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Chediak-Higashi Syndrome with early Developmental Delay resulting from Paternal Heterodisomy of Chromosome 1

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Abstract

Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disease characterized by variable oculocutaneous albinism, immunodeficiency, mild bleeding diathesis and an accelerated lymphoproliferative state. Abnormal lysosome-related organelle membrane function leads to the accumulation of large intracellular vesicles in several cell types, including granulocytes, melanocytes, and platelets. This report describes a severe case of CHS resulting from paternal heterodisomy of chromosome 1, causing homozygosity for the most distal nonsense mutation (p.E3668X, exon 50) reported to date in the *LYST/CHS1* gene. The mutation is located in the WD40 region of the CHS1 protein. The patient's fibroblasts expressed no detectable CHS1. Besides manifesting the classical CHS findings, the patient exhibited hypotonia and global developmental delays, raising concerns about other effects of heterodisomy. An interstitial 747kb duplication on 6q14.2 to 6q14.3 was identified in the proband and paternal samples by comparative genomic hybridization. SNP genotyping revealed no additional whole chromosome or segmental isodisomic regions or other dosage variations near the crossover breakpoints on chromosome 1. Unmasking of a separate autosomal recessive cause of developmental delay, or an additive effect of the paternal heterodisomy, could underlie the severity of the phenotype in this patient.

Keywords

Chediak-Higashi syndrome; *LYST/CHS1*; uniparental heterodisomy; SNP genotyping; lysosome-related organelle

INTRODUCTION

Chediak-Higashi syndrome (CHS; OMIM #214500) is a rare autosomal recessive disorder characterized by variable oculocutaneous hypopigmentation, mild bleeding tendency, severe immunodeficiency, and an accelerated lymphoproliferative phase that is usually fatal unless treated by bone marrow transplantation (BMT) [Huizing et al., 2001; Introne et al., 1999; Kaplan et al., 2008; White, 1966]. Patients who do not develop an accelerated phase or who survive after successful BMT develop late-onset cognitive impairment [Karim et al., 2002; Westbroek et al., 2007]. Characteristic large cytoplasmic granules representing abnormal lysosomes are found in bone marrow myeloblasts and promyelocytes, in melanocytes (giant melanosomes), and in platelets, which also exhibit scarce or absent dense bodies [Westbroek et al., 2007; Zarzour et al., 2005].

The disease is caused by mutations in the lysosomal trafficking regulator gene (*LYST/CHS1*, OMIM #606897) on 1q42.1-q42.2, identified after the murine homologue gene *beige* was identified [Nagle et al., 1996; Perou et al., 1996]. The gene contains 53 exons (51 coding) with an open reading frame of 11,406 bp, which encodes for a 429 kDa, 3801 amino acid protein, CHS1. This is a cytosolic protein with three recognized domains: an ARM/HEAT domain containing alpha helix repeats and sets of highly conserved hydrophobic residues, a BEACH (BEige And CHediak) domain containing the amino acid sequence WIDL (tryptophan, isoleucine, aspartic and leucine) and a series of seven WD40 (tryptophan–aspartic acid) repeats at the carboxyl terminus, predicted to be a protein-protein interaction domain [Nagle et al., 1996; Ward et al., 2003]. Although the current understanding of the function of the CHS1 protein is limited, studies suggest a role for CHS1 in regulating vesicle trafficking and determining the size of lysosomes and other organelles [Kaplan et al., 2008].

Genotype and phenotype association studies support a correlation between the severity of clinical manifestations and that of the corresponding mutations in the *LYST/CHS1* gene. Nonsense or frameshift mutations have been found in cases with infantile onset and development of hemophagocytic lymphohistiocytosis, while homozygous or compound heterozygous missense mutations are usually described in the adult-onset cases with subtle pigmentary changes, few or no infections and progressive neurological dysfunction [Kaplan et al., 2008; Karim et al., 2002; Westbroek et al., 2007].

