review

The discovery of microdeletion syndromes in the post-genomic era: review of the methodology and characterization of a new 1q41q42 microdeletion syndrome

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Purpose: The advent of molecular cytogenetic technologies has altered the means by which new microdeletion syndromes are identified. Whereas the cytogenetic basis of microdeletion syndromes has traditionally depended on the serendipitous ascertainment of a patient with established clinical features and a chromosomal rearrangement visible by G-banding, comparative genomic hybridization using microarrays has enabled the identification of novel, recurrent imbalances in patients with mental retardation and apparently nonspecific features. Compared with the "phenotype-first" approach of traditional cytogenetics, array-based comparative genomic hybridization has enabled the detection of novel genomic disorders using a "genotype-first" approach. We report as an illustrative example the characterization of a novel microdeletion syndrome of 1q41q42. **Methods:** We tested more than 10,000 patients with developmental disabilities by array-based comparative genomic hybridization using our targeted microarray. High-resolution microarray analysis was performed using oligonucleotide microarrays for patients in whom deletions of 1q41q42 were identified. Fluorescence in situ hybridization was performed to confirm all 1q deletions in the patients and to exclude deletions or other chromosomal rearrangements in the parents. **Results:** Seven cases were found with de novo deletions of 1q41q42. The smallest region of overlap is 1.17 Mb and encompasses five genes, including *DISP1*, a gene involved in the sonic hedgehog signaling pathway, the deletion of which has been implicated in holoprosencephaly in mice. Although none of these patients showed frank holoprosencephaly, many had other midline defects (cleft palate, diaphragmatic hernia), seizures, and mental retardation or developmental delay. Dysmorphic features are present in all patients at varying degrees. Some patients showed more severe phenotypes and carry the clinical diagnosis of Fryns syndrome. **Conclusions:** This new microdeletion syndrome with its variable clinical presentation may be responsible for a proportion of Fryns syndrome patients and adds to the increasing number of new syndromes identified with array-based comparative genomic hybridization. The genotype-first approach to identifying recurrent chromosome abnormalities is contrasted with the traditional phenotype-first approach. Targeting developmental pathways in a functional approach to diagnostics may lead to the identification of additional microdeletion syndromes. ***Genet Med*** **2007:9(9):607–616.**

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In 1963, Black and Carter[1] posited that the "elfin"-like facies characteristic of two clinical entities, supravalvular aortic stenosis and infantile hypercalcemia, suggested that the two disorders may be related. Nine years later, Beuren[2] demonstrated that supravalvular aortic stenosis and idiopathic infantile hypercalcemia, were, in fact, components of the same disorder,

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which might present with or without mental retardation. However, the genetic basis of the disorder, now commonly referred to as Williams-Beuren syndrome, remained unknown for almost 30 years. During those three decades, numerous loci were erroneously suggested as causative for the phenotype based on single case reports.[3–8] It was not until the finding of a translocation segregating with the supravalvular aortic stenosis phenotype and cloning of the breakpoints that the cytogenetic basis for Williams-Beuren syndrome was uncovered.[9]

Historically, patients with microdeletion syndromes were identified by a constellation of key clinical features that had evolved from the ascertainment of large collections of individuals with similar abnormalities. The cytogenetic bases of these syndromes was not known. The advent of G-banding and other chromosomal banding techniques in the 1970s allowed the identification of the alternating light- and dark-staining bands comprising each human chromosome, facilitating the detection of aneuploidies and large structural rearrangements such as deletions, duplications, and translocations. Thus, the cytogenetic basis of many syndromes was delineated. For example, although Langer-Giedion syndrome (LGS) was characterized by a recognizable phenotype as early as 1969,[10] its cytogenetic basis was unknown until high-resolution banding identified interstitial deletions in the long arm of chromosome 8 in patients with LGS[11,12]; the location of the deletion was subsequently determined to be 8q24.1.[13,14] Mapping of the deletion breakpoints in a cohort of LGS patients showed that 75% have cytogenetically detectable deletions of 8q24.1.[15]

The identification of patients with unbalanced translocations may facilitate the detection of submicroscopic deletions. This approach requires that the deleterious effects of each component chromosome of the unbalanced translocation be parsed. The association of DiGeorge syndrome with an interstitial deletion of the long arm of chromosome 22 was suggested by de la Chapelle and colleagues[16] following the identification of an unbalanced translocation resulting in a deleted chromosome 22 and comparison with published reports of monosomy 22. High-resolution G-banding and fluorescence in situ hybridization (FISH) in a large collection of patients later confirmed this hypothesis; more than 95% of patients have deletions of 22q11.2.[17,18]

