The human genome; you gain some, you lose some

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Appendix

1. MAPH / array-CGH request form
2. Colour pictures

N.B.: Please, send this form as attachment to
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Patient for MAPH and/or Array-CGH screening

Date of birth:	…… / …… / ……..	
DNA number /Isolation number:	D.. / ….	D1. ……
Gender:	M / F	
Severe developmental delay	YES / NO	
Mild developmental delay	YES / NO	
Dysmorphic features:	YES / NO	
Please, list:		
(Multiple) Congenital Abnormalities (MCA)	YES / NO	
Please, list		
Heart defects present:	YES / NO	
Positive family history:	YES / NO	
If yes, please specify		
Consanguinity:	YES / NO	
Perinatal onset growth retardation:	YES / NO	
Previously tested for:		
• Karyotyping:	YES / NO	P-number:
• Fragile X	YES / NO	
• microdeletion syndrome	YES / NO	
Outcome:		
MAPH screening :	YES / NO	
CGH screening:	YES / NO	
Responsible clinician:	Date:	

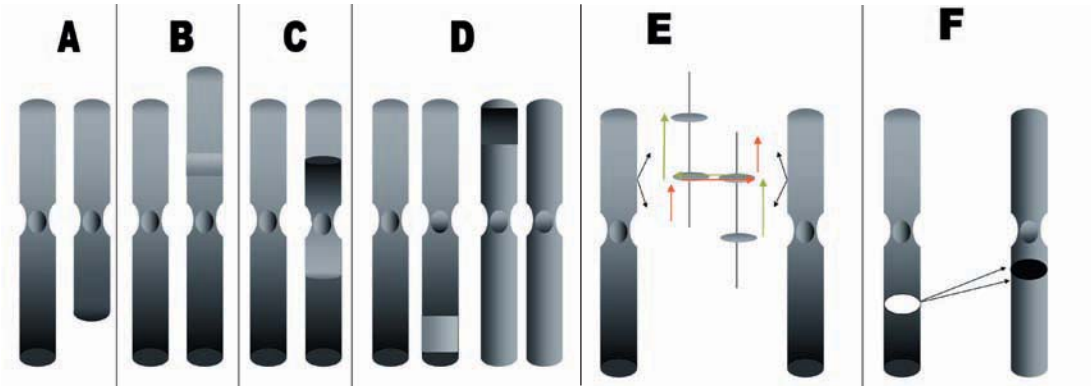
Colour pictures

Chapter I

p. 14 and 15

Figure 1. Deletion, duplication, inversion and balanced translocation.

Figure 2. Non-allelic homologous recombination and insertions.



A. Part of the long arm of the right chromosome is missing. The loss of genomic material is called a deletion.
B. A part of the short arm of the chromosome is present twice (right). This extra material is called a duplication. As the duplicated region is localised within the chromosome, this duplication is called an interstitial duplication.
C. The amount of genetic material in part C of this picture is similar to the unaffected left chromosome. However, a part of the chromosome is inverted. As the centromere is localised within the invertion, this situation is called a pericentromeric inversion.
D. Again the amount of genetic material is normal, however, a part of the information of the dark grey chromosome has been transported to the light grey chromosome and vice versa. This is called a balanced translocation.
E. Non allelic homologous recombination. The two alleles of a chromosome contain regions that are highly homologous (e.g. segmental duplications, low copy repeats or duplicons). The presence of these segmental duplications can result in misalignment of these regions and subsequently in non allelic homologous recombination. The green arrow shows the origin of a duplication of the region present between two highly homologous regions, whereas the red arrow indicates the origin of a deletion.
F. In this situation a part of the left chromosome is inserted in another chromosome. This is called an insertion.

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Figure 3. Current standard cytogenetic diagnostic tools and their characteristics.

