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Two Siblings with Alternate Unbalanced Recombinants Derived from a Large Cryptic Maternal Pericentric Inversion of Chromosome 20

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Abstract

Two brothers, with dissimilar clinical features, were each found to have different abnormalities of chromosome 20 by subtelomere fluorescence *in situ* hybridization (FISH). The proband had deletion of 20p subtelomere and duplication of 20q subtelomere, while his brother was found to have a duplication of 20p subtelomere and deletion of 20q subtelomere. Parental cytogenetic studies were initially thought to be normal, both by G-banding and by subtelomere FISH analysis. Since chromosome 20 is a metacentric chromosome and an inversion was suspected, we used anchored FISH to assist in identifying a possible inversion. This approach employed concomitant hybridization of a FISH probe to the short (p) arm of chromosome 20 with the 20q subtelomere probe. We identified a cytogenetically non-visible, mosaic pericentric inversion of one of the maternal chromosome 20 homologues, providing a mechanistic explanation for the chromosomal abnormalities present in these brothers. Array comparative genomic hybridization (CGH) with both a custom-made BAC and cosmid-based subtelomere specific array (TEL array) and a commercially-available SNP-based array confirmed and further characterized these rearrangements, identifying this as the largest pericentric inversion of chromosome 20 described to date. TEL array data indicate that the 20p breakpoint is defined by BAC RP11-978M13, ~900 kb from the pter; SNP array data reveal this breakpoint to occur within BAC RP11-978M13. The 20q breakpoint is defined by BAC RP11-93B14, ~1.7 Mb from the qter, by TEL array; SNP array data refine this breakpoint to within a gap between BACs on the TEL array (i.e. between RP11-93B14 and proximal BAC RP11-765G16).

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Keywords

FISH; subtelomere; array comparative genomic hybridization; array CGH; SNP; 20p; 20q; pericentric inversion; duplication; deletion

INTRODUCTION

Standard G-banded chromosome analysis has the limitation, even at a high level of resolution, of being unable to detect abnormalities involving fewer than approximately 5 Mb or rearrangements between regions with similar banding patterns. Panels of fluorescence *in situ* hybridization (FISH) probes, each located within 300 kb from the telomeric repeats, are clinically available for human subtelomeres [Knight et al., 1997]. These subtelomeric FISH probes have revealed cryptic subtelomeric abnormalities, which are now recognized in association with mental retardation and dysmorphic features [reviewed by Joyce et al., 2001]. Several subtelomere aberrations (such as deletions of 1p, 2q, 5q, 6p, 9q and 22q13.3 among others) are now recognized as distinct syndromes.

Inversions are intrachromosomal rearrangements, resulting from two breakage events occurring on the same chromosome with the subsequent reversal in the orientation of the piece between these breakpoints. When the breakpoints both occur on the same side of the centromere, the resulting inversion is termed paracentric. When the breakpoints occur on opposite sides of the centromere, the resulting inversion is termed pericentric. Pericentric inversions often lead to differences in arm length. In general, carriers of inversions are phenotypically normal, and are identified following either the birth of an abnormal child, as part of an infertility workup, or through routine prenatal testing for advanced maternal age. Between 85–90% of inversions identified in a proband are inherited from a parent [Kaiser, 1984; Pettenati et al., 1995]. The risk of having a child with an unbalanced chromosome aberration is dependent on both the type (para- or pericentric) and size of the inversion, with the risk being due to pairing and crossing over during meiosis. Individuals with a paracentric inversion are at a relatively low risk for unbalanced offspring, since an unbalanced meiotic outcome leads to dicentric or acentric recombinant chromosomes which are generally unstable. Individuals with a pericentric inversion are at a greater risk to have unbalanced offspring because the unbalanced recombinant chromosome would be monocentric; the risk of an unbalanced outcome generally increases or decreases depending on the size of the inversion. The probability of an odd number of recombination events depends mainly on the size of the chromosome and the inverted segment and has been estimated at between 0 (for very small inversions) and 50% (for very large inversions) [Kaiser, 1984].

Typically, an inversion can be identified by G-banding analysis as it produces a change in the banding pattern, or by FISH, in the case of a very large pericentric inversion, leading to change of signal pattern (i.e. the green signal, corresponding to pter, would appear to be on the q-arm, and the red signal, corresponding to qter, would appear to be on the p-arm). However, this can be difficult to distinguish in a metacentric chromosome, since the subtelomeric green (p) and red (q) signals are always at approximately the same distance from the centromere.

We report here two siblings with discordant phenotypes who were found to each have a different recombinant chromosome 20 arising from a large, cytogenetically undetectable, mosaic maternal pericentric inversion.

CLINICAL REPORTS

Patient 1

Patient 1 (Fig. 1) is a 7-year-5-month-old male born at 39 weeks via spontaneous vaginal delivery to a then 25-year-old primigravida mother and a 25-year-old father. The infant's birth weight was 3.36 kg (50–75th centile); length was 47 cm (10–25th centile). Pregnancy was complicated by early vaginal spotting and a kidney stone attack requiring treatment with Demerol at 37 weeks. There was no history of fever or infections or use of alcohol, tobacco, or drugs. He had an uncomplicated, 2-day stay in the newborn nursery. He was noted to be a “lazy” feeder as an infant, with poor suck.

