Published in final edited form as:

Am J Med Genet A. 2010 June ; 152A(6): 1488–1497. doi:10.1002/ajmg.a.33408.

Unbalanced der(5)t(5;20) translocation associated with Megalencephaly, perisylvian Polymicrogyria, Polydactyly and Hydrocephalus

Annemieke J.M.H. Verkerk¹, Rachel Schot², Laura van Waterschoot¹, Hannie Douben², Pino J. Poddighe², Maarten H. Lequin³, Linda S. de Vries⁵, Paulien Terhal⁶, Johanne M.D. Hahnemann⁷, Irenaeus F.M. de Co⁴, Marie-Claire Y. de Wit⁴, Leontien S. Wafelman⁸, Livia Garavelli⁹, William B. Dobyns¹⁰, Peter J. Van der Spek¹, Annelies de Klein², and Grazia M.S. Mancini²

¹Department of Bioinformatics Erasmus Medical Center, Rotterdam, The Netherlands

²Department of Clinical Genetics Erasmus Medical Center, Rotterdam, The Netherlands

³Department of Radiology Erasmus Medical Center, Rotterdam, The Netherlands ⁴Department of Child Neurology Erasmus Medical Center, Rotterdam, The Netherlands ⁵Department of Neonatology Wilhelmina Children's hospital, Utrecht Medical Center, Utrecht, The Netherlands

⁶Department of Medical Genetics Wilhelmina Children's hospital, Utrecht Medical Center, Utrecht, The Netherlands ⁷Kennedy Center, Glostrup, Denmark ⁸Department of Pediatrics, Albert Schweitzer Hospital, Dordrecht, The Netherlands ⁹Clinical Genetics Unit, Obstetric and Pediatric Department, S. Maria Nuova Hospital, Reggio Emilia, Italy ¹⁰Department of Human Genetics, University of Chicago, USA

Abstract

The combination of megalencephaly, perisylvian polymicrogyria, polydactyly and hydrocephalus (MPPH) is a rare syndrome of unknown cause. We observed two first cousins affected by an MPPH-like phenotype with a submicroscopic chromosome 5q35 deletion as a result of an unbalanced der(5)t(5;20)(q35.2;q13.3) translocation, including the *NSD1* Sotos syndrome locus. We describe the phenotype and the deletion breakpoints of the two MPPH-like patients and compare these with five unrelated MPPH and Sotos patients harboring a 5q35 microdeletion. Mapping of the breakpoints in the two cousins was performed by MLPA, FISH, high density SNP-arrays and Q-PCR for the 5q35 deletion and 20q13 duplication. The 5q35 deletion area of the two cousins almost completely overlaps with earlier described patients with an atypical Sotos microdeletion, except for the *DRD1* gene. The five unrelated MPPH patients neither showed submicroscopic chromosomal aberrations nor *DRD1* mutations. We reviewed the brain MRI of 10 Sotos patients and did not detect polymicrogyria in any of them. In our two cousins, the MPPH-like phenotype is probably caused by the contribution of genes on both chromosome 5q35 and 20q13. Some patients with MPPH may harbor a submicroscopic chromosomal aberration and therefore high-resolution array analysis should be part of the diagnostic workup.

Keywords

Megalencephaly; Polymicrogyria; Polydactyly; Hydrocephalus; microdeletion; 5q35.2; 20q13.3

INTRODUCTION

Polymicrogyria (PMG) is generally considered to be a post-migratory disorder of cortical organization mostly associated with motor and sensory defects and often with epilepsy and psychomotor retardation. Prenatal insults like intoxications, infections and hypoperfusion can cause PMG [Barth, 2003]. The recent discovery of *KIAA1279* mutations in Goldberg-Shprintzen syndrome with diffuse PMG and other familial PMG syndromes indicates that genetic factors play an important role [Piao et al., 2004; Brooks et al., 2005; Ouled Amar et al., 2009; Santos et al., 2008; Villard et al., 2002]. New chromosomal loci have been associated with PMG [Dobyns et al., 2008; Robin et al., 2006]. One of the PMG syndromes of unknown etiology is the recently described megalencephaly with perisylvian polymicrogyria, polydactyly and hydrocephalus (MPPH) [Mirzaa et al., 2004; Colombani et al., 2006; Garavelli et al., 2007; Tohyama et al., 2007]. Megalencephaly is thought to be a proliferation defect of the cerebral cortex. Although it has been associated with overgrowth syndromes like Sotos and cutis marmorata telangiectasia congenita (M-CM) syndrome, hamartomas like Pallister-Hall, Bannayan-Zonana syndrome and metabolic disorders, no gene mutations are known causing exclusively megalencephaly in humans [Barkovich et al., 2005; Guerrini et al., 2008]. Overlap between MPPH and M-CM has recently been suggested [Gripp et al., 2009].

We describe a family with two first cousins presenting with megalencephaly and polymicrogyria resembling MPPH syndrome. They both carry a derivative chromosome 5q as a result of the unbalanced segregations of a translocation t(5;20)(q35.2;q13.3) resulting in a chromosome 5q35.2-qter deletion encompassing the *NSD1* locus for Sotos syndrome, and a partial trisomy of chromosome 20q13.3-qter. We have fine-mapped the deletion breakpoints and compare these with the phenotype and breakpoints of Sotos microdeletion patients. We also genotyped five other MPPH patients using high resolution SNP array analysis and sequenced the *DRD1* gene as a possible candidate gene for MPPH.

CLINICAL REPORT

Patient 1 and 2 are first cousins, both from healthy Dutch parents (Fig. 1A). Their mothers are sisters.