Maternal isodisomy of chromosome 1 resulting in a homozygous nonsense mutation (c. 2620delT, p.Phe874fsX898) in the *LYST/CHS1* gene has been previously described in a 6 year old male with classic Chediak-Higashi syndrome and normal physical and neurocognitive development [Dufourcq-Lagelouse et al., 1999]. Additionally, chromosome 1 maternal or paternal iso- or heterodisomy has been reported in association with various isolated recessive disorders without other symptoms, such as growth or cognitive delays, suggesting a lack of imprinted genes on chromosome 1 [Benko et al., 2008; Fassihi et al., 2005; Field et al., 1998; Gelb et al., 1998; Miyoshi et al., 2001; Pulkkinen et al., 1997; Riveiro-Alvarez et al., 2007; Takizawa et al., 2000; Thompson et al., 2002; Turner et al., 2007; Zeng et al., 2006].

This report presents the molecular and cell biological findings in an unusually severe Chediak-Higashi patient with early global developmental delay caused by a homozygous nonsense mutation in exon 50 of the *LYST/CHS1* gene resulting from paternal heterodisomy of chromosome 1.

CLINICAL REPORT

Patient CHS-16, an 8-month-old male infant, was enrolled in a clinical protocol approved by the NHGRI Institutional Review Board. Parental consent was obtained for these studies.

CHS-16 is the first child of nonconsanguineous Caucasian parents. Pregnancy and delivery were uneventful and birth weight was within normal limits. At one month of age, the infant developed extensive eczema. Nystagmus and splenomegaly were noted at 3 months of age and the patient was referred to a tertiary Pediatric Center. A peripheral blood smear revealed large cytoplasmic granules in his neutrophils, lymphocytes, and eosinophils, while an ophthalmologic evaluation confirmed oculocutaneous albinism, suggesting the diagnosis of Chediak-Higashi syndrome.

The boy was subsequently treated for a MRSA superinfection of his eczematous rash at age 5 months. Investigations for hemophagocytic lymphohistiocytosis showed absent NK- and decreased cytolytic T-lymphocyte (CTL)- function, as well as a persistent increase of his serum interleukin 2 receptor levels (IL2R: 3,834 U/mL, normal 334-3026 U/mL). Ferritin, triglyceride levels and liver function tests were within normal limits. He was diagnosed with subclinical hypothyroidism (TSH 11.3 mU/L, T4 9.9 mcg/dl, negative thyroid antibodies) at age 5 months. A brain MRI showed mildly prominent lateral and third ventricles and extra-axial spaces but no other abnormalities. The intraorbital optic nerves appeared small, raising the question of optic nerve hypoplasia or atrophy.

Family history was significant for a mother with status post surgical repair of cleft lip and palate, seizures, and moderate cognitive delay (IQ 70), while his father had suspected learning difficulties and did not complete a high school education.

Previous genetic testing included a normal 46, XY karyotype at the 550 G-band level, negative FISH for 22q11.2 and negative mutation testing of the *UNC13D* and *syntaxis 11* genes associated with familial hemophagocytic lymphohistiocytosis 3 and 4 respectively, as well as *FOXP3* (IPEX: Immunodysregulation, Polyendocrinopathy, Enteropathy, X-linked) and *WAS* genes (Wiskott-Aldrich syndrome).

The patient was referred to the National Institutes of Health for confirmation of the diagnosis of Chediak-Higashi syndrome prior to undergoing bone marrow transplantation at the referring Institute. His physical growth, including head circumference, was within normal range. He had silvery blond hair, multiple hyperkeratotic, excoriated plaques on his extensor surfaces and a palpable spleen. Ophthalmologic evaluation revealed a slow velocity pendular nystagmus, minimal visual tracking, severe diffuse iris transillumination, a very blond fundus with no foveal reflex, and severe optic nerve pallor (Fig. 1A–B). Microscopic examination of his hair revealed the presence of small, homogeneously distributed pigment clumps (Fig. 1C). There were large cytoplasmic granules in his neutrophils, lymphocytes, and eosinophils and large Dohle bodies in his granulocytes (Fig. 1D–F), and whole mount electron microscopy of his platelets showed rare dense bodies (Fig. 1G–H). At the age of 8 months, the infant could smile, make cooing sounds, bring hands to his mouth and hold a rattle momentarily, but had significant truncal hypotonia, could not roll over or support his upper body on arms when supine, sit unsupported, reach intentionally or transfer objects between his hands. His hearing was not assessed although he quieted to the sound of his parents' voices. He was unable to localize to sounds or to his name being called. His significant development delay, as assessed with the Denver Developmental Screening Test II, was that of a 2-month-old.