At four months of age, his parents noticed developmental delay. At approximately 6 months of age, his height and weight were below the 5th centile. He rolled at six months, sat at 8 months and walked at 14 months. His preschool speech and adaptive milestones were moderately delayed. He used a spoon at 24 months of age, became toilet trained at age 4 to 5 years, and mastered unbuttoning at age 6 years. He did not speak in sentences until 48 months. Prior psychoeducational testing with the Wechsler Intelligence Scale for Children-Revised and the Vineland Adaptive Behavior Scales revealed cognitive and adaptive skills in the borderline to mildly mentally deficient range (WISC-R Verbal IQ 77, Performance IQ 67, Full Scale IQ 68; Vineland Adaptive Behavior Composite 65).

Developmental examination (7 years 4 months) revealed fair eye contact and subtle difficulty sustaining a verbal interchange with the examiner. Attention span and cooperation were appropriate. He pinches his hands when he is nervous and fixates on phrases or words he finds fascinating. His speech consisted of three to five-word phrases and short sentences. He had mild difficulties with language pragmatics. His academic skills were at the kindergarten level; he is currently in first grade. He had a score of 19.5 on the Childhood Autism Rating Scale. His clinical developmental diagnosis was borderline development to mild mental retardation, with mildly atypical features.

He had normal hearing, ophthalmologic and cardiac examinations. Growth hormone studies were also normal. Bone age scans at chronological ages 18 months and five years and one month indicated bone ages of 17 months and four years six months, respectively.

Height was 114.5 cm (5th centile). Weight was 20 kg (5–10th centile). Head circumference was 53 cm (50–75th centile). Dysmorphic features included: mild synophrys, long eyelashes, arched/thick eyebrows, mild hirsutism, thickened superior helices, slightly posteriorly rotated ears, thin upper lip, prominent philtrum, multiple sacral dimples and bilateral mild persistence of fetal pads on the fingers. Mouth, palate, uvula, chest, arms (extension/supination), nails, feet, and genitalia were normal. His inner canthal distance was 3 cm, outer canthal distance was 8.25 cm and interpupillary distance was 5.5 cm (75th centile). His inter-nipple distance to chest circumference ratio was 0.25 (75–90th centile). His palm length was 7.75 cm (25–50th centile) and middle finger length was 5 cm (<3rd centile, 50th for 4 years). The external genitalia were prepubertal. The right testis was descended, but the left testis was not palpable.

Patient 2

Patient 2 (Fig. 1) is the 1-year-3-month-old male sibling of Patient 1. He was born at 36 weeks by vertex vaginal delivery to a 31-year-old mother and a 31-year-old father. The infant's birth weight was 2.33 kg (50th centile). He had respiratory distress on day of life one, for which he received oxygen for 14 hours. He was subsequently diagnosed with right-sided choanal atresia, left-sided choanal stenosis and a deviated septum. A heart murmur

was noted; however, an echocardiogram was normal. He had a normal newborn hearing screen.

This patient had six surgeries before the age of four months to correct the choanal atresia. He continued to have mucus build up in his nose; this is treated with saline. He had a frenulectomy at 10 months of age. He had a normal head MRI; however, he has right-sided plagiocephaly for which he wore a helmet for four months. He has constipation that is treated with Miralax. He was a poor feeder. He had significant difficulty with swallowing and excess drooling. He choked and gagged on solid food and did not chew. A videofluoroscopic swallow study, performed at nine months of age, documented dysphagia characterized by a high incidence of laryngeal penetration on thin liquids, secondary to incoordination of suck-swallow-breath pattern.

Adaptive and language milestones were moderately delayed. By age 15 months, he was able to finger feed, but was not able to use a cup or spoon. Vocalizations were limited to occasional polysyllabic babbling (7–9 months). Gross motor skills were mildly delayed. He rolled prone to supine at seven months and supine to prone at 12 months. He sat at 12 months. He was able to pull to stand and cruise around furniture, but could not walk independently at 15 months. Socially, he was described by his family as “easygoing”, without atypical features. At 15 months, eye contact and attention span were appropriate for his developmental level. He was nonverbal except for occasional squealing. His fine motor, gross motor, and cognitive skills clustered around the 7 to 9 month level. He has problems bringing things to his midline. He receives physical, occupational, and speech therapy. His clinical developmental diagnosis was mild-moderate global developmental delay and dysphagia.

Height was 71.5 cm (<5th centile; 50th for 8 months). Weight was 9.41 kg (10–25th centile). Head circumference was 47 cm (25–50th centile). He had right-sided plagiocephaly and a closed fontanel. His eyes appeared mildly proptotic. Inner canthal distance was 2.75 cm, outer canthal distance was 7.25 cm, and interpupillary distance was 4.75 cm (50–75th centile). His ears appeared normal with no pits or tags and normal positioning. His inter-nipple distance to chest circumference ratio was 0.28 (>90th centile); his chest showed no pectus. His palm length was 5 cm (3rd–25th centile) and middle finger length was 3.5 cm (3rd–25th centile). His feet were slightly puffy and small (11 cm, <3rd centile). A prominent natal cleft was present. His neck, abdomen, hands, nails, and genitalia were normal. His receptive language and problem-solving skills are at the seven months level. His sensorimotor skills are at the seven-to-nine month level. Neurodevelopmental assessment suggests that his gross motor delay is consequent to his global cognitive delay; and is not suggestive of a primary motor system problem.

