SHORT COMMUNICATION

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Jacobsen Syndrome and Beckwith-Wiedemann Syndrome Caused by a Parental Pericentric Inversion inv(11)(p15q24)

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Summary

Here we report on a male infant presenting the typical pattern of Jacobsen syndrome including trigonocephaly, thrombocytopenia, congenital heart defect, urethral stenosis, and partial agenesis of the corpus callosum. Conventional karyotyping, FISH, SKY and CGH analyses showed that the region distal to the *MLL* locus on 11q23 was lost and replaced by the distal region of 11p, leading to a partial trisomy of 11p and a partial monosomy of 11q. According to ISCN (1995) the karyotype can be described as 46,XY,add(11)(q2?3). ish 11ptel(D11S2071x3),11qtel(VIJyRM2072x1). Array-CGH analysis allowed us to narrow down the breakpoints to 11p15.1 and 11q24.1. Methylation analyses of genes located on 11p showed an increased level of the non-methylated paternal allele of the *KCNQ1OT1* gene, confirming the concomitant presence of Beckwith-Wiedemann syndrome (BWS). The phenotype resulting from the 11q deletion seems to dominate the phenotype due to the distal 11p trisomy. Investigation of the parents revealed that this chromosomal rearrangement was caused by a paternal pericentric inversion inv(11)(p15q24).

Since chromosomal aberrations like the one described here can easily be overlooked during routine chromosome analysis, combined FISH analysis using subtelomeric and possibly additional probes should be applied if there is any doubt about the integrity of telomeric regions.

Keywords: Jacobsen syndrome, Beckwith-Wiedemann syndrome, 11q terminal deletion, pericentric inversion, parental chromosomal rearrangement, subtelomere FISH, inv(11)(p15q24)

Introduction

Jacobsen syndrome (OMIM 147791) was first described by Dr. Petrea Jacobsen in 1973 (Jacobsen *et al*. 1973). The characteristic pattern of dysmorphic features includes growth and mental retardation, trigonocephaly, facial dysmorphism (hypertelorism, epicanthus, ptosis, broad nasal bridge, short nose with anteverted nostrils, high arched palate, retrognathia, carp-shaped upper lip and low-set dysmorphic ears), abnormal brain structures, congenital heart defects, thrombocytopenia and genitourinary anomalies. The observed phenotype is usually the consequence of a terminal deletion in 11q (Schinzel *et al*. 1977; Reddy *et al*. 1986; Fryns *et al*. 1987). In the majority of cases the deletion occurs *de novo*. Even though the deletions vary in size, they are usually easy to detect by conventional chromosome analysis. The severity of the clinical appearance depends on the size of the deleted region (Penny *et al*. 1995).

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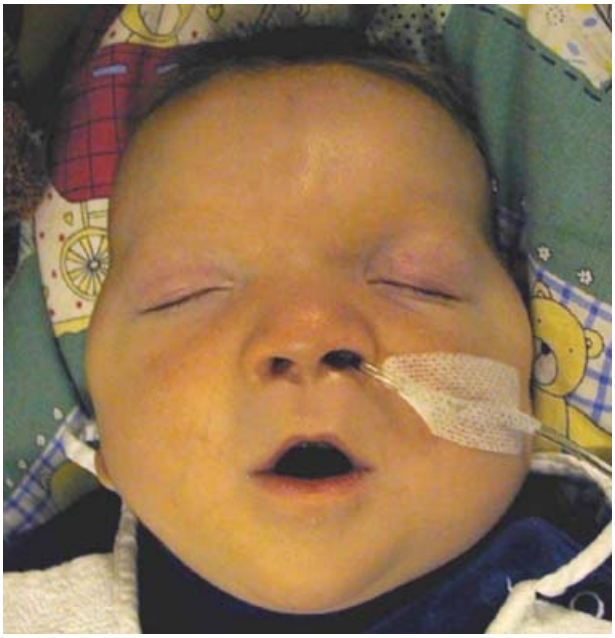

Figure 1 Face of patient at $3\frac{1}{4}$ months.