METHODS

Molecular analysis

Sequencing of exons 1–53 of the *LYST/CHS1* gene (NM_000081) and their intronic boundaries was performed by Transgenomic, Inc. (Omaha, NE) and verified by GeneDx

(Gaithersburg, MD). Parental samples were subsequently tested for the mutation identified in the proband.

For copy number real-time quantitative PCR, assays-by-design Taq-Man® MGB probes (FAM dye-labeled) specific to exons 41, 50 and 52 of the *LYST/CHS1* gene and an endogenous control, located in the *HPS6* gene on 10q24.32, *HPS6 3'-UTR-C*, were used (Applied Biosystems, Foster City, CA, USA). The reaction was conducted on an ABI-PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Copies of the *LYST/CHS1* gene were calculated using the formula: $\text{copynumber} = 2^{-\Delta C_t}$, as described [Livak and Schmittgen 2001].

To investigate uniparental disomy, genotyping of the proband and his parents was performed. Multicolor fluorescent dye technology ([FAM]TM, [VIC][®] and [NED]TM) was used to amplify chromosome-specific microsatellite markers by PCR using AmpliTaq Gold® PCR Master Mix (Applied Biosystems, Foster City, CA, USA). We selected 29 markers for chromosome 1 of the ABI Prism Linkage Mapping Set v2.5. The position of the markers was determined according to the CEPH genotype data. Labeled PCR products were electrophoresed on an ABI Prism 3130xl Genetic Analyzer, and analyzed with GeneMapper V3.7 software (Applied Biosystems, Foster City, CA, USA).

Cytogenetic analysis

High-resolution oligonucleotide array CGH was performed using the GenomeDx microarray v1.0, containing ~44,000 oligonucleotide probes (GeneDx, Gaithersburg, MD). Results were confirmed by quantitative real-time PCR.

Whole genome SNP genotyping was performed using the Illumina Infinium HumanHap1MDuo BeadChip Array according to the manufacturer's protocol (Illumina, Inc., San Diego, CA). Copy number analysis and loss of heterozygosity for breakpoint determination was performed for chromosome 1 by analyzing the B-allele frequency and log R ratio tracts of the accompanying Illumina BeadStudio software (ver. 3.3) as described [Peiffer et al., 2006; Steemers and Gunderson 2007].

Cell biology

Primary dermal fibroblasts from a forearm skin biopsy of the proband, CHS-16, and a previously described "classical" CHS patient, CHS-4, [Zarzour et al., 2005] were cultured as previously described [Westbroek et al., 2007]. Normal human dermal fibroblasts were purchased from the Coriell Cell Repository (Camden, NJ).

For immunofluorescence studies the following antibodies were employed: anti-LAMP-3/CD63 mouse monoclonal antibody (Developmental Studies Hybridoma Bank, Iowa City, IA), BODIPY 558/568 phalloidin (Invitrogen, Carlsbad, CA), secondary donkey anti-mouse antibodies conjugated to ALEXA-488 (Invitrogen, Carlsbad, CA), and a nuclear DAPI stain (Invitrogen, Carlsbad, CA). The cells were mounted in VectaShield (Vector Laboratories, Burlingame, CA) and imaged with a Zeiss 510 META confocal laser-scanning microscope (Carl Zeiss, Microimaging Inc., Germany) using a 488 argon and a 543 HeNe laser. Images were acquired using a Plan Apochromat 63X/1.4 oil DIC objective.

Western blot analysis was performed with the NuPAGE large protein analysis system according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). A mouse monoclonal antibody against α -tubulin (1:4000; Sigma, St. Louis, MO) and a rabbit polyclonal anti-CHS1 antibody were used (1:200; Quality Controlled Biochemicals, Hopkinton, MA). Production and characterization of the anti-CHS1 antibody is described in the Supplemental data.