Patient 1 (III-1 in Fig. 1A) is a girl examined at the age of 2 years for a severe psychomotor retardation, axial hypotonia, spasticity and epilepsy. The pregnancy was complicated by polyhydramnion, intrauterine growth retardation and pre-eclampsia. The delivery followed in the 37th week with a birth weight of 2180 grams (−2SD) and OFC of 33.0 cm (0 SD). The neonatal period was complicated by E.Coli sepsis, cholestasis and liver dysfunction. She also presented with a small ASD type II and hypothalamic hypothyroidism with low TSH values. In time she developed kyphoscoliosis, pectus carinatum and signs of rickets, which did not improve with vitamin D supplementation. She experienced several urinary infections caused by grade IV vesicoureteral reflux. Psychomotor development was delayed, but at the age of 1.5 years a status epilepticus caused regression. Examination at the age of 2 years showed macrocephaly (OFC at +3 SD, height at 0 SD), high broad forehead, large fontanel, hypertelorism with epicanthic folds, short upturned nose with hypoplastic nostrils, down turned corners of the mouth with thick vermilion of the lips, high arched palate, small pointed chin with a vertical groove, large low-set ears, barrel shaped chest with kyphoscoliosis, postaxial polydactyly of the 5th right toe. She had severe headlag, was unable to sit unsupported, showed a spastic tetraparesis, made eye contact and had no speech development. Brain MRI revealed megalencephaly, asymmetric mild dilatation of the lateral ventricles (right > left) and right perisylvian polymicrogyria (Fig. 2A). Family history showed two miscarriages, one concerning an anencephalic embryo.

Patient 2 (III-2 in Fig. 1A and Fig. 1B) was born to the sister of Patient 1's mother after a pregnancy complicated by polyhydramnios and fetal hydrops with hydrothorax. The delivery in the 35th week was complicated by perinatal asphyxia, shearing of umbilical cord, hemorrhage and fetal shock. She was macrosomic and macrocephalic at birth (weight 3160 grams, +2.5 SD; OFC 38 cm, +3.5 SD). The neonatal period was complicated by multiorgan failure, persistent hydrothorax, cholestasis, rickets, hypothalamic hypothyroidism with low TSH and FT4, vesicoureteral reflux causing urosepsis and a non-symptomatic cardiac ASD type II. She developed severe intractable epilepsy and tetraparesis and progressive hydrocephalus requiring a ventriculoperitoneal shunt at the age of 1 year. At that age, physical exam showed length at 0 SD, no psychomotor development, prominent forehead, deep-set eyes, short nose with hypoplastic nares, down turned mouth corners, pointed chin. Abdominal ultrasound showed hepatomegaly with foci suspected for hepatoma. She died of progressive respiratory and heart failure during pneumonia at the age of 1.5 years. Brain CT at the age of 1 year showed macrocephaly with generalized hydrocephalus, cavum septum pellucidum and abnormal gyral pattern in the perisylvian regions most consistent with PMG. (Fig. 2B). Family history revealed one miscarriage.

Patient 3 is a Dutch full term born girl with a birth weight of 2170 gram (− 2.5 SD), who was diagnosed with a large ASD, a perimembranous VSD and patent ductus arteriosus. OFC at the age of 1 week was 32.5 cm (−2.5 SD). Generalized hypotonia and developmental delay persisted after heart surgery, which justified neurological investigation. Brain MRI at the age of 5 months, 1 and 3 year showed prominent frontal lobes, deep sulci, delayed myelination of periventricular white matter, apparently normal cortical development (Fig. 2C). Her development is severely delayed. Hypercalcemia with nephrocalcinosis and severe hypotonia persist at the age of 2 years, with height and OFC at 0 SD. Facial features and congenital anomalies suggested the diagnosis of Sotos syndrome.

Patients 4, 5 and 6 have been clinically described by Mirzaa et al. [2004] (corresponding to LP95-025, LR02-064 and LR04-181) as affected by MPPH.

Patient 7 has been diagnosed by L.G. and W.B.D. as having features of MPPH. Clinical details are reported separately [Garavelli et al., 2007].

Patient 8 is a full term girl born with macrocephaly (OFC 41 cm) who showed squint and no visual awareness at the age of 3 months. Head circumference grows along the +4 SD. A brain MRI at the age of 3 months showed megalencephaly with mild ventricle dilatation, delayed myelination, periventricular white matter loss, cavum septum pellucidum, frontoparietal and perisylvian polymicrogyria (Fig. 2D). She did not present with polydactyly. The clinical and MRI findings are compatible with MPPH.

Clinical and radiology data of Sotos and MPPH patients are summarized in Table I.

METHODS

Karyotyping and MLPA

Standard G banding karyotyping was performed in all patients.

A subtelomeric MLPA to detect chromosomal imbalances using DNA from Patients 1 and 2 was performed using the P036 and P070 kits (MRC-Holland).

FISH analysis

Fish analysis was performed in Patients III-1, III-2, and their relatives and Patient 3. The BAC clones used were all selected from the University of California Santa Cruz UCSC

browser. The clones were purchased from BACPAC Resources. DNA was semi-automatically isolated with a AutoGenPrep 3000 robot (Autogen), after a whole genome amplification(WGA) (Rpli-g kit Qiagen), the DNA was digested and labelled with Bio-16-dUTP or dig -11-dUTP by a Random Prime labelling system (Invitrogen). Before use the probes were validated on metaphase cells from a healthy persons to confirm there cytogenetic location. The FISH experiments were performed according to standard protocols with minor modifications. FISH slides were analyzed with a Axioplan 2 Imaging microscope (Zeiss), images were captured using the fluorescent software Isis (MetaSystems).

Names and location of the BAC clones from 5pter: RP1-24H17; 5p13.2: RP11-253B9; 5qter: RP11-1240G13; NSD specific 5q35.3 probes: RP1-118M12 and RP11-47L17; 20pter: RP5-1061L1; 20qter: RP1-81F12.

