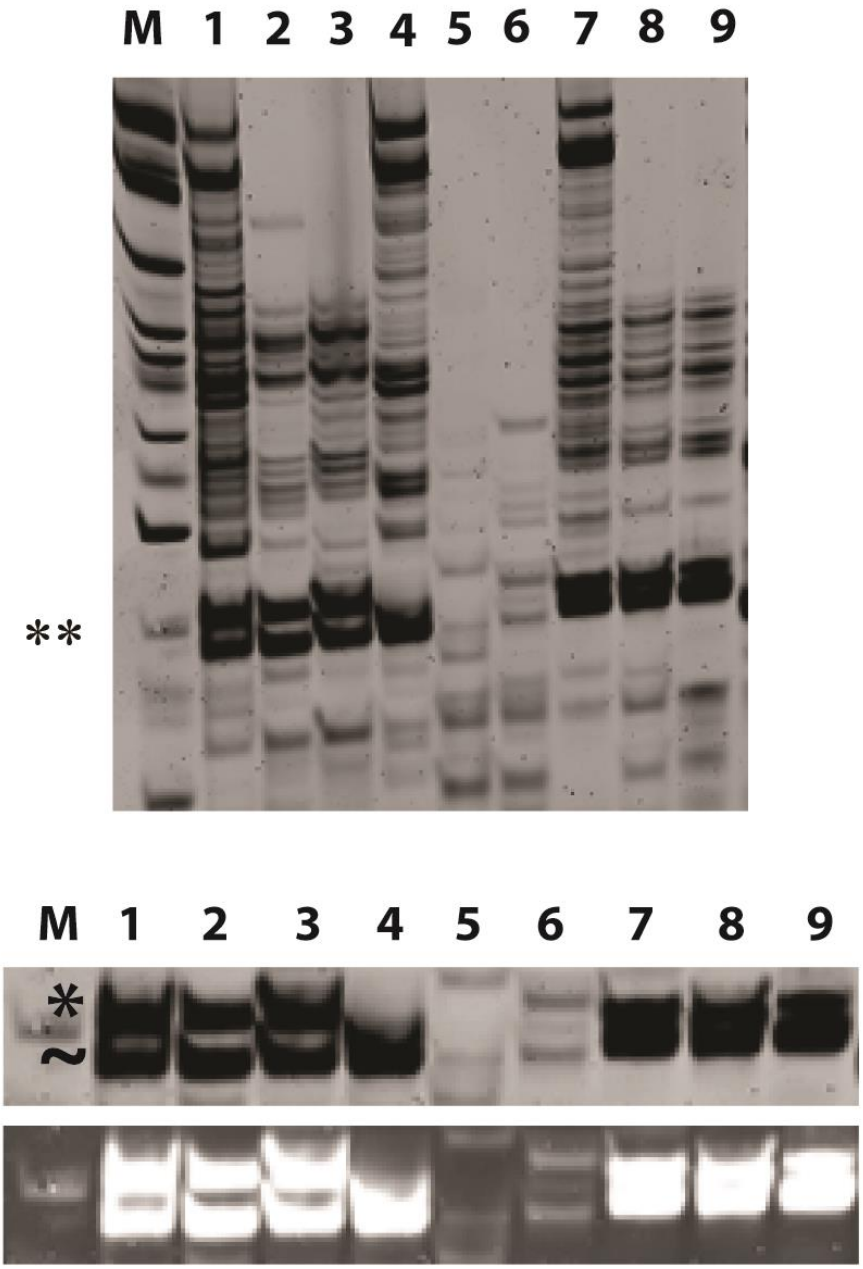
Supplementary figure 2 MLPA and *SHOX* specific qPCR

Copy number of the *SHOX* region was confirmed with a qPCR assay, with 11 amplicons within *SHOX* and the PAR1 region.[9] Patient 4 had gain of all amplicons and Patient 5 only of amplicons 2-8.



Supplementary figure 3 HUMARA assay

HUMARA analysis of genomic DNA of Patient 1 (lane 1-3), her father (lane 4-6) and mother (lane 7-9). Lane 1, 4 and 7 are undigested and the others HpaII digested samples of those individuals. In the upper panel the gel after electrophoresis is visualized, the polymorphic alleles of the androgen receptor are indicated (**) and enlarged in the lower panel. In the undigested Patient sample PCR analysis amplified two fragments, of which the band of higher molecular weight had a twofold intensity compared to the lower molecular weight band. This band therefore most likely represents the supernumerary X-chromosome, which is two times present in this 47,XXX patient and which therefore has a higher likelihood of being amplified. In the undigested control of the father (lane 4), only the lower molecular weight band was present, which is in accordance of a single X-chromosome in this male, whereas in the undigested control of DNA of the mother, two bands were present, with the larger fragment running at the same height as the band of the higher intensity found in patient 1. We conclude that the supernumerary X-chromosome of patient 1 is of maternal origin. HpaII digested samples of the patient had a similar pattern as the undigested control, with no change in intensity between the two fragments. Only background bands disappeared in the digested samples, which confirmed that HpaII digestion had digested the DNA. In the digested samples of the father almost no fragments were present, as expected from an active, and thus not methylated allele. In the digested samples of the mother the same two bands were identified as in the undigested control and also here no change in intensity between the upper and lower band was found. Therefore in both the mother (46,XX) and the patient (47,XXX) there is random XCI, with no skewed preference of inactivation of a particular X-chromosome.