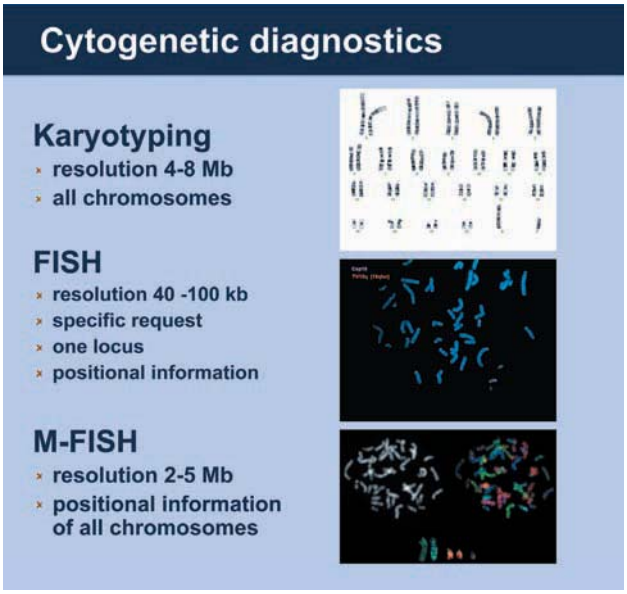

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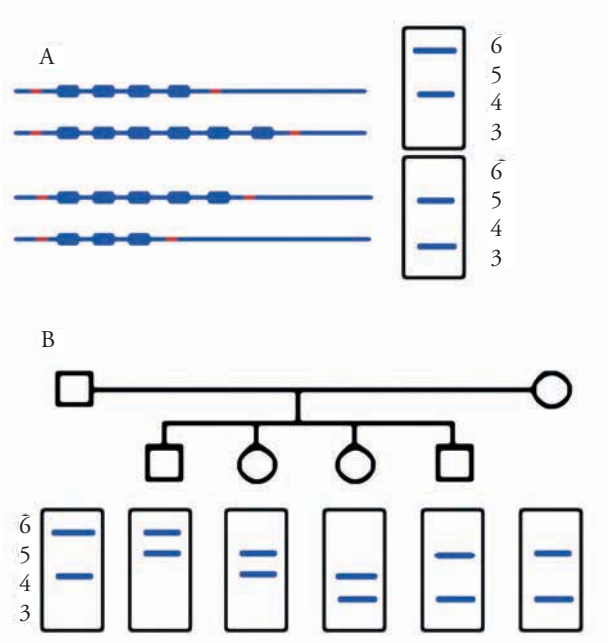
Figure 4. Identification of the parental origin of an allele.



A. Different VNTR lengths in both parents present on a specific region in the human genome. B.
B. One of the children has the identical combination of VNTR lengths as one of its parents. Uniparental disomy (of genetic material from the parent with identical VNTR lengths) or a deletion present at the allele inherited from the 'other' parent should be considered. Picture derived from www.geninfo.no.

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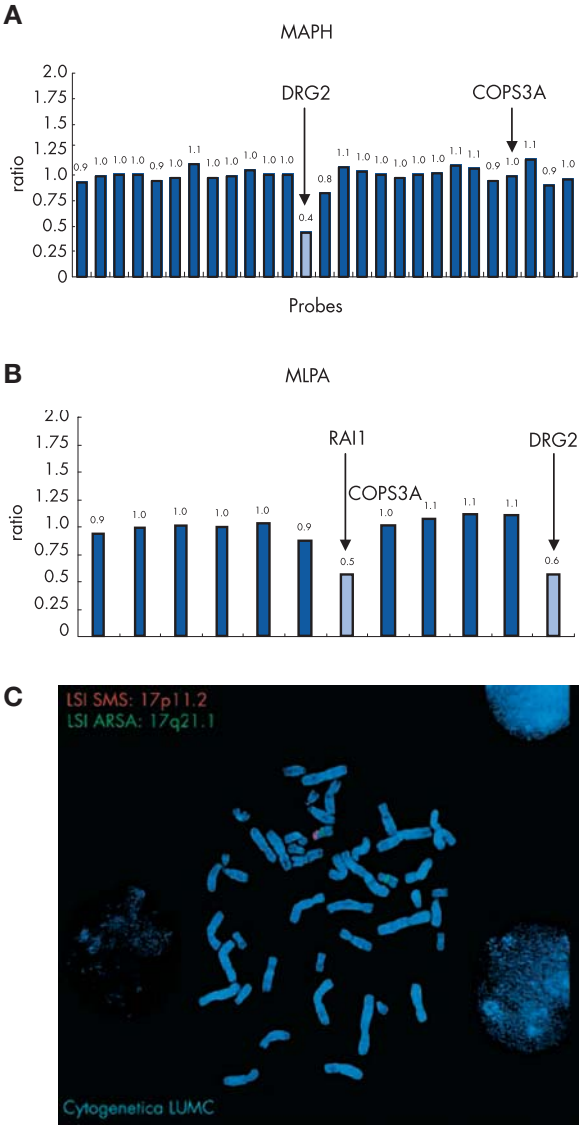
Figure 1. Results of case 3.