SNP-array analysis

Whole genome analysis was performed by microarray analysis using Affymetrix GeneChip 50 and 250K arrays. The assays were carried out according to the standard protocol. Briefly, 250 ng of total genomic DNA was digested with either HindIII (50K) or NspI (250K). Appropriate adaptors were ligated to the four base-pair overhang of the DNA fragments. A single primer was used for amplification and PCR products were purified. After fragmentation with DNase I, products were labeled with biotin and hybridized to the array. The arrays were washed and stained with streptavidin-phycoerythrin in the Affymetrix fluidics station 450 and scanned in the GeneChip Scanner 3000 G7. The images of the scans were collected and intensities measured in the Affymetrix GeneChip operating Software (GCOS). Genotype data analysis was performed in Affymetrix GeneChip Genotyping analysis software (GTYPE) using the Dynamic Model algorithm (DM) for 50K analysis and the BRLMM algorithm for 250K analysis.

Copy Number analysis

Copy number analysis was performed on the allele intensities of each SNP by using the software package: Copy Number Analyzer for Affymetrix GeneChip (CNAG version 3.0, Nannya et al, Cancer Res. 2005 Jul 15;65(14):6071–9)

For real-time PCR, primers and sequence analysis see Supplementary methods.

RESULTS

Cytogenetic, MLPA and FISH analysis

Standard G banding chromosome investigation (550 bands) was normal in all patients. MLPA analysis with subtelomere probe kits in Patients III-1 (Fig. 3A, right panel) and III-2 (not shown) and their mothers II-2 (Fig. 3B, right panel) and II-3 (not shown), respectively, revealed a loss of signal at the subtelomeric region of chromosome 5q35 and a gain of signal for chromosome 20q13 only for the Patients III-1 and III-2. FISH analysis was performed with chromosome 5qter and 5pter BAC probes, probes specific for the *NSD1* locus on chromosome 5q35.3 and chromosome 20 probes. Results confirmed an unbalanced translocation in both Patients III-1 and III-2 resulting in the karyotype 46,XX,der(5)t(5;20)(q35.2;q13.3) (Fig. 3C1, 3C2, 3C4). Further FISH analysis with probes on chromosomes 5q and 20 q revealed a balanced t(5;20)(q35.2;q13.3) translocation in both mothers II-1 (Fig. 3C3) and II-3 (not shown) of Patient III-1 and III-2, respectively, and the grandfather I-1 (not shown). Targeted FISH analysis of the sporadic Patient 3, performed in the suspicion of Sotos syndrome, showed a microdeletion with the *NSD1*-specific probe RP11-47L17 (not shown).

SNP array analysis

To define the deletion breakpoints in Patient 1 and to investigate the genome of Patients 3–8 for chromosomal abnormalities, DNA of these Patients was hybridized on either 100K or 250K SNP arrays (Affymetrix). Patient 1 showed a 5.7 Mb terminal deletion on chromosome 5q35.2-qter (Fig. 4A). The proximal deletion breakpoint on chromosome 5q35.2 is rs11953281, as indicated by copy number analysis (CNAG) [Ogawa et al, 2007]. The breakpoint, confirmed by Q-PCR analysis is between the genes *LOC645398* (present) and *DRD1* (absent) (Fig. 4B). In addition, an amplification of 8 Mb was seen for chromosome 20q13.3-qter (Fig. 4C). Copy number analysis indicated rs4811719 as the proximal SNP bordering the amplification, present in 2 copies, and rs6014787 as the first SNP present in 3 copies. The breakpoint was confirmed by Q-PCR between *LOC100131175* (2 copies) and the *TFAP2C* gene (3 copies; see supplementary data).

Comparison with other 5q35.3 microdeletion patients

Many of the symptoms of Patients 1 and 2 resembled Sotos syndrome; however overgrowth was not evident in Patient 1 and the phenotype was much more severe. Therefore we also studied in detail the genotype of Patient 3 with a similarly severe phenotype and lack of overgrowth. Japanese Sotos microdeletion patients have been described with broader and more severe phenotypic abnormalities than Caucasian microdeletions [Nagai et al., 2003; Tatton-Brown et al., 2005a and b; Mochizuki et al., 2008]. SNP array analysis for Patient 3 showed an interstitial 5q35.3 deletion spanning about 2 Mb, between rs9313730 and rs11745917, involving about 60 genes (Fig. 4A and B). This deletion is within the common microdeletion range of Caucasian Sotos patients [Tatton-Brown et al., 2005b; Kurotaki et al., 2003]. The most proximally deleted gene in Patient 3 is *THOC3* and the most distally (telomeric) located is *LOC653314*. We have aligned the deletions in our Patients 1–3 with the chromosome 5q deletions spanning the *NSD1* locus fine mapped by FISH, as reported by Tatton-Brown et al. [2005b] and deletions mapped by QMPSF by Saugier-Weber et al [2007]. All the described patients were diagnosed with Sotos syndrome, including Patient COG025 Tatton-Brown et al. [2005b], whose deletion extends until 5qter. Two other Patients, COG231 and COG342 [Tatton-Brown et al., 2005b], have the largest reported deletions extending on the proximal site and overlap with our Patients 1 and 2 (Fig. 4B). However, the most proximal probe (SOT21) used for the COG231 and COG342 patients gives uninformative results, possibly excluding *FLJ16171* and *DRD1* from the deleted area. Patients 14 and 15 from Saugier-Weber et al. [2007] also have unusually large deletions. Here the breakpoint is somewhere between the *FLJ16171* and *SFXN1* genes, also giving inconclusive results for *DRD1*. Clinical details in both studies from Tatton-Brown et al. [2005a and b] and Saugier-Weber et al. [2007] did not include MRI findings of the patients but the general conclusion was that, except for a more common deep learning disability and lack of overgrowth, the microdeletion patients presented with the same features of the patients with *NSD1* mutation.

In the five unrelated MPPH patients 4–8, SNP array analysis did not reveal pathogenic rearrangements (not shown). DNA of Patients 4,5,6 and 8 was analyzed by 100K SNP array, of patient 7 by 250K SNP array. Assuming that the MPPH phenotype of our patients 1 and 2 could be (in part) related to a larger 5q35 deletion than previously described in Sotos patients, we tested *DRD1* as a possible candidate and sequenced it in MPPH Patients 4–8. No mutations were found.