RESULTS

Molecular genetics

Sequencing of exons 1–53 and flanking regions of the *LYST/CHSI* gene in our patient revealed an apparently homozygous mutation c.11102G>T; p.E3668X in exon 50 (NM_000081.2 and NP_000072.2 respectively).

Of note, a lack of heterozygosity was observed throughout the *LYST/CHSI* gene, raising the possibility of hemizygosity, consanguinity, or uniparental disomy and leading to further molecular investigations in this case. Testing of the parental samples confirmed the mutation in the father, but not the mother of our patient (Fig. 2A).

CGH microarray analysis was performed to explore the possibility of a contiguous gene deletion encompassing the *LYST/CHSI* gene inherited from the mother leading to the unmasking of the *LYST/CHSI* gene mutation transmitted from his father. A 747 kb duplication extending from cytogenetic band 6q14.2 to 6q14.3 was found in the patient. This duplication was transmitted from his father, as confirmed by subsequent analysis by quantitative real-time-PCR for one of the genes located in this interval, *CYB5R4*, as well as by the SNP array analysis that allowed further characterization of the boundaries of the duplicated region. This copy number variation has not been reported previously. The genes located in that interval, namely *SNAP91*, *CYB5R4*, *MRAP2*, *KIAA1009* and *C6orf159*, have unclear biological function and are not associated with a clinical disorder to date.

In addition, an interstitial 1.9 Mb duplication extending from cytogenetic band 11q13.1 to 11q13.2 was identified in the mother that was not present in the patient. Larger duplications overlapping this region have been described to cause cognitive delay, microcephaly, arched palate, multiple craniosynostoses and heart defects [Jehee et al., 2007; Zarate et al., 2007]. This cytogenetic abnormality, if *de novo*, could underlie mother's history of cleft lip and palate, seizures and moderate developmental delay. In addition, we performed fragile X testing in the mother that was negative.

To further evaluate for hemizygosity of a part of the *LYST/CHSI* gene below the detection range of the CGH array, copy number real-time quantitative PCR of genomic DNA was performed for exons 41, 50 and 52 of the *LYST/CHSI* gene. Both parents and the proband carried two copies of all the exons tested, excluding a partial or complete deletion of the gene (data not presented).

Finally, we examined the possibility of uniparental disomy (UPD) by extended haplotype analysis using 29 highly polymorphic markers spanning the entire chromosome 1. The original analysis, with 13 markers, showed complete uniparental isodisomy for the paternal chromosome 1. When more markers were employed in an attempt to better characterize the telomeric regions of chromosome 1, it became apparent that the patient was heterozygous for small regions around the centromere and the p telomere of chromosome 1 (Fig. 3).

In our attempt to identify additional changes that would explain the severe phenotype of our patient, we employed the high-density 1M SNP Illumina BeadChip and PennCNV for data analysis. In addition to confirming the aberrations previously detected by array CGH in the proband and parental samples (Supplementary Fig. 1 and 2), we could explore in significantly improved detail the crossover breakpoints on chromosome 1 separating the iso- and heterodisomic regions, as well as exclude other areas of iso- or heterodisomy in the remaining chromosomes.

The 1p telomeric crossover breakpoint lies at 1p36.22 between SNPs rs1372106 (9,888,012) and rs11577757 (10,572,931) (Supplementary Fig. 3 and 4). This region corresponds to a

neuroblastoma tumor suppressor candidate region, containing several genes associated with susceptibility to neuroblastoma, including *UBE4B*, *DFFA*, *KIF1B* and *APTD1*, [Krona et al., 2003; Krona et al., 2004] as well as a locus linked with Leber's congenital amaurosis (LCA9) [Keen et al., 2003]. Our patient is heterodisomic for the whole 1p36 region, which is haploinsufficient in patients with the common terminal deletion syndrome, characterized by multiple congenital anomalies, including micro/brachycephaly, hearing loss, visual inattentiveness, cleft lip and palate, congenital heart disease, hypothyroidism, seizures and cognitive delay [Heilstedt et al., 2003; Redon et al., 2005].