M 1 2 3 4 5 6 7 8 9
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M 1 2 3 4 5 6 7 8 9
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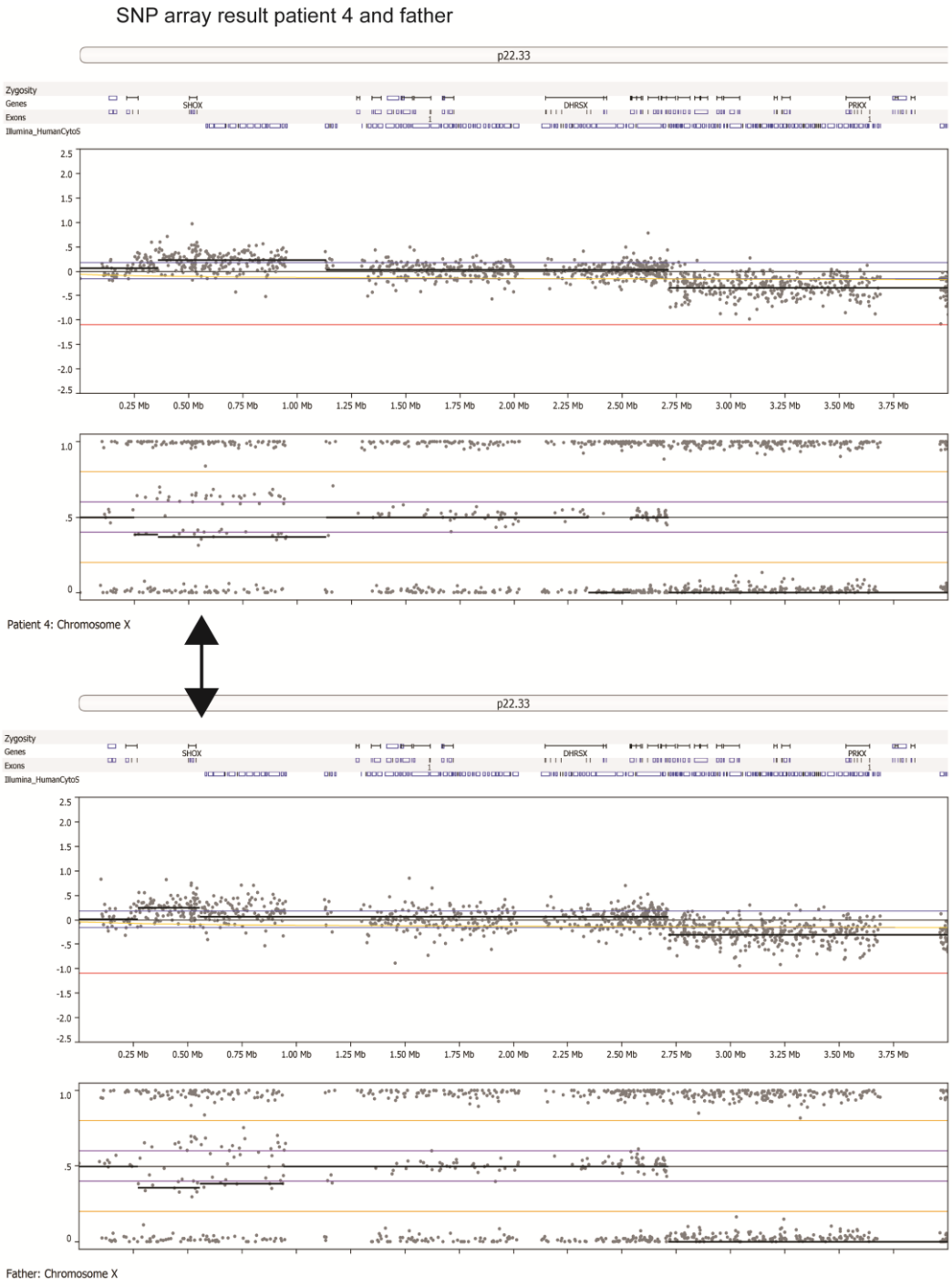
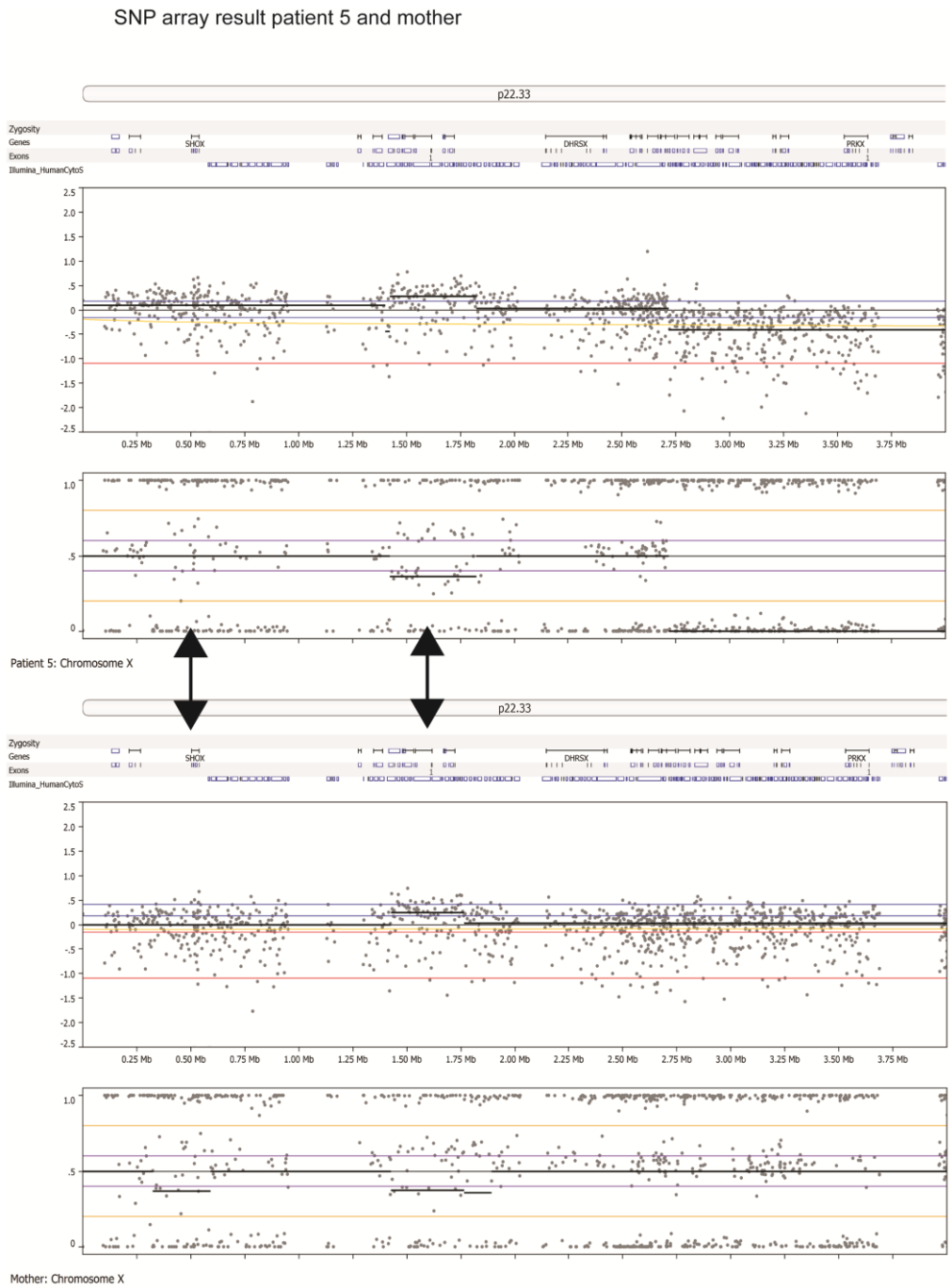
Supplementary figure 4 SNP-array results PAR1 region

Depicted are the PAR1 region represented on chromosome X of patient 4 (upper graph) and his father (lower graph) with a clear rise in LogR (upper tracks) and shift in B-allele frequency (lower tracks) indicative of a duplication.



Supplementary figure 5 SNP-array results PAR1 region

Depicted are the PAR1 region represented on chromosome X of patient 5 (upper graph) and his mother (lower graph) with a clear rise in LogR (upper tracks) and shift in B-allele frequency (lower tracks) indicative of a duplication.



Supplementary figure 6 ***SHOX*** **specific FISH**

DNA FISH of patient 5 with a BAC probe overlapping the SHOX gene (RP11-800K15; red) and a control probe Xq25 (RP11-49N19). RP11-800K15 only gives signals on chromosome X and chromosome Y.