Review of brain imaging in Sotos syndrome

Sotos syndrome has not been previously associated with polymicrogyria. After the observation of polymicrogyria in Patients 1 and 2 we reviewed brain MRIs of 10 Sotos patients with intragenic *NSD1* mutations and/or microdeletions detected by FISH, diagnosed

in our and other academic centers, including patients with a more severe phenotype like our Patient 3 and the patient described by Sogaard et al. [Patient 8 in Sogaard et al., 2005]. Almost constant MRI findings were megalencephaly with prominent frontal lobes, wide lateral ventricles, cavum septum pellucidum and wide Virchow-Robin perivascular spaces. In none typical polymicrogyria was observed (Table I). We therefore conclude that PMG does not belong to the phenotype of Sotos syndrome.

DISCUSSION

We describe two first cousins, with a submicroscopic deletion of a 5.7 Mb region on chromosome 5q35.2-qter, including the *NSD1* locus and an 8 Mb 20q13.3-qter amplification, as the result of a derivative chromosome 5 of a familial balanced translocation t(5;20)(q35.2;q13.3). Development and head circumference of the relatives bearing the balanced translocation are normal. The features of our patients overlap with the recently described MPPH syndrome, including the polydactyly in patient 1 and perisylvian polymicrogyria on brain MRI of both patients [Mirzaa et al., 2004]. No patients with chromosomal rearrangements at 5q and 20q have been described with these features.

Macrocephaly with megalencephaly and mild enlargement of the cerebral ventricles is typical of Sotos syndrome. Patients with large telomeric deletions of chromosome 5q35 including *NSD1* seem, with some exception [Stratton et al., 1994], to be severely affected, but no brain MRI has been reported [Kleczkowska et al. 1993; Joseph et al., 1990; Menten et al., 2006]. We reviewed the brain MRI of our Patient 3, Patient 8 of Sogaard et al. [2005], and eight other molecularly confirmed Sotos patients and consistently observed megalencephaly, mostly of frontal lobes, with apparently normal cortex, enlarged ventricles but no signs of high pressure hydrocephalus (Table I). Therefore we conclude that PMG and high-pressure hydrocephalus are not part of the Sotos phenotype, and the MPPH-like features of Patients 1 and 2 are probably not caused by *NSD1* haploinsufficiency.

Rare deletions distal to and excluding the *NSD1* locus have been associated with a relatively mild clinical phenotype [Stratton et al., 1994; Rauch and Dörr, 2007], but systematic analysis of the neuroimaging of the patients has not been reported.

The deletion breakpoint of the Patients 1 and 2 on chromosome 5 was mapped between the gene of unknown function *FLJ16171* (present) and *DRD1* (deleted), and it is to our knowledge the largest microdeletion in the 5q35.2qter area. In- or exclusion of *DRD1* in areas of other Sotos microdeletion patients was not clear from literature data; therefore we tested *DRD1* as a candidate for MPPH, but found no mutation in five unrelated MPPH patients. Nagai et al. [2003] reported severe phenotypic abnormalities in Japanese patients with microdeletions at the *NSD1* locus, including cardiac, urinary and endocrine problems compared to patients with intragenic *NSD1* mutations. This genotype-phenotype correlation, although less evident, was also confirmed in Caucasian Sotos patients [Saugier-Verber et al., 2007]. Low copy repeats (LCRs) have been considered responsible for non-allelic homologous recombination (NAHR) as a mechanism causing the microdeletion, supporting the definition of Sotos syndrome as a genomic disorder [Shaw and Lupski, 2004]. In the 5q35 area however, different mechanisms seem to be responsible for NAHR. LCRs (SoSPREP and SoSDREP) at 5q35.3 define the recurrent 1.9 Mb deletion in Sotos patients [Kurotaki et al., 2005]. However, atypical shorter non-recurrent deletions have been described [Tatton-Brown et al., 2005a and b] and in some of these Alu-mediated NAHR is suggested [Mochizuki et al., 2008]. In addition in the 5q35.1 area the contribution of highly homologous LINE elements seems to be responsible for induction of NAHR [Buysse et al., 2008].

In unbalanced translocations, the general assumption is that developmental defects are mostly the result of genetic haploinsufficiency. In a large series of over 5000 patients with developmental defects much fewer pathogenic microduplications than microdeletions have been detected [Shao et al., 2008]. In our Patients 1 and 2, however, the 20q13.3-qter amplification could also contribute to the phenotypic abnormalities.

The amplification breakpoint on chromosome 20q13.3 of Patient 1 and 2 was mapped between *LOC388799* (2 copies) and the known gene *TFAP2C* (3 copies).

Previously reported chromosome 20q amplifications are much larger than in our patients, being 20q11.2-qter [Shao et al., 2008] and 20q13.1-qter [Herens et al., 1990; Plotner et al., 2002]. Most of these are not pure trisomies, but are combined with complex rearrangements [Courtens et al., 2007]. Most common features in these patients are brachycephaly, bulging forehead, deep-set eyes, short nose, large ears, dimpled chin, heart defects, hydrocephalus, cortical atrophy, cerebellar atrophy [Herens et al., 1990; Plotner et al., 2002; Addor et al., 2002; Grange et al., 2005] and phenotypes vary from mildly to severely affected. Some of these features are easily recognizable in our Patients 1 and 2. A submicroscopic 20q13.1-qter duplication, extending 1Mb proximally of our patients' duplication [McMullan et al., 2009], was recently reported in a patient with mental retardation, without clinical detail.