Much like those with unbalanced translocations, patients with balanced translocations have helped identify novel microdeletions; typically, these deletions were too small to be easily visualized under the light microscope and necessitated the serendipitous ascertainment of a patient with a translocation segregating the deletion and recognizable clinical features. For example, Shaffer et al.[19] reported two family members with mental retardation and multiple congenital anomalies including multiple exostoses, enlarged parietal foramina, and craniofacial dysostosis. Karyotyping of the mother revealed an insertional translocation between chromosomes 11 and 13. Affected individuals inherited a deletion of 11p11.2 following malsegregation of the insertional translocation.[19] Additional patients were subsequently described with del(11)(p11.2p12) and multiple exostoses,[20,21] confirming that this constellation of features constituted a syndrome. In another example, three cases of Rubinstein-Taybi syndrome (RTS), a disorder consisting of mental retardation, characteristic facial features, and broad thumbs and toes, presented with balanced translocations involving chromosome 16 and 2, 7, or 20.[22] The breakpoint on chromosome 16 occurred in band 16p13.3, suggesting this as a location for the RTS locus. Subsequent analysis using a cosmid mapping to 16p13.3 of a cohort of 24 RTS patients showed microdeletions of this region in six cases, confirming that a substantial proportion of RTS result from microdeletions.[22]

In 1995, Flint et al.[23] developed a method for simultaneously interrogating all the unique human subtelomeres using FISH. These probe sets[24,25] could be used to detect submicroscopic chromosomal abnormalities in patients with idiopathic mental retardation but without features suggestive of a particular syndrome. Some of the abnormalities initially detected with subtelomere FISH have since been identified in a sufficiently large number of patients that the abnormality could be conclusively linked to the phenotype, which could in turn be delineated by comparison of clinical features among affected individuals. For example, one of the first syndromes discovered using subtelomere FISH resulted from deletion of 22q13.3[26]; once a common cause was established among a collection of patients, the clinical features could be delineated.[27] Other subtelomeric abnormalities have been identified in only one or a few patients and cannot be considered recurrent until a consistent collection of clinical features is delineated.

The use of subtelomere FISH panels has illustrated that, in the absence of specific clinical features suggestive of a syndrome, patients with mental retardation can be "screened" for a novel chromosomal abnormality. This development represented a shift from the traditional "phenotype-first" approach explained earlier, wherein a set of patients was grouped based on shared clinical features, to a "genotype-first" approach by which individuals can be characterized first by a common cytogenetic aberration and then as more patients with the same abnormality are ascertained, a clinical presentation can be delineated.

The development of comparative genomic hybridization (CGH), particularly CGH using microarrays (array CGH), broadened the scope and resolution at which the genotypes of patients with idiopathic mental retardation could be assayed. In contrast to subtelomere FISH, microarrays may be constructed with contiguous or noncontiguous coverage of the entire genome or with consideration of well-known microdeletion and microduplication syndrome loci; thus, the principles of subtelomere FISH panels could be applied to a much larger proportion of the genome. For example, in screening patients with unexplained overgrowth syndrome by whole-genome array CGH, Redon et al.[28] identified two individuals with de novo interstitial deletions of 9q22.32-q22.33. Although the breakpoints differed in each patient, the similarity of clinical features—both individuals presented with macrocephaly, overgrowth, trigonocephaly, and hyperactivity in addition to a constellation of distinct facial features—led the authors to suggest that deletion of 9q22.3 was a novel microdeletion syndrome.

In another example, three simultaneously published reports detailed the identification of a microdeletion syndrome encompassing 17q21.3.[29–31] In one of these reports, Sharp et al.[30] constructed a microarray targeted to potential "rearrangement hotspots" that, because of their genomic architecture containing segmental duplications, were likely to be affected by genomic instability through nonallelic homologous recombination.[32] Through the screening of 290 children with idiopathic mental retardation with or without associated congenital anomalies, Sharp et al.[30] identified de novo microdeletions of 17q21.31 in four individuals. In addition, the authors identified one patient with a de novo microdeletion of 15q24; three more patients with submicroscopic deletions of this region were subsequently identified by array CGH using either targeted or whole-genome BAC or oligonucleotide microarrays.[33]

In contrast to the Sharp et al.[30] publication, which targeted regions associated with segmental duplications, Ballif et al.[34] targeted the pericentromeric regions of the genome. These regions are inherently difficult to assess by chromosome analysis and are known to harbor microdeletions, including Williams, DiGeorge, and Prader-Willi syndromes. Array CGH analysis identified inherited copy-number variants (benign polymorphisms) and de novo deletions and duplications with potential clinical significance, including a recurrent de novo interstitial deletion of 16p11.2p12.2 in four patients. FISH and whole-genome microarray analysis of these four deletions showed that the breakpoints cluster at complex segmental duplications that flank the deletion region consistent with nonallelic homologous recombination mediating these rearrangements. The common clinical features of these patients suggested that deletion of 16p11.2p12.2 constitutes a novel microdeletion syndrome.