The plots correspond to the MAPH results showing (A) a deletion of the DRG2 gene, two normal copies of COPS3A (RAI1 not present), and the MLPA results; and (B) a deletion of RAI1, a deletion of DRG2, and a normal ratio of COPS3A. (C) The additional FISH analysis using the LSI-SMS probe specific for the Smith Magenis chromosomal region shows a normal signal on the short arm of only one copy of chromosome 17.

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Figure 3. Facial dysmorphism of case 6.

Note the microcephaly, ptosis of the left eye, flat philtrum, and thin upper lip.

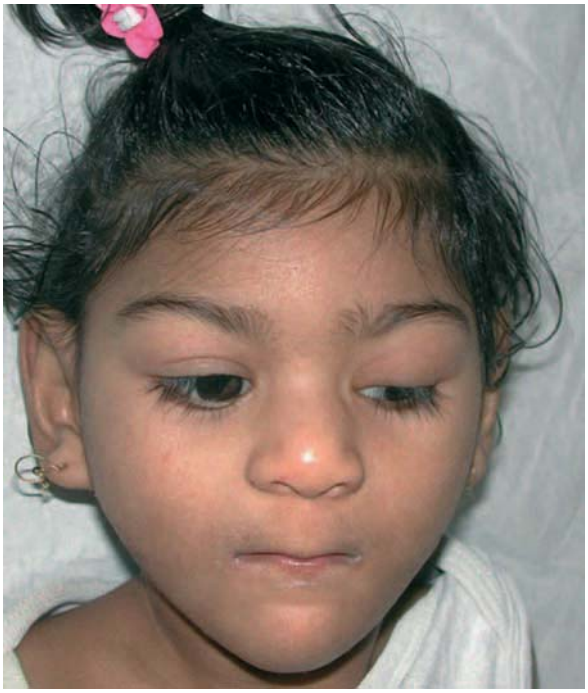

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Figure 2 Picture of the proband.

Note the microcephaly, myotonic facial expression, the proptosis of the eyes and the prominent simple ears.

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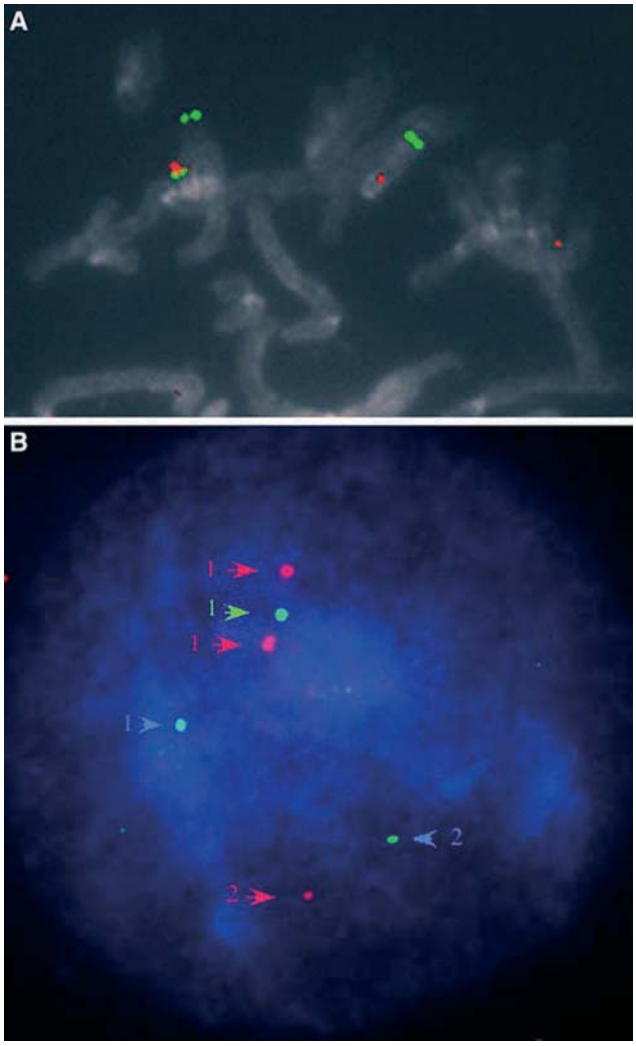
Figure 6. FISH analysis of chromosome 22.