The chromosome 20q13 area contains some genes subjected to genomic imprinting [Bonthron et al., 2000]. The best known is *GNAS1*, involved in the pathogenesis of Albright osteodystrophy [Rickard and Wilson, 2003]. It is not clear whether the extra maternal chromosome 20q13 material in our patients contributes to some of their phenotypic manifestations through imbalances in the imprinted *GNAS* cluster [Bastepe, 2008]. Paternally inherited deletions at the 20q13.3 locus including *GNAS* have been associated with major phenotypic abnormalities, suggesting that, besides the *GNAS* cluster, also other genes like i.e. *TF2PC* might influence imbalances between maternal and paternal alleles [Genevieve et al., 2005].

MPPH syndrome has been described so far in sporadic patients; therefore it is considered to result from *de novo* dominant mutations. We show MPPH-like features in two patients with a 5q35.3 deletion and 20q13.3 trisomy, but we excluded submicroscopic rearrangements in 5 typical MPPH patients. Our findings support the hypothesis that MPPH is a heterogeneous disorder [Gripp et al., 2009], with more mechanisms involved in its pathogenesis, some of which are apparently linked to the rearrangements of genes at terminal regions of chromosome 5q and 20q. We therefore advise high-resolution microarray analysis in the workup of patients with MPPH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the families of our patients, Dr. H.T. Bruggenwirth for NSD1 sequence analysis and Prof. Dr. F. Beemer for useful discussions. This work was partly supported a grant from the (U.S.A.) National Institutes of Health (1R01-NS058721) to W.B.D.

REFERENCES

Addor MC, Castagne C, Micheli JL, Schorderet DF. Partial trisomy 20q in a newborn with dextrocardia. *Genet Couns.* 2002; 13:433–440. [PubMed: 12558114]

- Barkovich AJ, Kuzniecky RI, Jackson GD, Guerrini R, Dobyns WB. A developmental and genetic classification for malformations of cortical development. *Neurology*. 2005; 65:1873–1887. [PubMed: 16192428]
- Barth, PG. International review of Child neurology series (ICNA). Mac Keith Press; London: 2003. Disorders of Neuronal Migration.
- Bastepe M. The GNAS locus and pseudohypoparathyroidism. *Adv Exp Med Biol*. 2008; 626:27–40. [PubMed: 18372789]
- Bonthron DT, Hayward BE. Characterization of TH1 and CTSZ, two non-imprinted genes downstream of GNAS1 in chromosome 20q13. *Hum Genet*. 2000; 107:165–175. [PubMed: 11030415]
- Brooks AS, Bertoli-Avella AM, Burzynski GM, Breedveld GJ, Osinga J, Boven LG, Hurst JA, Mancini GM, Lequin MH, de Coe RF, Matera I, de Graaff E, Meijers C, Willems PJ, Tibboel D, Oostra BA, Hofstra RM. Homozygous nonsense mutations in KIAA1279 are associated with malformations of the central and enteric nervous systems. *Am J Hum Genet*. 2005; 77:120–126. [PubMed: 15883926]
- Buyse K, Crepel A, Menten B, Patty F, Antonacci F, Veltman JA, Larsen LA, Tümer Z, de Klein A, van de Laar I, Devriendt K, Mortier G, Speleman F. Mapping of 5q35 chromosomal rearrangements within a genomically unstable region. *J Med Genet*. 2008; 45:672–677. [PubMed: 18628311]
- Colombani M, Chouchane M, Pitelet G, Morales L, Callier P, Pinard JP, Lion-Francois L, Thauvin-Robinet C, Mugneret F, Huet F, Guibaud L, Faivre L. A new case of megalencephaly and perisylvian polymicrogyria with post-axial polydactyly and hydrocephalus: MPPH syndrome. *Eur J Med Genet*. 2006; 49:466–471. [PubMed: 16807158]
- Courtens W, Wauters J, Wojciechowski M, Reyniers E, Scheers S, van Luijk R, Rooms L, Kooy F, Wuyts W. A de novo subtelomeric monosomy 11q (11q24.2-qter) and trisomy 20q (20q13.3-qter) in a girl with findings compatible with Jacobsen syndrome: case report and review. *Clin Dysmorphol*. 2007; 16:231–239. [PubMed: 17786114]
- Dobyns WB, Mirzaa G, Christian SL, Petras K, Roseberry J, Clark GD, Curry CJ, McDonald-McGinn D, Medne L, Zackai E, Parsons J, Zand DJ, Hisama FM, Walsh CA, Leventer RJ, Martin CL, Gajecka M, Shaffer LG. Consistent chromosome abnormalities identify novel polymicrogyria loci in 1p36.3, 2p16.1–p23.1, 4q21.21–q22.1, 6q26–q27, and 21q2. *Am J Med Genet Part A*. 2008; 146A:1637–1654. [PubMed: 18536050]
- Garavelli L, Guareschi E, Errico S, Simoni A, Bergonzini P, Zollino M, Gurrieri F, Mancini GM, Schot R, Van Der Spek PJ, Frigieri G, Zonari P, Albertini E, Giustina ED, Amarri S, Banchini G, Dobyns WB, Neri G. Megalencephaly and perisylvian polymicrogyria with postaxial polydactyly and hydrocephalus (MPPH): report of a new case. *Neuropediatrics*. 2007; 38:200–203. [PubMed: 18058629]
- Geneviève D, Sanlaville D, Faivre L, Kottler ML, Jambou M, Gosset P, Boustani-Samara D, Pinto G, Ozilou C, Abeguilé G, Munnich A, Romana S, Raoul O, Cormier-Daire V, Vekemans M. Paternal deletion of the GNAS imprinted locus (including *Gnasxl*) in two girls presenting with severe pre- and post-natal growth retardation and intractable feeding difficulties. *European Journal of Human Genetics*. 2005; 13:1033–1039. [PubMed: 15915160]
- Grange DK, Garcia-Heras J, Kilani RA, Lamp S. Trisomy 20q13 --> 20qter in a girl with multiple congenital malformations and a recombinant chromosome 20 inherited from a paternal inversion (20)(p13q13.1): clinical report and review of the trisomy 20q phenotype. *Am J Med Genet Part A*. 2005; 137A:308–312. [PubMed: 16092120]
- Gripp KW, Hopkins E, Vinkler C, Lev D, Malingier G, Lerman-Sagie T, Dobyns WB. Significant overlap and possible identity of macrocephaly capillary malformation and megalencephaly polymicrogyria-polydactyly hydrocephalus syndromes. *Am J Med Genet Part A*. 2009; 149A:868–876. [PubMed: 19353582]
- Guerrini R, Dobyns WB, Barkovich AJ. Abnormal development of the human cerebral cortex: genetics, functional consequences and treatment options. *Trends Neurosci*. 2008; 31:154–162. [PubMed: 18262290]
- Herens C, Verloes A, Laloux F, Van Maldergem L. Trisomy 20q. A new case and further phenotypic delineation. *Clin Genet*. 1990; 37:363–366. [PubMed: 2191794]