We have taken a directed, functional approach to identify individuals with novel microdeletions. In developing a targeted microarray for use with CGH,[35,36] we included regions commonly rearranged in chromosome abnormalities including microdeletion syndromes and the subtelomeric regions. In addition, to increase our coverage over the genome in a deliberate manner, we included genes in important developmental pathways, such as *PTCH*, *ZIC2*, and *DISP1*, which are involved in the sonic hedgehog (SHH) signaling pathway, the disruption of which has been implicated in holoprosencephaly (HPE). This microarray has been used to identify chromosome abnormalities in patients with developmental delay, mental retardation, seizures, and other developmental abnormalities[36,37] and has led to the identification of recurrent de novo deletions of 1q41q42 that include the *DISP1* locus.

MATERIALS AND METHODS

Subjects

At the time of this writing, more than 10,000 peripheral blood samples have been analyzed by our diagnostic laboratory (Signature Genomic Laboratories, LLC, Spokane, WA) by array CGH using the SignatureChip.[37] These samples were received from referring physicians (medical geneticists, pediatric neurologists, neonatologists, and general pediatricians) from the United States and abroad. The indications for referral were the same as those received for routine cytogenetic testing including developmental delay, mental retardation, dysmorphic features, failure to thrive, seizures, other developmental disabilities, and physical anomalies. Array CGH results for our first 1500 consecutive cases were recently published[36] and the results for a total case volume of 8789 are in press.[37]

Array CGH and FISH

All samples were tested by CGH with the SignatureChip diagnostic microarray using previously published methods.[35,36] There have been four versions of our microarray, but all have included bacterial artificial chromosomes (BACs) that contain the HPE or HPE-candidate loci of interest to this report. For this study, FISH was performed with published methods[38] using BAC RP11-139E20 containing the *DISP1* gene to confirm all 1q deletions in the subjects and to exclude deletions or other chromosomal rearrangements in the parents.

High-resolution microarray studies

Informed consent using a Washington State University Institutional Review Board–approved protocol and consent form was obtained through the referring physicians from parents of children in whom 1q41q42 deletions were identified, except for Subject 2 for whom informed consent was obtained using a Children's Hospital of Philadelphia Institutional Review Board–approved protocol and consent form. DNA samples were then sent to Children's Hospital of Philadelphia for high-resolution analysis. The microarray experiments were performed using the Affymetrix GeneChip 250K Sty chip (Affymetrix, Santa Clara, CA), as previously described.[39]

RESULTS

Approximately 7% of samples submitted for array CGH in our laboratory were found to have clinically relevant chromosome abnormalities.[36,37] Of these abnormal results, seven cases were identified with microdeletions of 1q41q42 that included the *DISP1* locus (Fig. 1; Table 1). In all cases, FISH was used to confirm the presence of a heterozygous deletion of the region. Parental analyses in all families demonstrated two copies of the *DISP1* locus; thus, these deletions of 1q41q42 are de novo in all tested cases. All seven deletions were identified in our laboratory, although one case (Subject 4) was previously published as a surviving Fryns syndrome patient with a normal karyotype.[40] The clinical features of another case (Subject 7) were published after deletion identification.[41]

The 1q41q42 deletions were analyzed further using the Affymetrix 250K SNP array platform to (1) determine the full extent of the deletions at a higher resolution and (2) define the smallest region of overlap (SRO) to identify candidate genes. Table 1 and Figure 2 show the results of this higher resolution analysis. Subject 6 has the largest deletion, which is ~9.07 Mb in size. The deletion in Subject 3 is the smallest at 2.72 Mb. Subject 7 was not tested by high-resolution analysis.