The mother is of Irish and German ancestry, 162.5 cm tall, and healthy. The father is of German ancestry, 180.3 cm tall, and healthy. There is no known consanguinity. The mother has a paternal cousin with mental retardation. The mother and father each have one sister who has no children by choice. The father has a half-brother, through the same father, who has one daughter who is doing well. The rest of the family history is non-contributory.

MATERIALS AND METHODS

Human Subjects

All individuals studied were enrolled under an IRB-approved protocol of informed consent.

Cytogenetic Analysis

Chromosomal analysis was performed on metaphase spreads prepared from seventy-two hour PHA stimulated cultured peripheral blood lymphocytes using standard methods. Banding at the 550-band level was minimally achieved for Patient 1 and 2 (partial karyotypes, Fig. 2) and their parents (partial karyotype of mother, Fig. 2). FISH was performed to analyze all subtelomeres using the Multiprobe Chromoprobe T System (Cytocell Technologies Ltd., Cambridge, U.K.); this analysis was carried out for Patients 1 and 2 as well as for their parents.

FISH was also performed with selected BACs and commercial subtelomere probes (Fig. 2) (Abbott Molecular, Des Plaines, IL) using standard protocols. Two BACs (RP11-48M7 and RP11-278L4) mapping to the terminal ~6 Mb of 20p were chosen to map the 20p breakpoint. Two BACs (RP11-560A15 and RP11-157P1) mapping to the terminal ~7.3 Mb 20q were chosen to map the 20q breakpoint. The map position of each BAC clone was determined according to the UCSC Human Genome Project (March 2006 assembly) (<http://genome.cse.ucsc.edu>). All BAC clones were obtained through CHORI BACPAC Resources (Oakland, CA). BAC DNA was isolated (Perfect Prep Plasmid XL, Eppendorf, Hamburg, Germany) and labeled by nick translation (Nick Translation Reagent Kit, Abbott Molecular, Des Plaines, IL) in the presence of Spectrum Green or Orange dUTP (Abbott Molecular, Des Plaines, IL). Commercial FISH probes (Abbott Molecular, Des Plaines, IL) for 20p telomere (TelVysion20p) and 20q telomere (TelVysion20q) were used. FISH probes were hybridized to metaphase preparations overnight; slides were then washed and counterstained with DAPI, using standard protocols.

ARRAY COMPARATIVE GENOMIC HYBRIDIZATION

BAC and cosmid-based subtelomeric array (TEL array)

The custom-made BAC and cosmid-based subtelomeric array (TEL array) has previously been described in detail [DeScipio et al., 2008]. Briefly, TEL array provides high resolution coverage of the most distal 10 Mb of each chromosome arm (excluding the satellite stalks of the acrocentric chromosomes 13, 14, 15, 21, and 22), and thus covers all of the subtelomeric regions likely to give rise to cryptic subtelomeric cytogenetic changes.

BACs were selected such that, together with the subtelomeric cosmids, complete (~1.5X coverage) tiling path coverage for the terminal 1 Mb region of each chromosome arm was achieved. Proceeding centromerically, one BAC clone was then selected for each 400 kb between 1 and 5 Mb from each telomere (~0.5X coverage), then one BAC clone for each 800 kb was selected between 5 and 10 Mb from each telomere (~0.25X coverage). For 6p and 9q, this standard coverage was supplemented with full tiling path coverage of BACs for the entire 5 Mb and 10 Mb subtelomere regions, respectively [DeScipio et al., 2008].

Single nucleotide polymorphism (SNP) array

SNP array genotyping was carried out by the Center for Applied Genomics (Children's Hospital of Philadelphia) using the Illumina Quad610 array and BeadStation Scanner and BeadStudio analysis software (Illumina, Inc., San Diego, CA). For each sample, 750 ng of DNA was isothermally amplified and enzymatically fragmented, precipitated, and resuspended, and the resulting product was hybridized to the Quad610 chip overnight. After hybridization and enzymatic extension, the products were fluorescently stained and visualized using the Beadstation Scanner and data collection software. Analysis was carried out using the BeadStudio Genotyping module (Illumina, Inc., San Diego, CA), and DNA copy number changes were visualized using B-allele frequency and Log₂R ratio. For quality control, both the genotyping yield (greater than 99.8%) and standard deviation of the Log₂R

ratio (less than 0.35) were met for further analysis. For the analysis, a subset of probes from the Quad610 array was used. Only copy number intensity (cnvi) probes on the Y chromosome and in the pseudoautosomal (XY) region were included, in addition to the SNP probes, resulting in a final set of 594,906 probes in total with an average spacing of 4.9 kb. The base pair of the first and last SNP deleted for each patient were reported.