- Joseph P, Kimm J, Kalyan-Raman UP, Nixon JP, Hiller J. Terminal deletion of the long arm of chromosome 5. *Am J Hum Genet.* 1990; 47(suppl):A31.
- Kleczkowska A, Fryns JP, van den Berghe H. A distinct multiple congenital anomalies syndrome associated with distal 5q deletion (q35.1qter). *Ann Genet.* 1993; 36:126–128. [PubMed: 8215219]
- Kurotaki N, Harada N, Shimokawa O, Miyake N, Kawame H, Uetake K, Makita Y, Kondoh T, Ogata T, Hasegawa T, Nagai T, Ozaki T, Touyama M, Shenhav R, Ohashi H, Medne L, Shiihara T, Ohtsu S, Kato Z, Okamoto N, Nishimoto J, Lev D, Miyoshi Y, Ishikiriya S, Sonoda T, Sakazume S, Fukushima Y, Kurosawa K, Cheng JF, Yoshiura K, Ohta T, Kishino T, Niikawa N, Matsumoto N. Fifty microdeletions among 112 cases of Sotos syndrome: low copy repeats possibly mediate the common deletion. *Hum Mutat.* 2003; 22:378–387. [PubMed: 14517949]
- Kurotaki N, Stankiewicz P, Wakui K, Niikawa N, Lupski JR. Sotos syndrome common deletion is mediated by directly oriented subunits within inverted Sos-REP low-copy repeats. *Hum Mol Genet.* 2005; 14:535–542. [PubMed: 15640245]
- McMullan DJ, Bonin M, Hehir-Kwa JY, de Vries BBA, Dufke A, Rattenberry E, Steehouwer M, Moruz L, Pfundt R, de Leeuw N, Riess A, Altug-Teber O, Enders H, Singer S, Grasshoff U, Walter M, Walker JM, Lamb CV, Val Davison E, Brueton L, Riess O, Veltman JA. Molecular karyotyping of patients with unexplained mental retardation by SNP arrays: A multicenter study. *Hum Mutat.* 2009; 30:1082–1092. [PubMed: 19388127]
- Menten B, Maas N, Thienpont B, Buysse K, Vandesompele J, Melotte C, de Ravel T, Van Vooren S, Balikova I, Backx L, Janssens S, De Paepe A, De Moor B, Moreau Y, Marynen P, Fryns JP, Mortier G, Devriendt K, Speleman F, Vermeesch JR. Emerging patterns of cryptic chromosomal imbalance in patients with idiopathic mental retardation and multiple congenital anomalies: a new series of 140 patients and review of published reports. *J Med Genet.* 2006; 43:625–633. [PubMed: 16490798]
- Mirzaa G, Dodge NN, Glass I, Day C, Gripp K, Nicholson L, Straub V, Voit T, Dobyns WB. Megalencephaly and perisylvian polymicrogyria with postaxial polydactyly and hydrocephalus: a rare brain malformation syndrome associated with mental retardation and seizures. *Neuropediatrics.* 2004; 35:353–359. [PubMed: 15627943]
- Mochizuki J, Saitsu H, Mizuguchi T, Nishimura A, Visser R, Kurotaki N, Miyake N, Unno N, Matsumoto N. Alu-related 5q35 microdeletions in Sotos syndrome. *Clin Genet.* 2008; 74:384–391. [PubMed: 18505455]
- Nagai T, Matsumoto N, Kurotaki N, Harada N, Niikawa N, Ogata T, Imaizumi K, Kurosawa K, Kondoh T, Ohashi H, Tsukahara M, Makita Y, Sugimoto T, Sonoda T, Yokoyama T, Uetake K, Sakazume S, Fukushima Y, Naritomi K. Sotos syndrome and haploinsufficiency of NSD1: clinical features of intragenic mutations and submicroscopic deletions. *J Med Genet.* 2003; 40:285–289. [PubMed: 12676901]
- Ogawa S, Nanya Y, Yamamoto G. Genome-wide copy number analysis on GeneChip platform using copy number analyzer for affymetrix GeneChip 2.0 software. *Methods Mol Biol.* 2007; 396:185–206. [PubMed: 18025694]
- Ouled Amar Ben Cheikh B, Baulac S, Lahjouji F, Bouhouche A, Couarch P, Khalili N, Regragui W, Lehericy S, Ruberg M, Benomar A, Heath S, Chkili T, Yahyaoui M, Jiddane M, Ouazzani R, Leguern E. A locus for bilateral occipital polymicrogyria maps to chromosome 6q16-q22. *Neurogenetics.* 2009; 10:35–42. [PubMed: 18758830]
- Piao X, Hill RS, Bodell A, Chang BS, Basel-Vanagaite L, Straussberg R, Dobyns WB, Qasrawi B, Winter RM, Innes AM, Voit T, Ross ME, Michaud JL, Descarie JC, Barkovich AJ, Walsh CA. G protein-coupled receptor-dependent development of human frontal cortex. *Science.* 2004; 303:2033–2036. [PubMed: 15044805]
- Plotner PL, Smith JL, Northrup H. Trisomy 20q caused by der(4) t(4;20) (q35;q13.1): Report of a new patient and review of the literature. *Am J Med Genet Part A.* 2002; 111A:71–75.
- Rauch A, Dörr HG. Chromosome 5q subtelomeric deletion syndrome. *Am J Med Genet Part C.* 2007; 145C:372–376. [PubMed: 17910075]
- Rickard S, Wilson LC. Analysis of GNAS1 and overlapping transcripts identifies the parental origin of mutations in patients with sporadic Albright hereditary osteodystrophy and reveals a model system in which to observe the effects of splicing mutations on translated and untranslated messenger RNA. *Am J Hum Genet.* 2003; 72:961–974. [PubMed: 12624854]