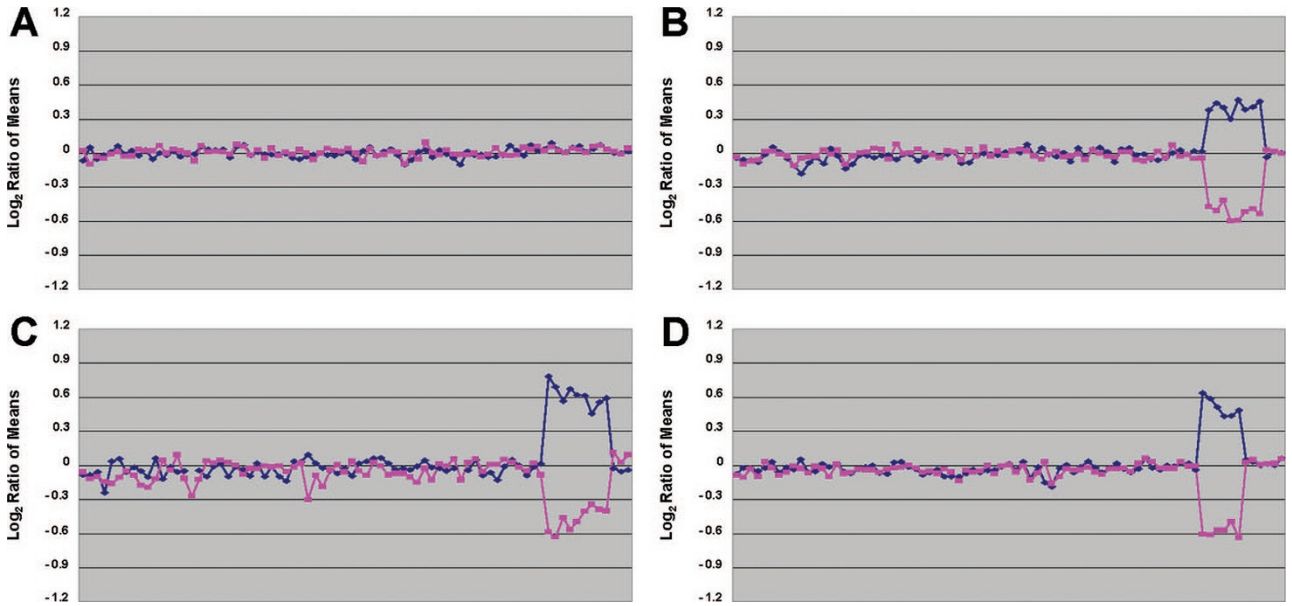

Fig. 1. Results for chromosome 1 using a targeted microarray. Each clone on the plot is arranged along the x-axis according to its location on the chromosome with the most distal 1p telomeric clones on the left and the most distal/telomeric 1q clones on the right. The dark blue line represents the control:subject fluorescence intensity ratios for each clone, whereas the pink line represents the fluorescence intensity ratios obtained from a second hybridization in which the dyes have been reversed (subject:control). (A) Normal plot for chromosome 1. Note that all data points are at a $\log_2$ ratio of zero. (B) Plot for Subject 2 showing a deletion from BAC RP11-1031M6 through BAC RP11-61M2. (C) Plot for Subject 3 showing a deletion from BAC RP11-1148E24 through BAC RP11-61M2. (D) Plot for Subject 1 showing a deletion from BAC RP11-208F18 through RP11-61M2. For each of the plots B, C, and D, the deletion is identified as a mirror image deviation from a $\log_2$ ratio of zero to a $\log_2$ ratio of 0.3 to −0.3.

Table 1
Results for subjects with deletions of 1q41q42

Subject	Deletion identified by SignatureChip analysis	Deletion defined by Affymetrix 250K chip[a]
1	RP11-208F18–RP11-61M2	chr1:218446383-222066752 (3.6 Mb)
2	RP11-1031M6–RP11-61M2	chr1:215990775-221174779 (5.2 Mb)
3	RP11-1148E24–RP11-61M2	chr1:219486921-222066752 (2.6 Mb)
4	RP11-1031M6–RP11-61M2	chr1:214186825-222703737 (8.5 Mb)
5	RP11-1031M6–RP11-61M2	chr1:215521497-220657758 (5.1 Mb)
6	RP11-1031M6–RP11-61M2	chr1:212019895-221091768 (9.07 Mb)
7	RP11-1031M6–RP11-61M2	~5 Mb[b]
Ref. 43	NT	chr1:217677120-227054747 (10 Mb)[c]

NT, not tested.
[a]Based on NCBI build 35, hg17, May 2004.
[b]Based on the publication by Kantarci et al.[41]
[c]Based on the publication by Slavotinek et al.[43]

The SRO between the seven deletions is ~1.17 Mb (Chr1: 219486921–220657758) (Fig. 3). There are five genes within the SRO (Table 2), based on the UCSC known genes track (http://genome.ucsc.edu; NCBI build35, May 2004), and four of these five genes have known functions.