Clinical Report

Here we report on a premature male infant born in the 32+5 week of pregnancy to healthy parents. The infant was admitted to the Department of Paediatrics with respiratory problems after emergency Caesarean section was initiated because of vaginal bleeding. Measurements at birth were: weight 3060 g (500 g above the 97th percentile), length 48 cm (97th percentile) and occipital circumference 32.5 cm (75th–90th percentile). He presented with facial dysmorphia including trigonocephaly, epicanthus, ptosis, broad nasal bridge, carp-shaped upper lip and posterior rotated low-set ears (Figure 1). Clinical examination revealed impairment of several organ systems, including tricuspidal valve failure with insufficiency, atrial septal defect with cross-shunt leading to pulmonary hypertension, and urethral stenosis with hydronephrosis on the right side. Cranial ultrasound examination showed a partial agenesis of the corpus callosum. Furthermore, blood count analysis revealed anemia (haemoglobin: 13.6g/dl) and thrombocytemia (18.000/μl). Measurements of the infant at the age of six months (adjusted to the fourth month) were: weight 6830g (adjusted 50th–75th percentile), length 63 cm (adjusted 10th–25th percentile) and occipital circumference 41 cm (adjusted 10th–25th percentile).

Materials and Methods

The parents gave their informed consent for all diagnostic procedures. All procedures were performed in accordance with the guidelines of the Institutional Review Board of Hannover Medical School.

Chromosome Analysis

Peripheral blood cells were cultured for 72 hours with the addition of phytohemagglutinin (PHA). Chromosome preparation and fluorescence R banding using chromomycin A3 were performed as previously described (Schlegelberger *et al*. 1999). Twenty-five metaphases were completely analysed. Karyotypes were described according to ISCN (1995).

Fluorescence in situ Hybridisation (FISH)

FISH analyses were performed on metaphases using the subtelomeric TelVision probes for 11p (SpectrumGreen) and 11q (SpectrumOrange), as well as the LSI MLL Dual Colour Break Apart Rearrangement Probe (Abbott, Diagnostics, Wiesbaden, Germany), as described previously (Schlegelberger *et al*. 1999). Analyses were performed using a fluorescence microscope (Axioskop, Carl Zeiss Jena GmbH, Jena, Germany) coupled to a CCD camera and FISHView EXPO 2.0 software (Applied Spectral Imaging, Ltd., Migdal HaEmek, Israel). Fifteen metaphase spreads were evaluated. FISH results were described according to ISCN (1995).

Spectral Karyotyping (SKY)

For SKY analysis, metaphase chromosomes were hybridised with the SKY probe mixture (Applied Spectral Imaging, Ltd., Migdal HaEmek, Israel) according to the manufacturer's instructions. For image acquisition and analysis, the SpectraCube^{TM} system was used, coupled to an epifluorescence microscope (Axioskop, Carl Zeiss Jena GmbH, Jena, Germany) with a CCD camera and SKYView software (Applied Spectral Imaging, Ltd., Migdal HaEmek, Israel). Ten metaphase spreads were evaluated.

Comparative Genomic Hybridisation (CGH)

Comparative genomic hybridisation (CGH) was performed as described previously (Tönnies *et al*. 2001)

with slight modifications. In brief, test and control DNA were differently labelled by nick translation with SpectrumGreen®-dUTP and SpectrumOrange®-dUTP (Vysis). 200ng of labelled test DNA, 200 ng reference DNA, and 12.5μg Cot-1 DNA were coprecipitated, denatured, and hybridised to normal denatured metaphase spreads. After incubation at 37°C for 3 days, standard posthybridization washes were performed. Metaphase images were evaluated using an epifluorescence microscope (Axiophot, Carl Zeiss Jena GmbH, Jena, Germany) fitted with a cooled CCD camera (Hamamatsu, Japan) and appropriate single band pass filter sets. Image analysis and karyotyping were performed using the ISIS analysis system (Metasystems, Germany).

Array-CGH

A DNA chip containing 6251 individual BAC/PAC clones was used. Clone selection and spotting, as well as labelling and hybridisation of DNA probes, were performed as described previously (Zielinski *et al.* 2005).

Image analysis was performed using a dual laser scanner and the GenePix Pro 4.0 imaging software (GenePix 4000 A; Axon Instruments, Union City, CA, USA). Data normalisation and analysis were carried out using software packages marray and aCGH from R software3 (http://www.r-project.org). Raw fluorescence intensity values were normalised by applying the print-tip LOESS normalisation function. Spot quality criteria were set as foreground to background >3.0 and SD of triplicates <0.2. For breakpoint calling the aCGHSmooth software was used (Jong *et al.* 2004).

Analysis of the Methylation Status of the *KCNQ1OT1* and H19 Genes

Modification of genomic DNA was carried out using the CpGenome™ DNA Modification Kit (Ampligene/ONcor kit, Gaithersburg, MD, USA) according to the manufacturer's recommendations.