- Robin NH, Taylor CJ, McDonald-McGinn DM, Zackai EH, Bingham P, Collins KJ, Earl D, Gill D, Granata T, Guerrini R. Polymicrogyria and deletion 22q11.2 syndrome: Window to the etiology of a common cortical malformation. *Am J Med Genet Part A*. 2006; 140A:2416–2425. and others. [PubMed: 17036343]
- Santos NF, Secolin R, Brandão-Almeida IL, Silva MS, Torres FR, Tsuneda SS, Guimarães CA, Hage SR, Cendes F, Guerreiro MM, Lopes-Cendes I. A new candidate locus for bilateral perisylvian polymicrogyria mapped on chromosome Xq27. *Am J Med Genet Part A*. 2008; 46A:1151–1157. [PubMed: 18384144]
- Saugier-Verber P, Bonnet C, Afejar A, Drouin-Garraud V, Coubes C, Fehrenbach S, Holder-Espinasse M, Roume J, Malan V, Portnoi MF, Jeanne N, Baumann C, Héron D, David A, Gérard M, Bonneau D, Lacombe D, Cormier-Daire V, Billette de Villemeur T, Frébourg T, Bürglen L. Heterogeneity of NSD1 alterations in 116 patients with Sotos syndrome. *Hum Mutat*. 2007; 28:1098–1107. [PubMed: 17565729]
- Shao L, Shaw CA, Lu XY, Sahoo T, Bacino CA, Lalani SR, Stankiewicz P, Yatsenko SA, Li Y, Neill S, Pursley AN, Chinault AC, Patel A, Beaudet AL, Lupski JR, Cheung SW. Identification of chromosome abnormalities in subtelomeric regions by microarray analysis: a study of 5,380 cases. *Am J Med Genet Part A*. 2008; 146A:2242–2251. [PubMed: 18663743]
- Shaw CJ, Lupski JR. Implication of human genome architecture for rearrangement based disorders: the genomic basis of disease. *Hum Mol Genet*. 2004; 13:R57–64. Spec No. [PubMed: 14764619]
- Sogaard M, Tümer Z, Hjalgrim H, Hahnemann J, Friis B, Ledaal P, Pedersen VF, Baekgaard P, Tommerup N, Cingoz S, Duno M, Brondum-Nielsen K. Subtelomeric study of 132 patients with mental retardation reveals 9 chromosomal anomalies and contributes to the delineation of submicroscopic deletions of 1pter, 2qter, 4pter, 5qter and 9qter. *BMC Med Genet*. 2005; 6:21–31. [PubMed: 15904506]
- Stratton RF, Tedrowe NA, Tolworthy JA, Patterson RM, Ryan SG, Young RS. Deletion 5q35.3. *Am J Med Genet*. 1994; 51:150–152. [PubMed: 8092192]
- Tatton-Brown K, Douglas J, Coleman K, Baujat G, Cole TR, Das S, Horn D, Hughes HE, Temple IK, Faravelli F, Waggoner D, Turkmen S, Cormier-Daire V, Irrthum A, Rahman N for the Childhood Overgrowth Collaboration. Genotype-phenotype associations in Sotos syndrome: an analysis of 266 individuals with NSD1 aberrations. *Am J Hum Genet*. 2005a; 77:193–204. [PubMed: 15942875]
- Tatton-Brown K, Douglas J, Coleman K, Baujat G, Chandler K, Clarke A, Collins A, Davies S, Faravelli F, Firth H, Garrett C, Hughes H, Kerr B, Liebelt J, Reardon W, Schaefer GB, Splitt M, Temple IK, Waggoner D, Weaver DD, Wilson L, Cole T, Cormier-Daire V, Irrthum A, Rahman N, the Childhood Overgrowth Collaboration. Multiple mechanisms are implicated in the generation of 5q35 microdeletions in Sotos syndrome. *J Med Genet*. 2005b; 42:307–313. [PubMed: 15805156]
- Tohyama J, Akasaka N, Saito N, Yoshimura J, Nishiyama K, Kato M. Megalencephaly and polymicrogyria with polydactyly syndrome. *Pediatr Neurol*. 2007; 37:148–51. [PubMed: 17675034]
- Villard L, Nguyen K, Cardoso C, Martin CL, Weiss AM, Sifry-Platt M, Grix AW, Graham JM Jr, Winter RM, Leventer RJ, Dobyns WB. A locus for bilateral perisylvian polymicrogyria maps to Xq28. *Am J Hum Genet*. 2002; 70:1003–1008. [PubMed: 11822025]
- Waters JJ, Gourley DS, Aitken DA, Davison BC. Trisomy 20q caused by der (X)t(X;20)(q28;q11.2). *Am J Med Genet*. 1990; 36:310–312. [PubMed: 2363429]

Figure 1.

Pedigree of family with Patient 1 (III-1) and 2 (III-2).

(A) Pedigree, with two affected first cousins, Patient 1 and 2 (filled symbols), carrying an unbalanced 46,XXder(5)t(5;20)(q35.3;q13.3) translocation. Black dots indicate carriers of the balanced t(5;20)(q35.2;q13.3) translocation. (B) Patient 2 at the age of 3 months.

Figure 2.