The common clinical features found in our seven subjects are shown in Table 3. Figure 4 shows a composite of five subjects identified to have deletions of 1q41q42. Although none of the subjects has frank HPE, they show some common clinical features including significant developmental delay and distinct facial dysmorphism (frontal bossing, deep-set eyes, broad nasal tip, depressed nasal bridge, anteverted nares). Some subjects showed coarse facies in infancy, microcephaly, cleft palate, clubfeet, seizures, and short stature. Two subjects (Subjects 4 and 7) had diaphragmatic hernia and lung hypoplasia and the clinical diagnosis of Fryns syndrome.[40,41]

DISCUSSION

Traditional cytogenetic approaches to syndrome detection rely on the acumen—and, with many rare novel syndromes, luck—of the physician and cytogeneticist. Because of the relative rarity of some of these novel syndromes, it is difficult for one physician to ascertain sufficient patients with similar phenotypes to draw meaningful conclusions about a pattern of concurrent clinical features. This assumes that the clinical features are distinctive enough to establish a phenotypic relationship among patients. Thus, the physician or cytogeneticist is limited by human subjectivity in discerning subtle gradations of clinical presentations. The phenotypic overlap between distinct syndromes may also confound diagnoses; many cases of monosomy 1p36, for example, were initially diagnosed as Prader-Willi syndrome because of shared manifestations.[43] Furthermore, basing the clinical picture on a single index case may also mask unappreciated or unknown variability in the phenotype.

High-resolution molecular cytogenetic technologies such as array CGH have altered the means by which new microdele-