RESULTS

Standard G-banded karyotypes were normal for Patients 1 and 2 (partial karyotype, Fig. 2) and both of their parents (partial karyotype of mother, Fig. 2). Fluorescence *in situ* hybridization (FISH) analysis of all subtelomeres was subsequently performed on both brothers (Patients 1 and 2). The proband (Patient 1) was found, by FISH, to have a subtelomeric 20p deletion and a 20q duplication (Fig. 2); his karyotype was interpreted as 46, XY.ish der(20)(20PTTEL18-,20QTEL14++). Subtelomeric FISH studies were also performed on the proband's brother (Patient 2), who was found to have a subtelomeric 20p duplication and 20q deletion (Fig. 2). His karyotype was initially reported as: 46, XY.ish der(20)(20PTTEL18++,20QTEL14-).

G-banding and subtelomeric FISH studies were performed on maternal and paternal chromosomes and initially interpreted as normal: 46, XX.ish subtel(41×2) and 46, XY.ish subtel(41×2), respectively. We then hypothesized that one parent had either germline mosaicism for each of the der(20) chromosomes or carried a large pericentric inversion of chromosome 20 that was not cytogenetically visible by either G-banding or by subtelomeric FISH studies. Since chromosome 20 is a metacentric chromosome, a large pericentric inversion would likely not be apparent by a shift in the position of the centromere relative to the green (p-arm) and red (q-arm) subtelomere FISH probe signals.

To test the second hypothesis, that one parent carried a large pericentric chromosome 20 inversion, we needed to determine the orientation of the arms of chromosome 20 in the parental samples, relative to the subtelomeres. FISH was used to further characterize the parental chromosomes using a probe from the short (p) arm of chromosome 20 (RP11-48M7, labeled in green) in conjunction with the 20q subtelomere probe (20QTEL14, labeled in red) (Abbott Molecular, Des Plaines, IL) (Fig. 2). FISH studies on paternal chromosomes were normal while a maternal inversion of chromosome 20 was identified. On one chromosome 20, the red (q) and green (p) signals were present on opposite arms of the chromosome while on the other chromosome 20, the red and green signals were present on the same arm, demonstrating that the 20q telomere was inverted relative to the 20p13 probe (RP11-48M7) (Fig. 2). Interestingly, this was identified in about half of maternal cells studied (7/13, or 53%). This positioned the p arm breakpoint distal to BAC clone RP11-48M7 which maps from ~3.7 to 3.9Mb from the 20p terminus (pter) (UCSC Human Genome Project, March 2006 assembly).

To further localize the breakpoint on each arm, we utilized a probe from the long (q) arm of chromosome 20 (RP11-157P1, labeled in red) in conjunction with the 20p subtelomere probe (20PTTEL18, labeled in green) (Abbott Molecular, Des Plaines, IL). These results confirmed a pericentric inversion of chromosome 20, revealed by the presence of red (RP11-157P1, 20q13.3) and green (20PTTEL18, 20p subtelomere) signals on the same arm (Fig. 2). This positioned the q arm breakpoint distal to BAC clone RP11-157P1 which maps from ~2.0 to 2.2Mb from the 20q terminus (qter) (UCSC Human Genome Project, March 2006 assembly).

These FISH data have mapped the duplicated/deleted regions in the proband and his brother. The breakpoint on 20p is distal to RP11-48M7, with the proximal end of this BAC mapping

3.9 Mb from 20pter. The breakpoint on 20q is distal to RP11-157P1, with the distal end of this BAC mapping 2.2 Mb from 20qter.

To determine if other members of the family carry this inversion, FISH analysis was also performed on the proband's maternal grandparents and maternal aunt. FISH was carried out with the 20q subtelomere probe (20QTEL14) in combination with RP11-48M7 (20p13) probe. Twenty metaphase spreads from each individual were analyzed by FISH; no abnormalities were identified. It is therefore most likely that this inversion was a new event that occurred in the mother of Patients 1 and 2. The other possibility is that one of her parents is a germline mosaic for this inversion; however, this is unlikely since she herself is mosaic.

Based on these data, the karyotypes for the mother, proband (Patient 1) and sibling (Patient 2) were reinterpreted. The proband's karyotype: 46, XY.ish rec(20)dup(20q)inv(20)(p13q13.3)(20PTEL18-,20QTEL14++)mat; his brother's (Patient 2) karyotype: 46, XY.ish rec(20)dup(20p)inv(20)(p13q13.3)(20PTEL18++,20QTEL14-)mat. The maternal karyotype: 46, XX.ish inv(20)(p13)(20PTEL18-, RP11-48M7+,20QTEL14+)(q13.3)(20PTEL18+, RP11-157P1+,20QTEL14-)[7]/46, XX.ish 20p13(20PTEL18X2, RP11-48M7X2),20q13.1(20QTEL14X2, RP11-157P1X2)[6]*de novo*.