The methylation status of the *KCNQ1OT1* and *H19* genes was analysed by amplifying differentially methylated DNA segments of these genes. For each gene, two rounds of PCR with nested primers were performed (primer sequences and PCR conditions are available on request). Afterwards PCR products were digested with *Taq*I, so that only the PCR products representing the methylated alleles would be digested. BWS patients with a paternal duplication at 11p15 are expected to show a reduced level of methylated *KCNQ1OT1* alleles and an increased level of methylated *H19* alleles. After digestion DNA fragment size was determined by agarose (2.5%) gel electrophoresis.

Results

Chromosome analysis revealed a subtle structural aberration of the long arm of chromosome 11. The karyotype was described as 46,XY,add(11)(q2?3) (Figure 2). SKY analysis showed no material derived from another chromosome (data not shown). FISH analysis using the *MLL* probe showed a regular signal constellation (data not shown). Further investigations using the subtelomeric probes for 11q and 11p showed the loss of the telomere-associated region of 11q on one chromosome 11, which was replaced by additional material from the telomere-associated region of 11p (Figure 3). The karyotype was described as 46,XY,add(11)(q2?3).ish 11ptel(D11S2071x3),11qtel(VIJyRM2072x1).

The CGH analyses performed in parallel confirmed the loss of 11q23 to 11qter and the gain of 11p15 to 11pter described as: 46,XY,rev ish enh(11p15pter),dim(11q23qter) (data not shown). In addition, array-CGH was performed in order to define the breakpoints more precisely and to rule out other chromosomal rearrangements. The breakpoints were determined from 11p15.1 to 11pter (RP11-4B7 to CTC-908H22) and 11q24.1 to 11qter (RP11-166D19 to RP11-469N6) (data not shown). No other imbalances were seen.

To investigate whether this chromosomal aberration is due to a balanced parental chromosome rearrangement, chromosome analyses and FISH studies were performed on peripheral blood lymphocytes from the parents. The mother had a normal karyotype. The father showed a subtle change in 11p15, which was shown to be derived from a pericentric inversion inv(11)(p15q24) by FISH. Simultaneous hybridisation of the 11pter, 11qter and *MLL* probes showed that on one copy of chromosome 11 that signal for the *MLL* locus in 11q23 was next to the 11pter signal instead of the 11qter signal (Figure 4). Thus, the partial trisomy 11p and the partial monosomy

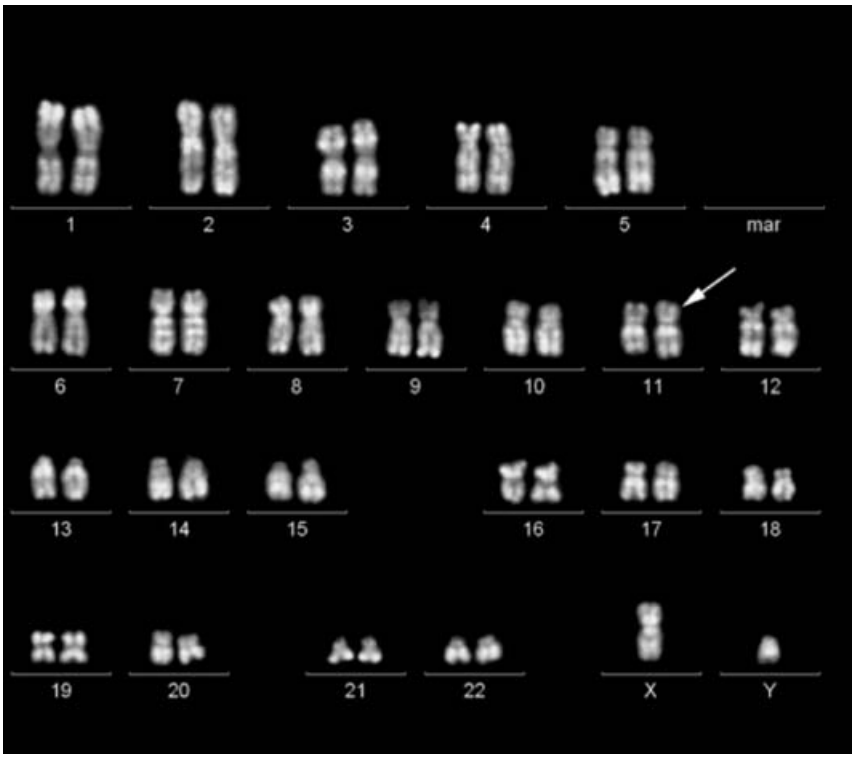

Figure 2 Karyotype of the patient: 46,XY,add(11)(q2?3). The arrow indicates the chromosome 11 with a subtle structural aberration.