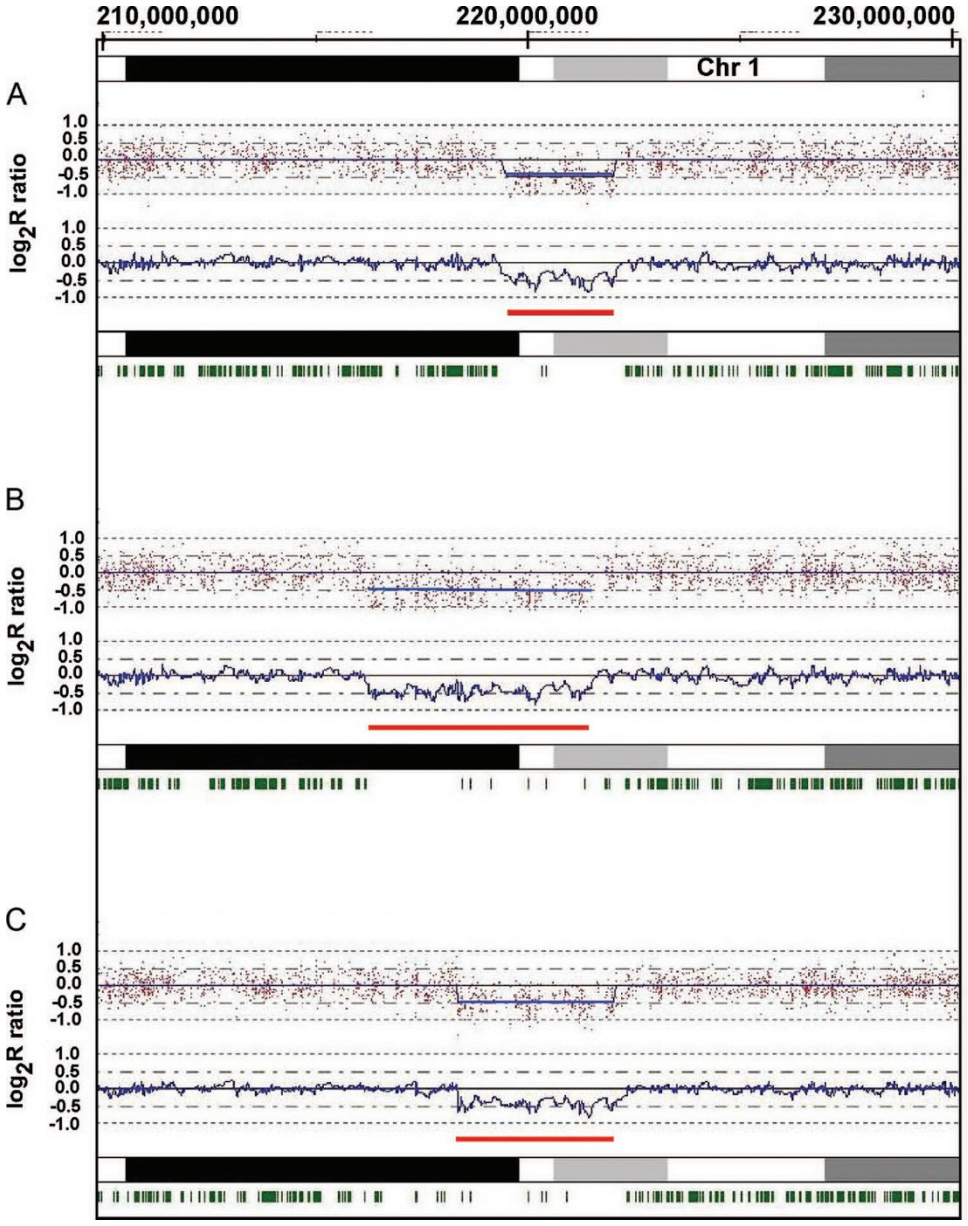

Fig. 2. Results for chromosome 1 using a high-density oligonucleotide microarray. Copy number data for a selected region of chromosome 1 (210,000,000–230,000,000, NCBI build 35) is shown for Subject 1(A), Subject 2 (B), and Subject 3 (C). The plots show the signal intensity ratio ($\log_2$ ratio) of each probe on the Affymetrix 250K Sty chips resulting from analysis with CNAG software.[42] Dots represent raw $\log_2$ ratio values for each SNP. Lines represent copy number inferences based on local mean analysis for five consecutive single nucleotide polymorphisms (SNPs). Heterozygous SNP calls are shown as bars below the chromosome. For probes that are a normal copy number, the signal intensity ratio of the subject versus controls is expected to be 1 and $\log_2$ ratio should be around 0.0 ($\log_2 1 = 0$). The deletions detected in Subjects 1 to 3 based on $\log_2$ ratio are underlined. Loss of copy number due to deletion in the subjects results in a negative $\log_2$ ratio (mean $\log_2$ ratio ~ -0.5).

tion syndromes are discovered by introducing an objective means of collecting a cohort of patients. Thus, a common phenotype among a group of patients may be delineated only after a common genotype has been isolated. The subjects in our study show common clinical features including significant developmental delay and distinct facial dysmorphism (frontal bossing, deep-set eyes, broad nasal tip, depressed nasal bridge, anteverted nares) (Fig. 4). Some subjects showed coarse facies in infancy, microcephaly, cleft palate, clubfeet, seizures, and short stature. Two subjects (Subjects 4 and 7) had diaphragmatic hernia and lung hypoplasia and the clinical diagnosis of Fryns syndrome.[40,41]

Deletions of 1q41q42 have been infrequently reported in the literature[41,44–46]; these cases showed a similar constellation of clinical features as the cases reported here, such as congenital diaphragmatic hernia[41,44] and possible Fryns syndrome.[41] The patient reported by Slavotinek et al.[44] had a 10-Mb deletion of 1q41q42 that includes our SRO and the *DISP1* gene (Table 1). This patient had diaphragmatic hernia, pulmonary hypoplasia, heart defects, bilateral cleft lip and palate, facial dysmorphism,

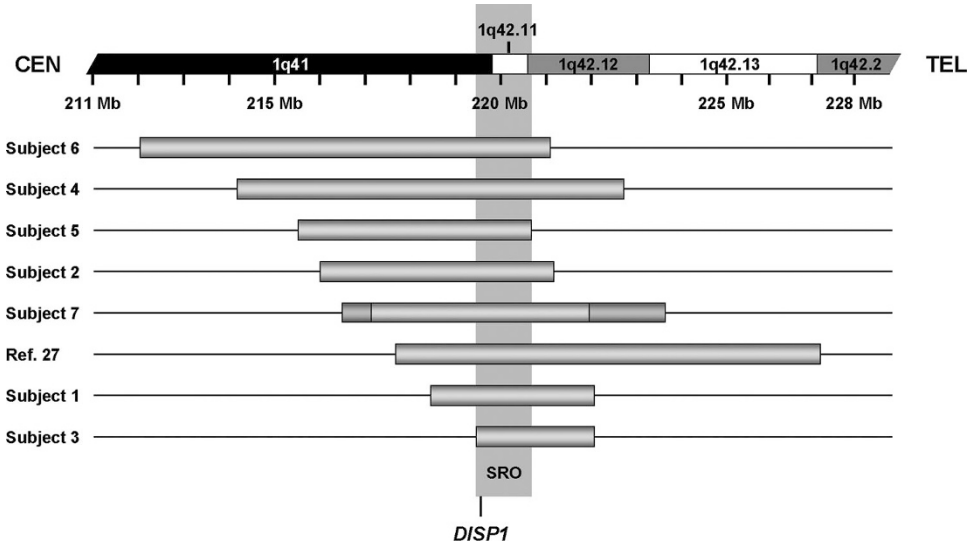

Fig. 3. Breakpoint locations for subjects with deletions of 1q41q42. The chromosome bands of the 1q42 region are shown with the distance from the 1p telomere in Mb along the top (based on the UCSC May 2004 draft of the human genome). Yellow bars indicate the deleted regions for each patient based on the array CGH data of Table 1. Gray bars for Subject 7 indicate the regions containing the breakpoints based on the data in the publication by Kantarci et al.[41] The region shaded in gray indicates the smallest region of overlap (SRO) defined by the breakpoints in these subjects and contains the *DISP1* locus.