The 1q telomeric crossover breakpoint lies in the 1q43-1q44 region, between SNPs rs2502349 (241,138,321) and rs4658673 (243,108,935). Patients with 1q42-q44 deletion have variable delays, seizures and congenital anomalies, including cleft palate, congenital heart disease, midline defects, agenesis of the corpus callosum and microcephaly [Boland et al., 2007; Dobyns et al., 2008].

The pericentromeric crossover breakpoints were located at 1p12, between SNPs rs1353132 (120,350,254) and rs2603977 (120,440,096), and 1q23.1, rs536192 (153,904,285) and rs7532302 (154,926,142) respectively. The crossover on 1q is located more distally to the regions implicated in the thrombocytopenia, absent radius (TAR) syndrome and the recently described 1q21.1 microdeletion/microduplications syndromes [Brunetti-Pierrri et al., 2008; Klopocki et al., 2007].

CHS-16 does not express CHS1 protein

To detect the relative protein expression of CHS1, we performed Western blotting with a custom-made polyclonal anti-CHS1 antibody. CHS-16 fibroblasts did not express CHS1 protein (~430 kDa) in contrast to normal cells (Fig. 2B) and similar to our negative control cells from a previously published classical CHS patient, CHS-4. (Westbroek and others 2007; Zarzour and others 2005) The specificity of the custom-made anti-CHS1 antibody was verified by LC/MS-MS (see Supplemental material).

CHS-16 fibroblasts have enlarged lysosomes

Previously, we showed that fibroblasts derived from classical CHS patients have enlarged lysosomes in the perinuclear area [Westbroek et al., 2007]. By using confocal laser scanning microscopy we examined the size and localization of lysosomes in CHS-16 fibroblasts. Control fibroblasts had normal sized lysosomes, marked by the lysosome-specific marker LAMP-3, that were distributed in the perinuclear region as well as the periphery (Fig. 4A + B, arrows). CHS-16 fibroblasts exhibited many enlarged lysosomes that were localized in the perinuclear area (Fig. 4C+D, arrows), with only few normal sized lysosomes in the peripheral area (Fig. 4D, arrowheads).

DISCUSSION

Patient CHS-16 carries a nonsense mutation at exon 50, out of 53, of the *CHS1* gene; this could be expected to allow for partial synthesis of the CHS1 protein and result in a mild phenotype. In contrast, along with a severe "classical" clinical and cell biological Chediak-Higashi phenotype, our patient showed a severe delay in the acquisition of motor skills and overall neurocognitive development and an unusual degree of visual impairment that was deemed out of proportion to his albinism.

The most distal mutation reported to date in the *LYST/CHS1* gene is a homozygous p.K3465fsX3467 in exon 46 observed in a Turkish infant with classic Chediak-Higashi syndrome [Karim et al., 1997]. Protein truncation studies in a series of "classical" Chediak-Higashi syndrome cases revealed 3 more patients carrying stop codons in the WD40 domain

of the protein [Certain et al., 2000]. These mutations suggest that the presence of the C-terminal part of the CHS1 protein is significant for protein function, though insufficient data exist on protein expression in the reported patients, due to the lack of a reliable antibody against the CHS1 protein.

Our patient carries a premature termination (stop) codon (PTC) in exon 50, at amino acid position 3668, out of the 3801 amino acids of the CHS1 protein. This premature stop occurs in the WD40 domain (amino acids 3553–3779) of the 3801 amino acid CHS1 protein and represents the most distally located nonsense mutation described in the *LYST/CHS1* gene, based on the LYSTbase, last updated June 2007 (Curator Mauno Vihinen, Institute of Medical Technology, Finland; URL: <http://bioinf.uta.fi/LYSTbase/>), the Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff, release 2008.4 (<http://www.hgmd.cf.ac.uk/ac/index.php>) and literature review. This change is located 37 bp before the exon-exon junction with exon 51, upstream of two introns before the poly-A tail and, hence, it is expected to be recognized for nonsense mediated decay (NMD) [Chang et al., 2007; Khajavi et al., 2006]. The lack of a partially translated product was verified in our case with a novel CHS1 polyclonal rabbit antibody. The absent protein expression is consistent with our patient's severe CHS phenotype, while it also reduces the possibility of a toxic partially translated product with dominant negative or gain-of-function effect, as the reason for his unusual neurocognitive delay. We have observed the same mutation in compound heterozygous state in one more of our patients with a classical CHS phenotype (unpublished data).