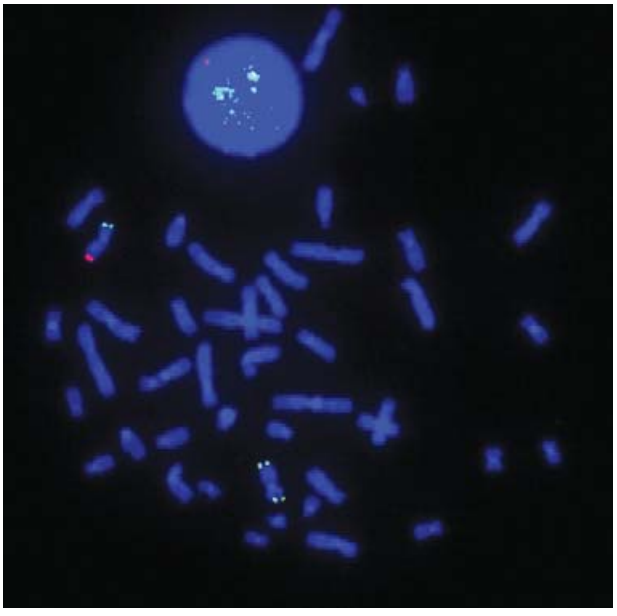

Figure 3 FISH analysis with subtelomeric probes for 11q (orange) and 11p (green). The normal chromosome 11 has one orange and one green signal for 11q and 11p, respectively, whereas the aberrant chromosome 11 has green signals for 11p on both ends and no orange signal.

11q in the child resulted from unequal crossing-over during paternal meiosis.

Since the critical region of 11q was lost, the diagnosis of Jacobsen syndrome was confirmed. Moreover, knowledge of the cytogenetic results led us to assume that the child could additionally be affected with Beckwith-Wiedemann syndrome (BWS, OMIM 130650). BWS is characterised by large body size, macroglossia, visceromegaly and an increased risk for embryonal tumors (e.g. Wilms tumor, hepatoblastoma, neuroblastoma, rhabdomyosarcoma) (Pettenati *et al*. 1986). Duplication of 11p15 was the first described cytogenetic abnormality associated with BWS (Waziri *et al*. 1983). In about half of the patients, loss of methylation of genes located in this region is the cause of the disease and in 10–20% of patients it is due to paternal uniparental disomy of 11p15 (Bliek *et al*. 2001, Weksberg *et al*. 2001). At birth, measurements of the child were at high percentile levels, length at the 97th percentile, occipital circumference at 75th–90th percentile and weight even above the 97th percentile. No further abnormalities

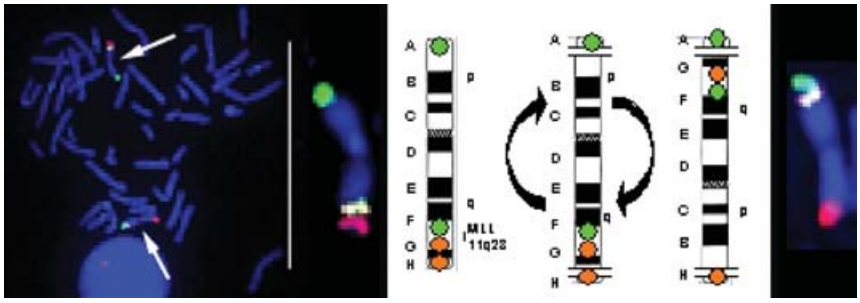

Figure 4 FISH analysis on paternal chromosomes using the subtelomeric probes for 11q (orange) and 11p (green), together with the *MLL* probe (green/orange fusion signal). The normal chromosome 11 shows the fusion signal for the *MLL* locus in 11q23 next to the orange signal for 11qter, as expected, whereas the aberrant chromosome 11 shows the fusion signal for the *MLL* locus next to the green signal for 11pter. This signal constellation indicates the presence of a pericentric inversion with one breakpoint distal to the *MLL* locus and the other in 11p.

such as exomphalos or macroglossia, which are indicative of BWS, were observed. The analysis of the methylation status of the *KCNQ1OT1* gene revealed an increased level of non-methylated alleles (data not shown), which is in agreement with the presence of a duplication of paternal origin in the proband. The somewhat less quantitative properties of this assay for the *H19* gene did not allow the clear visualisation of a higher ratio of paternal alleles.