To further refine the chromosome 20 p and q breakpoints, we performed array comparative genomic hybridization using both the TEL array and the Illumina Quad610 SNP array. TEL array is a BAC/cosmid-based, high-resolution array of the terminal 10 Mb of each telomeric and subtelomeric chromosomal region [DeScipio et al., 2008]. The chromosome 20 p and q subtelomere deletions/duplications in Patients 1 and 2 were confirmed, and the breakpoints were refined. The TEL array data revealed that BAC clone RP11-978M13, which maps 900 kb from the 20p terminus, defined the 20p breakpoint in both Patients 1 and 2 (UCSC Human Genome Browser, March 2006 assembly) (Fig 2). The BAC clone RP11-93B14, which maps 1.7 Mb from the 20q terminus defined the 20q breakpoint in Patients 1 and 2 (UCSC Human Genome Browser, March 2006 assembly) (Fig 2). Therefore, Patient 1 is monosomic for the most terminal 900 kb of chromosome 20p and trisomic for the most terminal 1.7 Mb of 20q. Patient 2 is trisomic for the most terminal 900 kb of chromosome 20p and monosomic for the most terminal 1.7 Mb of 20q. According to the UCSC Human Genome Browser (March 2006 assembly), at least 20 known or hypothetical genes map to this 20p region and at least 70 known or hypothetical genes map to this 20q region (Fig. 2). The mother's DNA was not analyzed on the TEL array; since she was mosaic for the inversion (~50%) that was also thought to be balanced.

SNP array (Illumina Quad610) analysis was also performed on both brothers (Patients 1 and 2) and their mother. SNP array data agreed with and further refined the p and q breakpoints defined by the TEL array and revealed additional details regarding the position of the chromosome 20 breaks in the two brothers and the mother's mosaic chromosome 20 aberration. SNP array data revealed that the 20p breakpoints in both Patient 1 and 2 occurred within the BAC (RP11-978M13) identified by the TEL array while the 20q breakpoints occurred in a gap between the two BACs (RP11-765G16 and RP11-93B14) identified by the TEL array (data not shown). Interestingly, the SNP array revealed that the pericentric, mosaic chromosome 20 inversion in the mother is in fact unbalanced, with small duplications at both the p (~116 kb) and q (~60 kb) breakpoints (data not shown). Additionally, the mother's chromosome 20 aberration was detected at the same level of mosaicism (~50%) by SNP array as in metaphase FISH studies. In concordance with the p and q breakpoints of the mother's duplications, the two brothers (Patients 1 and 2) actually have different p and q breakpoints corresponding to the proximal or distal end of the p or q duplication identified by the mother's SNP array analysis.

DISCUSSION

We describe here what is, to our knowledge, the largest pericentric inversion of chromosome 20 described to date. This inversion was identified following the birth of siblings with different apparently *de novo* abnormalities, each involving chromosome 20. Through the use of an anchored fluorescence *in situ* hybridization (FISH) methodology, we identified the mother as a mosaic carrier of a large pericentric inversion with breakpoints in the subtelomeric regions of chromosome 20. Additionally, utilizing two separate microarray CGH platforms, we confirmed and further characterized the 20p and 20q subtelomere breakpoints, thereby fine-mapping the implicated chromosome regions in the proband, his brother, and his mother. Furthermore, SNP array analysis revealed small duplications (~116 kb and ~60 kb, p and q arms respectively) at each of the 20p and 20q inversion breakpoints in the mother. The 20p and 20q breakpoints in the brothers also differed, depending on whether they occurred at the proximal or distal boundary of these duplications at the mother's inversion breakpoints.

The mother was found to be a mosaic carrier of the pericentric inversion of chromosome 20 in ~50% of her leukocytes (by FISH and SNP array analysis). This inversion was large, such that there was no cytogenetically visible change in the G-banding pattern (Fig. 2) and the change in the placement of the centromere by subtelomeric FISH (Fig. 2) could have been easily overlooked. Counseling this patient for future pregnancies was difficult, since her risk to have a child with an unbalanced chromosome rearrangement was high, but difficult to exactly predict due to the unknown significance of the inverted chromosome 20 with respect to pairing and crossing-over during meiosis, as well as the unknown level of mosaicism in her germ cells.

The use of subtelomeric FISH has lead to the recognition of many microscopically cryptic rearrangements, including large parental inversions leading to offspring with unbalanced recombinant chromosomes. However the previous reports of large inversions were identified in submetacentric chromosomes, allowing unambiguous identification of the carrier parent. Anderlid *et al.* reported a brother and sister, each with 6p subtelomere trisomy and 6q subtelomere monosomy inherited from their father who carried a pericentric inversion of chromosome 6 [Anderlid *et al.*, 2002]. Clarkson *et al.* reported a female with 11p subtelomere trisomy and 11q subtelomere monosomy; the father carried a pericentric inversion of chromosome 11 [Clarkson *et al.*, 2002]. In these cases, the short and long arms were easily distinguished, with the reversal of green (p subtelomere) and red (q subtelomere) FISH signals clearly visible on the inverted chromosome in each parent. To our knowledge, this is the first report of a large pericentric inversion involving a metacentric chromosome that was not detected by G-banding and was originally overlooked by subtelomere FISH analysis.