A A partial metaphase of the patient, hybridised with the telomere probe RP11-3018K1 (*green*; chromosome region 22q13), N25 (*red*; VCFS/DGS region) and RP11-66F9 (*green*; CES region). On the right chromosome, green signals of RP11-3018K1 (telomeric side of chromosome 22) and a red signal N25 corresponding to the VCFS/DGS region are present; however, the signal of RP11-66F9 is lacking, indicating a deletion of the CES region. On the left chromosome, in addition to the green signals of RP11-3018K1, a red signal corresponding to the VCFS/DGS regions and a green signal corresponding to the CES region are both present. These latest two signals are partly overlapping. On this chromosome, the signal of N25 is stronger than the signal on the right chromosome, suggesting a duplication of the VCFS/DGS region. These findings are confirmed by the result of the interphase nucleus depicted in part b of this figure. B The different chromosomes 22 are marked 1 and 2. The signal of LSI ARSA, corresponding to the telomeric side of chromosome 22, is indicated with a *blue arrow*. The *red arrow* indicates the N25 signal (corresponding to the VCFS/DGS region), which is duplicated in chromosome 22 nr.1 (two red signals). The *green arrow* indicates the signal of RP11-66F9 (corresponding to the CES region). This signal is missing on chromosome 22 nr.2, demonstrating the deletion of the CES region.

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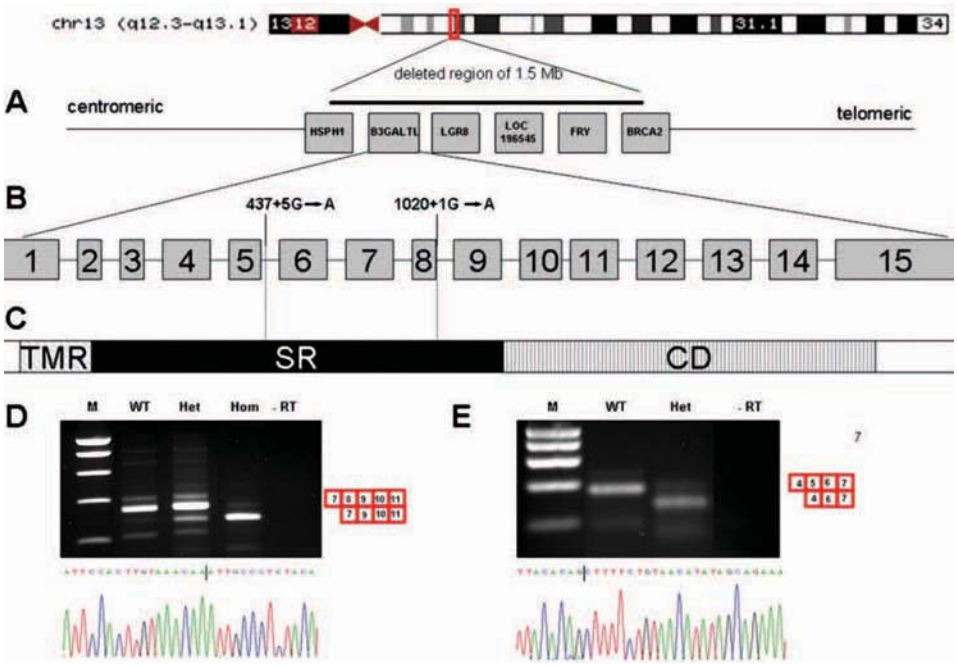
Figure 1. Overview of the location of the mutations in the *B3GALTL* gene and the results of the RT-PCR of RNA isolated from fibroblasts.