The lack of identification of the mutation in the mother led to a series of studies to exclude hemizygoty, which allowed us to identify paternal heterodisomy as the underlying molecular mechanism for this patient's apparently homozygous nonsense mutation.

Our initial haplotype analysis using 13 markers spread across chromosome 1 showed that the patient was homozygous for all the markers tested for chromosome 1. It was not until more markers were employed (total 29) that we identified small regions of heterodisomy that were further verified with the SNP genotyping array. It is possible that heterodisomy is more common than previously reported, as many of the studies, reviewed here only for chromosome 1, use fewer than 15 markers [Dufourcq-Lagelouse et al., 1999; Fassihi et al., 2005; Turner et al., 2007]. The use of SNP arrays in population-based studies will allow further delineation of the incidence and clinical implications of iso- or heterodisomy.

The paternally derived chromosomes 1 in this case were heteroallelic around the centromeres and at the telomeric regions, with the remainder of the chromosome showing homoallelism/isodisomy. This pattern is compatible with a non-disjunction event during paternal meiosis I resulting to primary heterodisomy, where the uniparental chromosomes contain the centromeric region of both homologues of the respective parent [Kotzot 2008]. The areas of homozygosity (secondary isodisomy) are caused by chromatid exchanges during the metaphase of meiosis I. The disomic gamete containing both paternal homologues is either fertilized with a normal gamete from the other parent, followed by a postzygotic loss (trisomy rescue) of the excess chromosome, or can be fertilized with a nullisomic gamete for that chromosome from the other parent (gamete complementation) in order to give a viable conceptus. Such events are extremely rarely and the risk for recurrence is negligible, which has significant implications for the genetic counseling of such families.

Since the first case in 1997, [Pulkkinen et al., 1997] at least 19 cases of uniparental hetero- or isodisomy of chromosome 1 have been reported resulting in various autosomal recessive conditions with a typical phenotype, suggesting the lack of imprinted genes affecting growth and development on either the paternal or maternal alleles on that chromosome [Benko et

al., 2008; Dufourcq-Lagelouse et al., 1999; Fassihi et al., 2005; Field et al., 1998; Gelb et al., 1998; Miyoshi et al., 2001; Pulkkinen et al., 1997; Riveiro-Alvarez et al., 2007; Takizawa et al., 2000; Thompson et al., 2002; Zeng et al., 2006) reviewed in a recent report [Turner et al., 2007].

However, recent studies in ovarian and breast cancers showed that *NOEY2* (*ARHI*, aplasia Ras homologue member 1) represents a maternally imprinted tumor suppressor gene on 1p31, [Feng et al., 2008; Yu et al., 2003; Yu et al., 1999] and loss of imprinting of p73 on 1p36.33 is described in lung, esophageal, and renal carcinoma [Cai et al., 2000; Mai et al., 1998a; Mai et al., 1998b; Moll and Slade 2004]. In addition, there are approximately 17 predicted imprinted genes, maternal and paternal, on chromosome 1, based on computational approaches [Luedi et al., 2007]. Whether there is an increased risk for cancer related to isodisomy of chromosome 1 is difficult to assess, given the rarity of this genetic alteration.

Moreover, two recent reports present cases of chromosome 1 complete or partial UPD and an unusual phenotype. Maternal heterodisomy was found in a child with autism [Wassink et al., 2005], while complete paternal UPD of chromosome 1 was reported in a case with unusually severe haemolytic uremic syndrome [Fremaux-Bacchi et al., 2007]. Taken together with the present study, these reports suggest that there may be additional effects caused by iso- or heterodisomy of chromosome 1, exclusive of aberrant genomic imprinting, that modify the severity of the phenotype of the patients. Examples could include the co-existence of another unrecognized autosomal recessive disorder, microdeletions or duplications below the detection threshold of the methods employed or low-level mosaicism.