Table 2
Candidate genes identified in the smallest region of overlap on 1q41q42

Gene symbol	Description	Function
DISP1	Dispatched A	Required in the SHH signaling pathway
SUSD4	Sushi domain containing 4	Unknown
CAPN2	Calpain 2, large subunit	Calcium-regulated protease
TP53BP2	Tumor protein p53 binding protein, 2	Regulation of apoptosis and cell growth
FBXO28	F-box protein 28	Ubiquitination and degradation

SHH, sonic hedgehog.

and clubfeet.[44] Given the midline defects found in Fryns syndrome (diaphragmatic hernia, orofacial clefting), limb malformations (nail and finger hypoplasia), central nervous system anomalies (seizures, agenesis of the corpus callosum), and dysmorphic features (coarse facies, anteverted nares, hypertelorism)[47] and the findings of 1q41q42 abnormalities in some Fryns patients (Patient 4 in this study[40]; Subject 7 in this study[27,41]), we propose that deletion of 1q41q42 has a variable presentation, with the extreme end of the spectrum demonstrating a Fryns syndrome phenotype.

The common features found in these cases support our hypothesis that deletion of 1q41q42 is a new microdeletion syndrome. However, in extrapolating a common set of features from a small cohort of patients, one must be wary of confirmation bias, by which characteristics that confirm a hypothetical phenotype are selected over outliers. The recent report of a novel genomic disorder on 2p15 illustrates the inherent difficulty in delineating a phenotype in a small sample of cases.[48] To illustrate, Subject 4 in our cohort has a Fryns syndrome phenotype but also one of the largest deletions (~8.5 Mb) among our subjects (Fig. 3). The clinical variability that we observed may be attributed to either variable penetrance due to haploinsufficiency of a single gene in the SRO, modifiers within the larger deletion or elsewhere in the genome, or haploinsufficiency of genes outside the SRO, shared by these Fryns syndrome subjects. Repeated occurrences of the same alteration will be necessary to clarify the phenotype.

Although our study illustrates the difficulty in establishing a common phenotype in a small cohort of patients after the identification of a common genotype, it also highlights the inherent limitations of traditional phenotype-first cytogenetic disease identification. Whereas previous investigations of HPE candidate loci focused on patients with varying degrees of HPE and apparently normal karyotypes,[49–51] we tested individuals with a variety of phenotypes who did not necessarily present with HPE. The role that these genes play in the SHH signaling pathway, however, suggested that they have critical functions in development and are logical targets for investigation. Surprisingly, although many of our patients had other midline defects (cleft palate, diaphragmatic hernia), seizures, distinct dysmorphic features, and mental retardation or developmental delay, none had frank HPE, as might be expected from deletion of genes involved in the SHH pathway. However, because none of these patients has HPE, subjects such as these would not have been enrolled in any of the previous HPE screening studies,[49–51] *preventing* their identification using traditional means.

Although we have five candidate genes in the SRO (Table 2), we propose that haploinsufficiency of *DISP1* is a reasonable cause for some of the developmental defects found in this deletion syndrome. *DISP1* is essential in the SHH signaling pathway[52,53] and belongs to a family of 12-pass transmembrane proteins that all have a sterol-sensing domain consisting of about 180 amino acids that form five membrane-spanning

Table 3
Clinical features found in subjects with deletion of 1q41q42

	Subjects						
	1	2	3	4[a]	5	6	7[b]
Gender	F	M	M	M	M	M	F
Age at diagnosis	17 y	20 mo	15 y	16 y	5 y, 7 mo	5 y, 10 mo	Birth
Facial features							
Coarse facies	+	+		+	−	+	+
Head	Microcephaly	Microcephaly	Dolichocephaly			Microcephaly, trigonocephaly	
Hair					Recessed hairline with sparse eyebrows	Mildly rough texture	
Ears					Low set	Normal	Low set
Frontal bossing		+	+	+	+	+	
Prominent brow	+		+		−	−	
Depressed nasal bridge	+	+	+	+	−	+	+
Broad nasal tip	+		+		−	+	+
Anteverted nares	+			+	−	+	+
Narrow bitemporal diameter	+		+		−	+	
Hypertelorism		+			−	+	+
Palpebral fissures		Up-slanting		Down-slanting	Normal	Inner canthal folds present	
Deep-set eyes	+		+		−	+	
Prominent mandible/maxilla	+		+				
Mouth		Thin upper lip	Short, upturned, protruding upper lip		Full lips, prominent philtrum	Full, thick lips, wide-spaced teeth	Short philtrum, tented upper lip
Palate	High and narrow				Normal	Mildly high	
Cleft palate	+			+	−	−	+
Trunk	Mild pectus excavatum		Accessory nipple, mild scoliosis	Diaphragmatic hernia, lung hypoplasia	Normal	Accessory nipple	Diaphragmatic hernia, lung hypoplasia, VSD
Extremities		Hip dysplasia		Contractures		Mild limb shortening, hypermobile knees	Mild upper arm shortening
Fingers	Slender, mild webbing between 3rd and 4th fingers			Short, triangular, distal phalanges		Mildly short	
Nails						Hypoplastic 5th toenails	Hypoplasia