Thus, unbalanced recombination of the paternal pericentric inversion caused concomitant Jacobsen syndrome and Beckwith-Wiedemann syndrome in the proband.

Discussion

In the majority of cases, Jacobsen syndrome is caused by a *de novo* deletion in 11q. The breaks most often occur in expanded CCG-trinucleotide repeats within the folate-sensitive fragile site FRA11B, located in 11q23.3 (Jones *et al*. 1994, 2000). These repeats may induce increased chromosomal instability, thus leading to terminal deletions of 11q.

However, as recently reported by Riegel *et al*. (2005), complex chromosomal rearrangements may underlie what appears at first glance to be a "simple" translocation. Because these complex rearrangements are sometimes subtle, and cannot be detected by routine methods, their frequency may be underestimated. Here we present a case with a complex chromosomal rearrangement leading to loss of the terminal region of 11q, due to a paternal pericentric inversion inv(11)(p15q24). This finding has been confirmed both by FISH analyses using subtelomeric probes and by CGH analysis. Performing SKY and array-CGH analysis, further interchromosomal rearrangements or chromosomal imbalances could be ruled out. Array-CGH allowed us to further narrow down the breakpoints, and confirmed the loss of the critical region for Jacobsen syndrome. The child showed the typical symptoms of Jacobsen syndrome, but carries the partial 11q monosomy in addition to a partial 11p trisomy.

Duplication of the paternal allele of 11p15 leads to BWS. The subject's body measurements at birth, presenting with high percentile levels, were in accordance with the expected macrosomia associated with BWS. However, measurements at the age of six months indicated growth retardation. This could be explained by counterbalancing the overgrowth typical for BWS with the reduced growth typical for Jacobsen syndrome (Grossfeld *et al*. 2004). Because BWS is associated with an increased risk for childhood tumors, extensive attempts were undertaken to contact the parents and to advise them to undergo regular follow-up examinations. Unfortunately, the child was lost for follow-up because the parents refused any further contact.

In the case of Jacobsen syndrome the presence of different symptoms and the severity of the phenotype correlate with the size of the deleted region (Penny *et al*. 1995). Detailed investigation of cases with small

deletions, using new methods such as array-CGH and SNP (single nucleotide polymorphism)-chips, will further help to narrow down the critical regions in 11q and to identify the genes responsible for the different features of Jacobsen syndrome.

Inherited chromosomal rearrangements leading to an 11q deletion have seldom been reported. To our knowledge, only one other similar case has been described. Clarkson *et al*. (2002) reported on a 16-year-old girl with a rather mild phenotype of Jacobsen syndrome. Further investigation revealed that the recombinant chromosome 11 was due to a paternal inversion inv (11)(p15.5q24.3).

Since chromosomal aberrations like the one described here can easily be overlooked during routine chromosome analyses, FISH analyses using subtelomeric probes for 5q, 7p, 7q, 9p and 11q should be routinely performed in addition to a high-resolution karyotype, in the presence of a trigonocephaly sequence in a child with multiple congenital anomalies.

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References

Bliek, J., Maas, S. M., Ruijter, J. M., Hennekam, R. C., Alders, M., Westerveld, A. & Mannens, M. M. (2001) Increased tumor risk for BWS patients correlates with aberrant H19 and not KCNQ1OT1 methylation: occurrence with KCNQ1OT1 hypomethylation in familial cases of BWS. *Hum Mol Genet* **10**, 467–476.

Clarkson, B., Pavenski, K., Dupuis, L., Kennedy, S., Meyn, S., Nezarati, M. M., Nie, G., Weksberg, R., Withers, S., Quercia, N., Teebi, A. S. & Teshima, I. (2002) Detecting rearrangements in children using subtelomeric FISH and SKY. *Am J Med Genet* **107**, 267–274.

Fryns, J. P., Kleczkowska, A., Smeets, E. & Van den Berghe, H. (1987) Distal 11q deletion: a specific clinical entity. *Helv Pediatr Acta* **42**, 191–194.

Grossfeld, P. D., Mattina, T., Lai, Z., Favier, R., Jones, K. L., Cotter, F., Jones, C. & the 11q Consortium. (2004) The 11q terminal deletion disorder: A prospective study of 110 cases. *Am J Med Genet* **129A**, 51–61.