A, Genes present in the 1.5-Mb deletion found in two brothers with Peters Plus syndrome. *B,* 15 exons of the *B3GALTL* gene, with the localization of the mutations. *C,* B3GALTL protein, which consists of a transmembrane region (TMR), a stem region (SR), and a catalytic domain (CD). Both mutations (c.1020 1GrA and c.437 5GrA) are located in the stem region. *D,* Result of the nested RT-PCR of exons 7–11 of the *BGALTL* gene, with RNA derived from myoblasts (WT), RNA from fibroblasts of a father heterozygous for the c.1020 1GrA mutation (Het), and RNA from fibroblasts of his affected son with c.1020 1GrApat/delmat (Hom). The patient shows a smaller band compared with the WT band, which indicates a skip of exon 8. Sequence analysis of this band is shown. The vertical line indicates the end of exon 7 and the beginning of exon 9. The RT-PCR of the father shows, in addition to the WT band, a skipped product with much less intensity. *E,* Result of the RT-PCR encompassing exons 4–7 of the *BGALTL* gene, with RNA derived from lymphocytes of a control individual (WT) and a patient with a c.1020 1GrAmat/c.437 5GrApat genotype (Het). In addition to a faint WT band, the patient shows a smaller product that lacks exon 5. The sequence analysis of this smaller band confirms the skip of exon 5.

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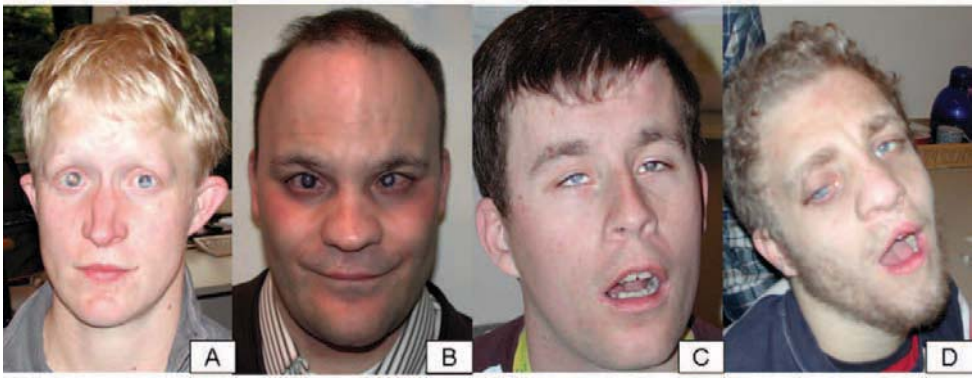
Figure 2. Facial features of four patients with Peters Plus syndrome.



Patients A and C are homozygous for the c.1020+1G→A mutation. Patient B has the c.1020+1G→A_{mat}/c.437+5G→A_{pat} genotype, and patient D has the c.1020+1G→A_{pat}/del_{mat} genotype. Note the Peters anomaly of the eyes, the long face, and the Cupid's bow shape of the upper lip in all patients. Patients B and D have a repaired cleft lip and/or palate. Patient A is female; the rest are male.

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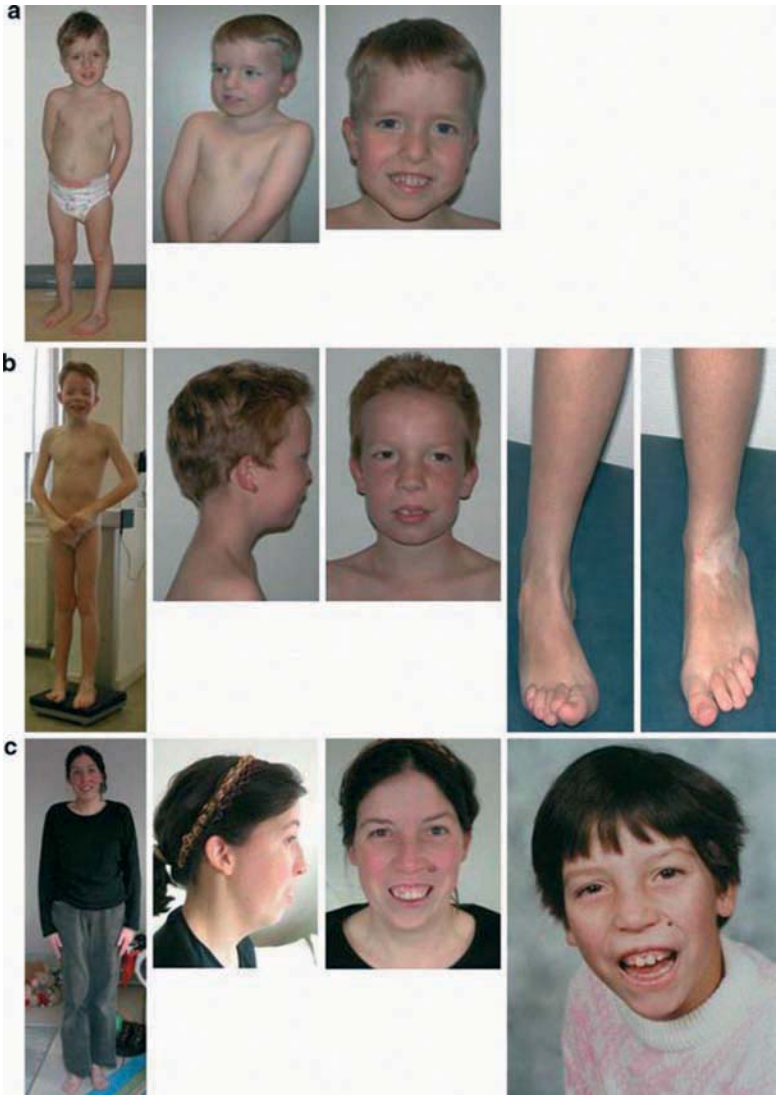
Figure 1. Three unrelated patients.