There have been several reports describing recombinant aneuploidy resulting from pericentric inversions of chromosome 20. However, these previous reports of pericentric inversions of chromosome 20 have been cytogenetically visible by G-banding analysis and their recombinant outcomes have therefore resulted in larger deleted and duplicated regions of 20p and 20q [Bown *et al.*, 1986; Grange *et al.*, 2005; Lucas *et al.*, 1985; Molina-Gomes *et al.*, 2006]. Our review will focus on submicroscopic aberrations of 20p and 20q, as is the case in the two individuals we present here. Furthermore, we will focus on pure, isolated submicroscopic terminal aberrations of 20p or 20q as the phenotype due to the given chromosome 20 aberration is complicated when accompanied by deletion or duplication of another chromosomal region (as in derivative chromosomes resulting from an unbalanced translocation).

The proband (Patient 1), originally referred to rule out a diagnosis of mild Cornelia de Lange syndrome, was identified with monosomy for the 20p subtelomere and trisomy for the 20q subtelomere, leading to a relatively milder phenotype when compared to his brother (Patient 2). Sixteen cases of submicroscopic 20p subtelomere deletions have been reported [Ravnan et al., 2006; Roberts et al., 2004; Baker et al., 2002; Baroncini et al., 2005; Ballif et al., 2007; Shaffer et al., 2006; Toruner et al., 2007]. Among these sixteen cases, five were *de novo*, one was paternally inherited, one was maternally inherited from a similarly affected mother, and the inheritance of the remaining nine cases was not known. Clinical information was reported for eight of these sixteen cases of 20p submicroscopic subtelomere deletion; these findings are summarized and compared with Patient 1 in this report (Table I). The most consistent finding among these individuals is developmental delay/mental retardation. Craniofacial dysmorphism is also common; however, there is limited clinical overlap between the reported individuals. It is important to note that the size of the deleted 20p subtelomere region is not reported in any of these eight cases; however, all deletions were submicroscopic. To the best of our knowledge, there have not been reports of pure, terminal cryptic duplications of 20q subtelomere reported in the literature.

The brother of the proband (Patient 2) was identified as having the “opposite” of the chromosomal imbalance of his brother (Patient 1). Patient 2 has a duplication of 20p and a deletion of 20q. To the best of our knowledge, there have not been reports of pure duplication of 20p subtelomere reported in the literature. Six individuals have been reported with pure 20q subtelomere deletion [Ravnan et al., 2006; Roberts et al., 2004; Beri-Deixheimer et al., 2007; Bena et al., 2007]. Clinical and cytogenetic findings in each of these six individuals are summarized and compared to our Patient 2 (Table II). The most consistent shared feature among these individuals is developmental delay. Dysmorphic features are also common; however, significant clinical overlap between individuals is not overwhelming. Ardalan *et al.* described a child with a recombinant chromosome 20 derived from a familial pericentric insertion with a resultant 4.7 Mb duplication of the terminal segment of 20p and a 1.6 Mb deletion of 20q; this resultant deletion and duplication is therefore significantly larger than in Patient 2 [Ardalan et al., 2005].

In summary, this family illustrates that a large, cryptic pericentric inversion in a metacentric chromosome can be over-looked, even when subtelomere FISH analysis is performed. Initial parental studies were thought to be normal due to the metacentric nature of the inverted chromosome, further complicated by mosaicism. Molecular cytogenetic analysis utilizing BAC probes within the 20p13 (and subsequently the 20q13 region) to “anchor” the arm, along with one subtelomeric probe allowed detection of this inversion. We suggest that in the case of a subtelomeric duplication and deletion in a metacentric chromosome, parental studies be performed utilizing an internal chromosome arm control to assess for pericentric inversions. Furthermore, SNP array analysis unexpectedly revealed small duplications at the inversion breakpoints in the mother. The advent of high resolution microarray CGH analysis will likely reveal many unbalanced chromosomal aberrations in individuals previously described as having “balanced” aberrations; the incidence of such cases is yet to be determined.