ISCN. (1995) *An International System for Human Cytogenetic Nomenclature*. (editorF. Mitelman). S. Karger, Basel.

Jacobsen, P., Hauge, M., Henningsen, K., Hobolth, N., Mikkelsen, M. & Philip, J. (1973) An (11;21) translocation in four generations with chromosome 11 abnormalities in the offspring. A clinical, cytogenetical and gene marker study. *Hum Hered* **23**, 568–585.

Jones, C., Slijepcevic, P., Marsh, S., Baker, E., Langdon, W. Y., Richards, R. I. & Tunnacliffe, A. (1994) Physical linkage of the fragile site FRA11B and a Jacobsen syndrome deletion breakpoint in 11q23.3. *Hum Mol Genet* **3**, 2123–2130.

Jones, C., Mullenbach, R., Grossfeld, P., Auer, R., Favier, R., Chien, K., James, M., Tunnacliffe, A. & Cotter, F. (2000) Co-localisation of CCG repeats and chromosome deletion breakpoints in Jacobsen syndrome: evidence for a common mechanism of chromosome breakage. *Hum Mol Genet* **9**, 1201–1208.

Jong, K., Marchiori, E., Meijer, G., van der Wart, A. & Ylstra, B. (2004) Breakpoint identification and smoothing of array comparative genomic hybridization data. *Bioinformatics* **20**, 3636–3637.

Penny, L. A., Dell'Aquila, M., Jones, M. C., Bergoffen, J., Cunniff, C., Fryns, J.-P., Grace, E., Graham, J. M., Kousseff, B., Mattina, T., Syme, J., Voullaire, L., Zelante, L., Zenger-Hain, J., Jones, O. W. & Evans, G. A. (1995) Clinical and molecular characterization of patients with distal 11q deletions. *Am J Hum Genet* **56**, 676–683.

Pettenati, M. J., Haines, J. L., Higgins, R. R., Wappner, R. S., Palmer, C. G. & Weaver, D. D. (1986) Wiedemann-Beckwith syndrome: presentation of clinical and cytogenetic data on 22 cases and review of the literature. *Hum Genet* **74**, 143–154.

Reddy, K. S., Thomas, I. M. & Narayanan, H. S. (1986) Partial deletion of the long arm chromosome 11 in Jacobsen syndrome. *Indian J Pediatr* **53**, 123–126.

Riegel, M., Baumer, A., Suss, J. & Schinzel, A. (2005) An unusual reciprocal translocation detected by subteleomeric FISH: interstitial and not terminal. *Am J Med Genet* **135A**, 86–90.

Schinzel, A., Auf der Maur, P. & Moser, H. (1977) Partial deletion of long arm of chromosome 11[del (11)(q23)]: Jacobsen syndrome. Two new cases and review of the clinical findings. *J Med Genet* **14**, 438–444.

Schlegelberger, B., Metzke, S., Harder, S., Zuhlke-Jenisch, R., Zhang, Y. & Siebert, R. (1999) Classical and molecular cytogenetics of tumor cells. In: *Diagnostic cytogenetics* (editorR.-D. Wegner), pp 151–185. Berlin Heidelberg, Springer-Verlag, New York.

Tonnies, H., Stumm, M., Wegner, R. D., Chudoba, I., Kalscheuer, V. & Neitzel, H. (2001) Comparative genomic hybridization based strategy for the analysis of different chromosome imbalances detected in conventional cytogenetic diagnostics. *Cytogenet Cell Genet* **93**, 188–194.

Waziri, M., Patil, S. R., Hanson, J. W. & Bartley, J. A. (1983) Abnormality of chromosome 11 in patients with features of Beckwith-Wiedemann syndrome. *J Pediatr* **102**, 873–876.

Weksberg, R., Nishikawa, J., Caluseriu, O., Fei, Y. L., Shuman, C., Wei, C., Steele, L., Cameron, J., Smith, A., Ambus, I., Li, M., Ray, P. N., Sadowski, P. & Squire, J. (2001) Tumor development in the Beckwith-Wiedemann syndrome is associated with a variety of constitutional molecular 11q15 alterations including imprinting defects of KCNQ1OT1. *Hum Mol Genet* **10**, 2989–3000.

Zielinski, B., Gratias, S., Toedt, G., Stange, D. E., Radlwimmer, B., Lohmann, D. R. & Lichter, P. (2005) Detection of chromosomal imbalances in retinoblastoma by matrix-based comparative genomic hybridization. *Gene Chromosome Cancer* **43**, 294–301.

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