Brain imaging. (A) T1 weighted axial brain MRI of patient 1 at the age of 2 years showing asymmetry of lateral ventricles with right-sided porencephalic cystic dilatation and perisylvian polymicrogyria (arrows). (B) Brain CT scan of Patient 2 at the age of 1.5 years showing hydrocephalus and bilateral wide sylvian fissures and cortical anomaly compatible with perisylvian polymicrogyria. (C) T1 weighted axial brain MRI of Patient 3 at the age of 3 years, showing prominent frontal lobes and no polymicrogyria. (D) T2 weighted axial brain MRI of Patient 8 at the age of 3 months, showing enlarged lateral ventricles and bilateral cortical polymicrogyria most prominent in perisylvian areas.

Figure 3.

MLPA and FISH analysis on DNA of Patient III-1 and her mother II-2.

(A and B) Ideograms, partial karyotype of chromosome 5 and 20 and the results of the MLPA P036B telomere analysis of (A) Patient 1 (III-1 in Fig 1) and (B) her mother (II-2 in Fig 1) are shown. (C) Fluorescence in situ hybridization experiments on metaphases obtained from cultured lymphocytes of the same Patient 1 (panels 1,2,4) and her mother (panel 3) hybridized with: C.1: 5pter (RP1-24H17) in red and 20qter (RP1-81F12) in green. C.2: 5pter (RP1-24H17) in red and 5q35 (NSD1 RP11-47L17) in green; C.3: 5p13.2 (RP11-253B9) and 5qter (RP-1240G13) in green and 20pter RP5-1061L1, 20qter (RP1-81F120) in red; C.4: 5p13.2 (RP11-253B9) in green and 5q35.3 (NSD1 RP1-118M12) in red. Note the unbalanced pattern in the patient (panels C1, C2 and C4) and the balanced pattern in the carrier of the balanced translocation (panel C3).

Figure 4.

Overview of rearrangements with breakpoints in MPPH-like and Sotos patients. (A) view of SNP array data of chromosome 5 from Patient 1 with MPPH-like disease and Patient 3 with Sotos syndrome. (B) Scheme of 5q35 area with the deletions (in red) observed in Patient 1, 3 and published Patients COG025 and COG231 [Tatton-Brown et al., 2005b], and Patients 14 and 15 [Saugier-Weber et al., 2007]. Uninformative areas are indicated in grey. Genes and SNP locations in the breakpoint areas are indicated.

(C) view of SNP array data and amplification (in green) of chromosome 20q13.3-qter from Patient 1.

Table I

Patient with Sotos or MPPH	Laboratory diagnosis	Brain MRI (age)	Clinical presentation	Reference
Patient 1 MPPH	del	Megalencephaly, wide ventricles, unilateral perisylvian polymicrogyria	Macrocephaly, PMR, epilepsy, spastic tetraparesis, postaxial polydactyly right foot, VUR, hypothyroidism, rickets, ASD II	This report
Patient 2 MPPH	del	Megalencephaly, hydrocephalus, abnormal perisylvian gyral pattern on CT, cavum septum pellucidum	Macrocephaly, macrosomia, profound PMR, epilepsy, spastic tetraparesis, no polydactyly, hypothyroidism, hepatoma, cholestasis, VUR, ASD II	This report
Patient 4 MPPH	-	asymmetric megalencephaly, bilateral perisylvian PMG, short CC, hydrocephalus	Macrocephaly, profound PMR, seizures, spastic quadriplegia; no polydactyly	LP95-025 in Mirzaa et al., 2004
Patient 5 MPPH	-	megalencephaly, bil. perisylvian PMG, hydrocephalus, enlarged LV	Macrocephaly, profound PMR; postaxial polydactyly 4 limbs	LR02-064 in Mirzaa et al., 2004
Patient 6 MPPH	-	Symmetric megalencephaly, perisylvian PMG, hydrocephalus	Macrocephaly, profound PMR, seizures, hypotonia, polydactyly 4 limbs	LR01-181 in Mirzaa et al., 2004
Patient 7 MPPH	-	megalencephaly, hypoplastic CC, bilateral perisylvian PMG	Macrocephaly, large fontanelle, hypertelorism, depressed nasal bridge; postaxial polydactyly 3 limbs	Garavelli et al., 2007
Patient 8 MPPH	-	megalencephaly, bilateral frontoparietal PMG, delayed myelination	Macrocephaly at birth, squint, no visual awareness at 3 months	This report
Sotos 1 (Patient 3 in the text)	del	Dolichocephalic skull, prominent frontal lobes (8 m)	Severe PMR, hypotonia	This report
Sotos 2	mut	Prominent perivascular spaces, cerebellar arachnoidal cyst (11y)	Motor delay	This report
Sotos 3	mut	Cavum septum pellucidum, ventriculomegaly (5 y)	PMR, epilepsy from 2 yr.	This report
Sotos 4	mut	Cavum septum pellucidum, prominent perivascular spaces (3 y)	Mild PMR	This report
Sotos 5	mut	Ventriculomegaly, cerebellar arachnoidal cyst (3 y)	Behavioral problem, mild LD	This report
Sotos 6	mut	Ventriculomegaly (5 months)	PMR and hypotonia at age 5 months	This report
Sotos 7	mut	Ventriculomegaly, thin corpus callosum, diffuse atrophy, prominent frontal lobes (1.5 y)	PMR, intrauterine asphyxia, neonatal hypoglycaemia	This report
Sotos 8	mut	Ventriculomegaly, thick corpus callosum (7 months)	VSD, West syndrome, moderate PMR	This report
Sotos 9	del	Ventriculomegaly, diffuse atrophy (1 y)	Moderate PMR	patient 8 in ref. Sogaard et al., 2005
Sotos 10	mut	Ventriculomegaly, diffuse atrophy, gliosis of WM (8 y)	Mild PMR, mild hemiplegia	This report

PMR= psychomotor retardation, LD= learning disability, WM= white matter, del = *NSD1* microdeletion by FISH, mut= intragenic *NSD1* mutation, y=year