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References

- Anderlid BM, Schoumans J, Anneren G, Sahlen S, Kyllerman M, Vujic M, Hagberg B, Blennow E, Nordenskjold M. Subtelomeric rearrangements detected in patients with idiopathic mental retardation. *Am J Med Genet.* 2002; 107:275–284. [PubMed: 11840483]
- Ardalan A, Prieur M, Choiset A, Turleau C, Goutieres F, Girard-Orgeolet S. Intrachromosomal insertion mimicking a pericentric inversion: molecular cytogenetic characterization of a three break rearrangement of chromosome 20. *Am J Med Genet A.* 2005; 138A:288–293. [PubMed: 16158442]
- Baker E, Hinton L, Callen DF, Altree M, Dobbie A, Eyre HJ, Sutherland GR, Thompson E, Thompson P, Woollatt E, Haan E. Study of 250 children with idiopathic mental retardation reveals nine cryptic and diverse subtelomeric chromosome anomalies. *Am J Med Genet.* 2002; 107:285–293. [PubMed: 11840484]
- Ballif BC, Sulpizio SG, Lloyd RM, Minier SL, Theisen A, Bejjani BA, Shaffer LG. The clinical utility of enhanced subtelomeric coverage in array CGH. *Am J Med Genet A.* 2007; 143A:1850–1857. [PubMed: 17632771]
- Baroncini A, Rivieri F, Capucci A, Croci G, Franchi F, Sensi A, Battaglia P, Aiello V, Calzolari E. FISH screening for subtelomeric rearrangements in 219 patients with idiopathic mental retardation and normal karyotype. *Eur J Med Genet.* 2005; 48:388–396. [PubMed: 16378923]
- Bena F, Bottani A, Marcelli F, Sizonenko LD, Conrad B, Dahoun S. A de novo 1.1–1.6 Mb subtelomeric deletion of chromosome 20q13.33 in a patient with learning difficulties but without obvious dysmorphic features. *Am J Med Genet A.* 2007; 143A:1894–1899. [PubMed: 17632785]
- Beri-Deixeimer M, Gregoire MJ, Toutain A, Brochet K, Briault S, Schaff JL, Leheup B, Jonveaux P. Genotype-phenotype correlations to aid in the prognosis of individuals with uncommon 20q13.33 subtelomere deletions: a collaborative study on behalf of the ‘association des Cytogeneticiens de langue Francaise’. *Eur J Hum Genet.* 2007; 15:446–452. [PubMed: 17290276]
- Bown N, Cross I, Davison EV, Burn J. Partial trisomy 20p resulting from a recombination of a familial pericentric inversion. *Hum Genet.* 1986; 74:417–419. [PubMed: 3793104]
- Clarkson B, Pavenski K, Dupuis L, Kennedy S, Meyn S, Nezarati MM, Nie G, Weksberg R, Withers S, Quercia N, Teebi AS, Teshima I. Detecting rearrangements in children using subtelomeric FISH and SKY. *Am J Med Genet.* 2002; 107:267–274. [PubMed: 11840482]
- DeScipio C, Spinner NB, Kaur M, Yaeger D, Conlin LK, Ambrosini A, Hu S, Shan S, Krantz ID, Riethman H. Fine-mapping subtelomeric deletions and duplications by comparative genomic hybridization in 42 individuals. *Am J Med Genet A.* 2008; 146A:730–739. [PubMed: 18257100]
- 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 A.* 2005; 137A:308–312. [PubMed: 16092120]
- Joyce CA, Dennis NR, Cooper S, Browne CE. Subtelomeric rearrangements: results from a study of selected and unselected probands with idiopathic mental retardation and control individuals by using high-resolution G-banding and FISH. *Hum Genet.* 2001; 109:440–451. [PubMed: 11702226]
- Kaiser P. Pericentric inversions. Problems and significance for clinical genetics. *Hum Genet.* 1984; 68:1–47. [PubMed: 6389316]
- Knight SJ, Horsley SW, Regan R, Lawrie NM, Maher EJ, Cardy DL, Flint J, Kearney L. Development and clinical application of an innovative fluorescence in situ hybridization technique which detects submicroscopic rearrangements involving telomeres. *Eur J Hum Genet.* 1997; 5:1–8. [PubMed: 9156314]
- Lucas J, Le Mee F, Le Marec B, Pluquailec K, Journal H, Picard F. [Trisomy 20p derived from a maternal pericentric inversion and brachymesophalangy of the index finger]. *Ann Genet.* 1985; 28:167–171. [PubMed: 3879151]
- Molina-Gomes D, Nebout V, Daikha-Dahmane F, Vialard F, Ville Y, Selva J. Partial trisomy 20p resulting from recombination of a maternal pericentric inversion: case report of a prenatal diagnosis by chorionic villus sampling. *Prenat Diagn.* 2006; 26:239–241. [PubMed: 16485319]

- Pettenati MJ, Rao PN, Phelan MC, Grass F, Rao KW, Cosper P, Carroll AJ, Elder F, Smith JL, Higgins MD, Lanman JT, Higgins RR, Butler MG, Luthardt F, Keitges E, Jackson-Cook C, Brown J, Schwartz S, van Dyke DL, Palmer CG. Paracentric inversions in humans: a review of 446 paracentric inversions with presentation of 120 new cases. *Am J Med Genet.* 1995; 55:171–187. [PubMed: 7717416]
- Ravnan JB, Tepperberg JH, Papenhausen P, Lamb AN, Hedrick J, Eash D, Ledbetter DH, Martin CL. Subtelomere FISH analysis of 11 688 cases: an evaluation of the frequency and pattern of subtelomere rearrangements in individuals with developmental disabilities. *J Med Genet.* 2006; 43:478–489. [PubMed: 16199540]
- Roberts AE, Cox GF, Kimonis V, Lamb A, Irons M. Clinical presentation of 13 patients with subtelomeric rearrangements and a review of the literature. *Am J Med Genet Part A.* 2004; 128A: 352–363. [PubMed: 15264280]
- Shaffer LG, Kashork CD, Saleki R, Rorem E, Sundin K, Ballif BC, Bejjani BA. Targeted genomic microarray analysis for identification of chromosome abnormalities in 1500 consecutive clinical cases. *J Pediatr.* 2006; 149:98–102. [PubMed: 16860135]
- Toruner GA, Streck DL, Schwalb MN, Dermody JJ. An oligonucleotide based array-CGH system for detection of genome wide copy number changes including subtelomeric regions for genetic evaluation of mental retardation. *Am J Med Genet A.* 2007; 143A:824–829. [PubMed: 17366576]

Fig. 1.
Photograph of the proband (Patient 1) and brother of the proband (Patient 2).