H.N. (a), P.V. (b) and F.T. (c) showing a mild mental retardation (IQ 50–60), a severe delay in active language ability, some typical facial features like downslanted palpebral fissures, mild hypertelorism, a broad nasal tip and small ears and a short neck with webbing, which is most pronounced in a and b. Patient H.N. and P.V. both show pectus carinatum. This was also observed for patient F.T. (not shown). H.N. also has an operated clubfoot on the left, while patient P.V.'s right foot is turned inside, the other foot showing a cafe´-au-lait spot. c Patient F.T. has a short neck and small ears. On the outer right a photograph is shown of the patient at age 11. The karyotype was normal in all patients and hematological analysis showed a persistent microcytic hypochromic anemia without iron deficiency

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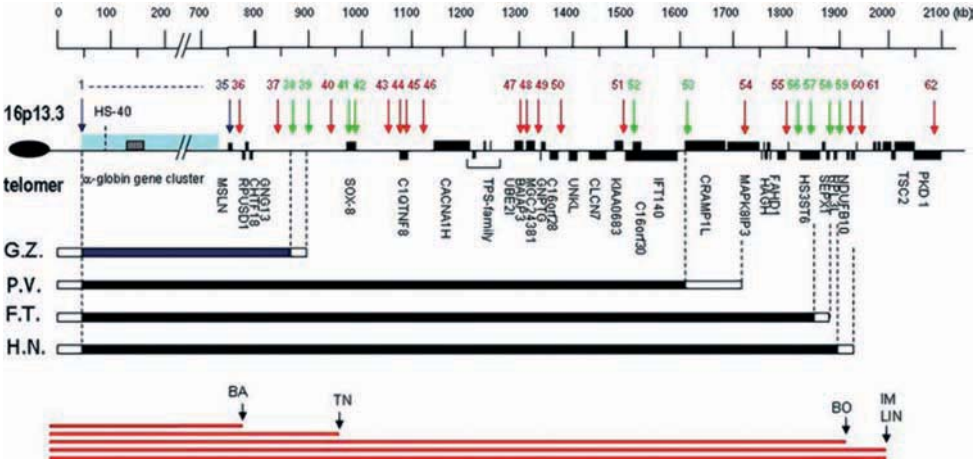
Figure 2 Schematic presentation of short arm of chromosome 16 (16p13.3), showing a 2 Mb region from the telomere containing the α-globin gene cluster up to the *TSC* and *PKD* genes.