Given that the father had borderline learning difficulties, it is also possible that the small duplication on chromosome 6q14.2 to 6q14.3 is implicated in our patient's phenotype. Moreover, mother's mild developmental delay may be attributed to the large duplicated region on chromosome 11, not shared with the proband, or considered multifactorial, suggesting similar effects on the child. The mild hypothyroidism of the proband could also have affected his development. In summary, although the developmental delay in this case is probably multifactorial, the simultaneous presence of large regions of homozygosity in the very gene-rich chromosome 1 makes unmasking of another recessive mutation in this region affecting mental function highly likely.

Lastly, our case underlines the advantage of a SNP genotyping array over the CGH array platform in providing loss-of-heterozygosity analysis, which enables the detection of copy-number neutral chromosomal aberrations such as uniparental disomy, as suggested by various prior studies [Altug-Teber et al., 2005; Andersen et al., 2007; Bruce et al., 2005; Middleton et al., 2006; Pei et al., 2006; Ting et al., 2007; Walker and Morgan 2006]. Although the changes responsible for the developmental delay in this case remain obscure, implementing advanced genotyping technologies in the work-up of individual patients can provide useful clinical information [Deardorff et al., 2008; Gijsbers et al., 2008; Strauss et al., 2008], and help define their advantages and limitations as diagnostic tools in the emerging era of personalized medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1. Clinical findings of patient CHS-16

(A) Iris transillumination (caused by light that is transmitted back through the iris when it is shone through the pupil) in CHS-16, indicating a lack of pigment; (B) Hypopigmentation of the retina in CHS-16; (C) Microscopic examination of the hair revealed the presence of small, homogeneously distributed pigment clumps (arrows); (D-F) Wright stains of peripheral smear showing an eosinophil, polymorphonuclear leukocyte and lymphocyte with giant intracytoplasmic granules, as well as Dohle bodies (E) (100 $\times$). (G) Whole mount electron microscopy of platelets from the patient's blood showing absent dense bodies (10,000 $\times$). (H) Only two normal-sized dense bodies (arrows) were present in one of the normal-sized platelets (13,000 $\times$).

FIG. 2. Mutation analysis and protein expression

(A) Sequencing of the *CHS1* gene revealed an apparently homozygous G>T nucleotide substitution in exon 50 (arrow) in patient CHS-16, resulting in the replacement of a glutamic acid (GAA), with a stop codon (TAA), p.E3668X mutation. This change was found in the heterozygous state in the paternal, but not the maternal sample; (B) Western blot of protein extracts derived from CHS-16, control and CHS-4 fibroblasts labeled with anti-rabbit anti-CHS1 and anti-mouse anti-alpha-Tubulin Ab (loading control, 55 kDa). CHS-16 fibroblasts do not express CHS1 protein (~430 kDa, lane 1).

FIG. 3. Haplotype analysis using chromosome 1 polymorphic markers show primary heterodisomy of chromosome 1

A total of 29 polymorphic markers on chromosome 1 were employed for haplotype analysis. Small areas of heterozygosity were observed at the 1p telomere and around the centromere, where markers from both the paternal homologues were detected (solid black bars: paternal allele 1; solid gray bars: paternal allele 2; dashed/stripped bars: maternal alleles; solid lines combining the bars represent areas where the markers were non-informative, i.e. the father was homozygous for the respective marker. Asterisk represents a single marker that does not appear to derive from the father, and may indicate variability due to slippage during replication.

FIG. 4. CHS-16 fibroblasts have enlarged lysosomes

(A) LAMP-3 staining (green) in control fibroblasts shows lysosomes distributed throughout the cell (arrows). (B) Enlarged view of LAMP-3 in control fibroblasts. (C) LAMP-3 (green) in CHS-16 fibroblasts shows accumulation of enlarged lysosomes in the perinuclear area (arrows). (D) Enlarged view of LAMP-3 in CHS-16 fibroblasts, showing some normal sized lysosomes in the peripheral area (arrowheads). Co-staining with BODIPY 558/568 phalloidin (red) visualizes the cell boundaries; the DAPI stain (blue) visualizes the nucleus. Scale bars 20 μ m.