Fig. 2.
G-banded partial karyotypes of chromosome 20, metaphase spreads showing FISH with subtelomere and BAC probes and TEL array chromosome 20 data for Patient 1, Patient 2 and their mother.

TABLE I

Individuals With Submicroscopic Isolated Terminal 20p Deletions

	1	2	3	4	5	6	7	8	
Reference	1	1	1	1	1	2	3	4	This report
Originally reported as	Case 133	Case 134	Case 135	Case 136	Case 137	Patient 11	Case 6	Case 5	Patient 1
Size of deletion									0.9 Mb del 20p 1.7 Mb dup 20q
Inheritance	var (pat)	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>		<i>de novo</i>	mat	<i>de novo</i>	mat
Sex	M	M	F	M	M	M	M	M	M
Age at report (years)	2 4/12	1 11/12	3 9/12	1	1	3	10	10	7 5/12
Clinical Indication	Features of SMS	DD	DD, DF, growth delay	Growth delay, hypotonia, kidney hypoplasia	DD	DD, macrocrania	Moderate global intellectual disability (WISC-III)	Moderate MR	? Mild Cornelia de Lange syndrome Borderline development to mild MR
Height						10 th	25 th	short	5 th
Weight						50–75 th			5–10 th
Head						>95 th	2 nd		50–75 th
Craniofacial dysmorphism						Frontal bossing, high forehead	Microcephaly, long face, deep-set eyes, small mouth, up-slanting palpebral fissures	Trigonocephaly, high nasal bridge	Mild synophrys, long eyelashes, arached/thick eyebrows, thin upper lip
External ear anomalies						Large lobes			Thickened superior helices, slight posterior rotation
Philtrum							Short		prominent
Hand anomaly						Left single palmar crease			BL mild persistence of fetal pads
Foot anomaly						Partial 2,3 syndactyly	Pes planus		Normal
Hypotonia				Y		Y			No
Hirsutism						Y			Mild
Unique features						Prone to diarrhea	Generalized epilepsy, delayed secondary dentition	PDA, dextrocardia, brachydactyly	Multiple sacral dimples

(1) Ravnan et al. [2006], (2) Roberts et al. [2004], (3) Baker et al. [2002], (4) Baroncini et al. [2005]

Var, variant; SMS, Smith-Magenis syndrome; DD, developmental delay; DF, dysmorphic features; MR, mental retardation; PDA, patent ductus arteriosus; Blank, not reported

TABLE II

Individuals With Submicroscopic Isolated Terminal 20q Deletions

	1	2	3	4	5	6	
Reference	1	1		3		4	This report
Originally reported as	Case 142	Case 143	Patient 12	Patient 1	Patient 2		Patient 2
Size of deletion				1.33 MB	1.33 MB	1.1–1.6 MB	0.9 Mb del 20q 1.7 Mb dup 20p
Inheritance	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	mat
Sex	M	M	M	F	F	F	M
Age at report (years)	11 1/2	7 8/12	9	7 10/12	4	6 8/12	1 3/12
Clinical Indication	MCA	DD, MR	Global DD	Idiopathic MR	Idiopathic MR	Speech delay, strabismus	DD, minor dysmorphism
Height			25 th	+0.3 SD		50 th	<5 th , 50 th for 8 mon
Weight			75 th	−0.5 SD		75 th	10–25 th
Head			50 th	−2.3 SD		25–50 th	25–50 th
Craniofacial dysmorphism			Short/smooth philtrum, arched eyebrows, BL crumpled ear helices, high palate, bulbous nose	Micro/brachycephaly, prominent metopic suture, down-turned corners of mouth, anteverted nares	Epicanthic folds, trigonocephaly		Mildly proptosis, Rt plagiocephaly
Developmental delay (DD)		Y	Global DD	Mild global delay	Severe global delay, delayed motor miles	Learning difficulties	Mild-moderate, global DD
Mental retardation (MR)		Y	Mild	Mild psychomotor			
Language delay				Mildly retarded w/ phonological abnormalities	Absent at 28 mon	Speech delay (simple but complete sentences)	Moderately delayed
Temporal narrowing				Y	Y		
Up-slanting palpebral fissures			Y	Y			
Mild hypertelorism				Y	Y		
Thin upper lip			Y	Y	Y		
Constipation			Y				Y
Unique features			2,3 syndactyly, BL 5 th finger clinodactyly,	Nystagmus	Thin corpus callosum, great vessels transposition, autistic behaviours, stereotypies of hands,	Alternating convergent strabismus, hyperopia, Rt	Prominent natal cleft, dysphagia, frenulectomy, foot length <3rd, choanal

1	2	3	4	5	6	
		single café au lait, frontal upsweep of the hair, eczema		irregular sleep (nocturnal wake- up)	eye astigmatism, joint laxity, anxious, flat feet	atresia Rt, choanal stenosis Lt

(1) Ravnan et al. [2005], (2) Roberts et al. [2004], (3) Beri-Deixheimer et al. [2007], (4) Bena et al., 2007
MCA, multiple congenital anomalies; DD, developmental delay; MR, mental retardation; BL, bilateral; Rt, right; Lt, left; Blank, not reported