The *arrows* and *numbers* represent the location of the probes. The deletions found by MLPA are shown as *bars* below the figure. Large deletions previously described are indicated as *red bars.*

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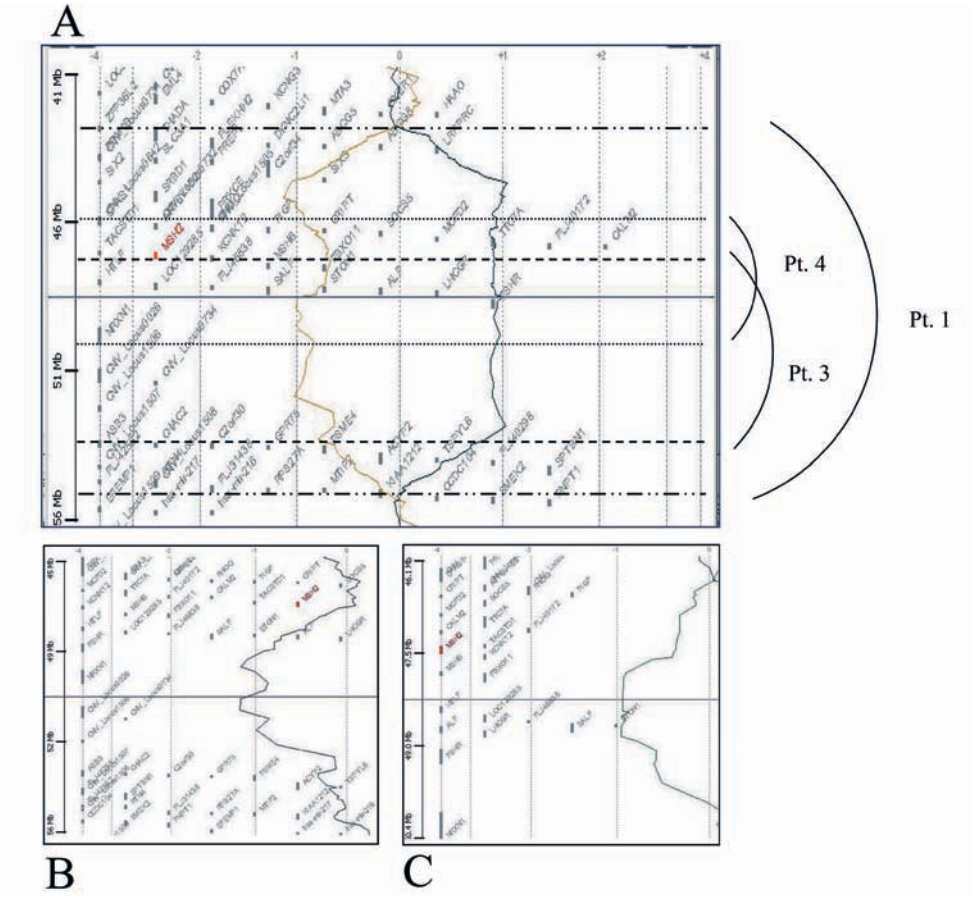
Figure 1. The result of patient 1 using the Agilent microarray platform.



(A) Due to the dye swap, the deletion is depicted in two colors resulting in a symmetrical profile pattern. All genes localized within the deleted region are visualized using the Agilent software tool. The deleted region of patient 3 (B) and patient 4 (C) are outlined by the dashed line and the dotted line, respectively. For the size of the deletion see table 2. Pt= patient.

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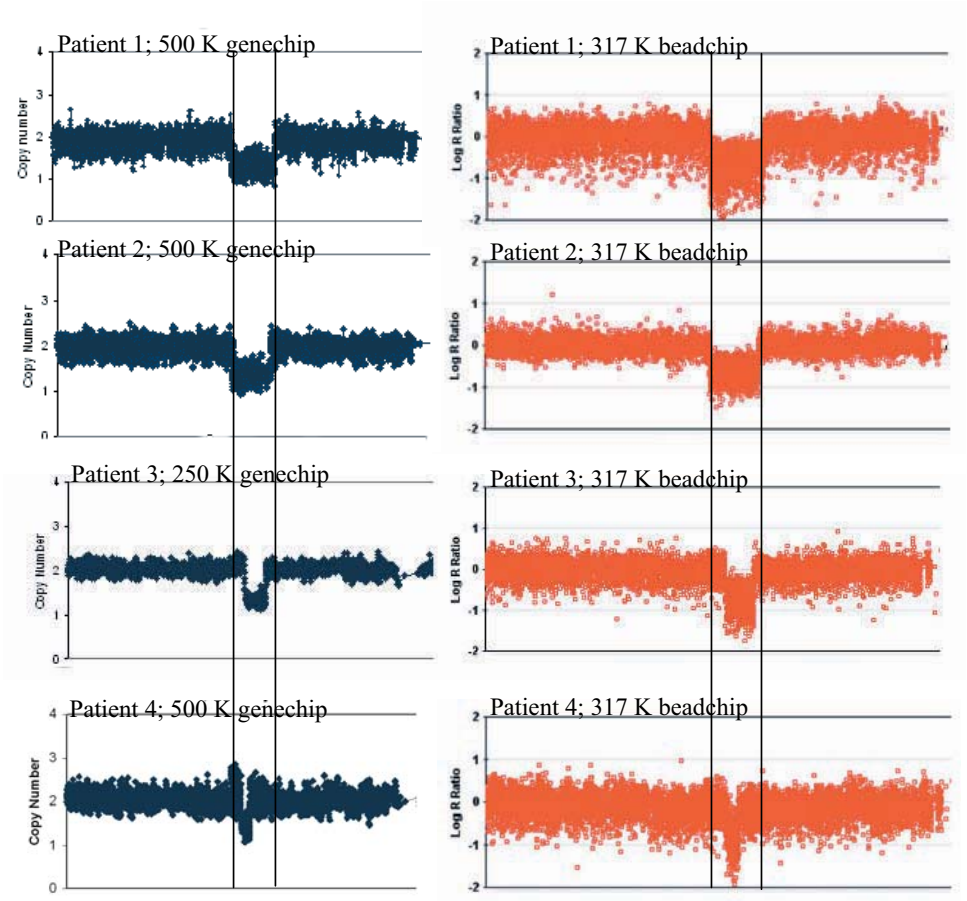
Figure 2. The interstitial 2p deletion of the four patients analysed by Affymetrix genechip (left) and the beadchip of Illumina (right).



The deletions of the different patients are shown separately. Patient 3 was only analysed using 250K NspI genechip. A normal copy number of two is represented by a copy number between 1.6 and 2.4 for the Affymetrix genechip or by a LogR ratio between –0.3 and +0.3 for the beadchip of Illumina. The vertical lines represent the size of the largest deletion. In general, the variation of the data points obtained by the beadchip is larger than that of the genechip. Especially in patient 3, the difference in variation is remarkable.

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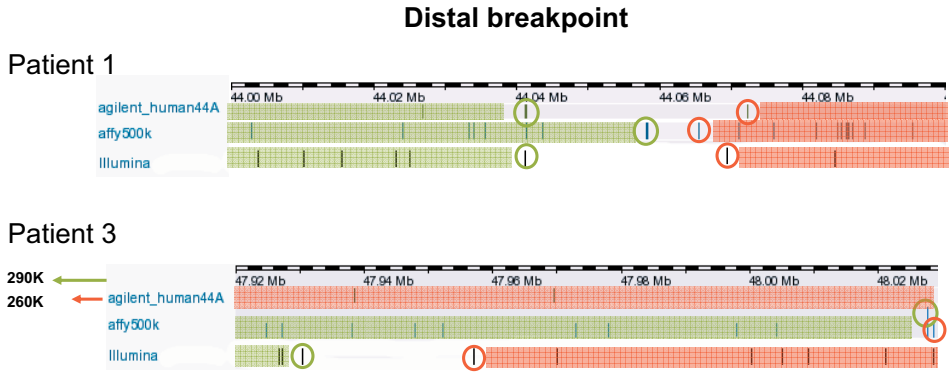
Figure 3. Overview of the distal breakpoints of patient 1 and patient 3 defined by Agilent, the Affymetrix genechip and the beadchip of Illumina.



The deleted region is depicted in red, whereas regions showing two copies are depicted in green. A green circle represents the last data point that showed a normal copy of two. A red circle represents the first data point that showed a deletion.
The number of data points per platform is comparable at the location of the distal breakpoint of patient 1 and 3. In patient 1, the breakpoint mapping of all platforms is concordant. In contrast, there is a huge difference in breakpoint mapping in patient 3. According to the results obtained by Agilent platform, the distal breakpoint of the deletion is located 290-260K outside the most distal point of the picture (47,92 Mb) (green and red arrow). The results of the Affymetrix platform show that the deleted region starts more proximally at ~48.03Mb (black arrow). The beadchip of Illumina defines the distal breakpoint of the deletion between these two points.