(*Continued*)

Table 3
Continued

	Subjects						
	1	2	3	4[a]	5	6	7[b]
Feet	Wide space between 1st and 2nd toes, high arches	Bilateral club feet		Bilateral club feet		Pes planovalgus, wide space between 1st and 2nd toes	Bilateral clubfeet
Neurologic	Seizures, normal brain MRI	Seizures, normal EEG, normal brain MRI	Seizures, enlarged ventricles on MRI	Seizures, abnormal EEG, small cerebellum by CT scan, gyral malformations by MRI	Seizures, abnormal EEG, normal MRI	Seizures, abnormal EEG, normal MRI, enlarged ventricles prenatally	
Measurements at last evaluation							
Height	3rd %ile	<5th %ile	25th–50th %ile		4th %ile	<3rd %ile	
Weight	3rd %ile	<5th %ile	10th %ile		14th %ile	<3rd %ile	
FOC	2nd %ile	<2nd %ile	50th %ile		20th %ile	<5th %ile	

VSD, ventricular septal defect; MRI, magnetic resonance imaging; EEG, electroencephalography; CT, computed tomography; FOC, frontal occipital circumference; %ile, percentile; +, feature present; −, feature not present; blank, subject was not examined for that feature.
[a]Based on the publication by Van Hove et al.[40]
[b]Based on the publication by Kantarci et al.[41]

Fig. 4. Five subjects identified to have deletion of 1q41q42. (A) Subject 1. (B) Subject 3 at age 6. (C) Subject 3 at age 16. (D) Subject 4. (E) Subject 5. (F) Subject 6.

domains.[54] This sterol-sensing domain is shared with other family members including Ptch1 and several proteins that regulate cholesterol biosynthesis or trafficking.[55–57] A mouse knockout of *Disp1* shows forebrain defects consistent with HPE.[58] The function of DISP1 is conserved from *Drosophila* to mouse, making it likely to be important for normal development of the brain and limbs.[57] Given the evidence that DISP1 is involved in the SHH signaling pathway, haploinsufficiency through mutations or deletions may be expected to cause HPE or other malformations such as midline defects or limb abnormalities. Cognitive deficits, developmental delay, or mental retardation would also be expected.

We have used a functional approach, using knowledge of important developmental pathways, such as the SHH signaling pathway, to target developmentally important genes in children with developmental delay, mental retardation, seizure, and dysmorphism. Our analysis of more than 10,000 clinical cases yielded seven patients with deletion of 1q41q42, a region containing the *DISP1* gene. The variable phenotypes suggest that deletion of the SRO is sufficient for the developmental delay, mental retardation, seizures, and dysmorphic features. Larger deletions result in cleft palate, clubfeet, and, in two cases, diaphragmatic hernia. Identification and analysis of additional cases of deletion 1q41q42 or mutations in *DISP1* should prove useful for delineating the genes responsible for the various features of this syndrome and will help define whether this is a contiguous gene syndrome or a single gene disorder with variability in phenotypic presentation.

SUMMARY

History shows that banded chromosomes have been important in the identification of the chromosomal basis of some syndromes (e.g., Prader-Willi syndrome,[59,60] Williams-Beuren syndrome [9,61] DiGeorge syndrome[16,62]) and in the identification of new syndromes (e.g., Potocki-Shaffer syndrome,[19] monosomy 1p36[63]). However, it took many decades to dis-

cover the etiology of most well-known microdeletion syndromes. Since the advent of array CGH as a routine research and diagnostic tool in the past several years, numerous new syndromes have been identified,[28–31,33,34,48,64] and the molecular basis for some known syndromes has been uncovered.[65–68] The discovery of novel deletions in multiple individuals allows for delineation of critical regions in which to search for genes causing the features of the syndrome. In some cases, true contiguous gene syndromes are identified (e.g., Potocki-Shaffer syndrome,[20,21] LGS[69]), whereas in others, a single gene results in the complex phenotype (e.g., Alagille syndrome[70]). We anticipate that large-scale molecular testing methods such as array CGH will continue to play an important role in identifying new microdeletion syndromes and in delineating the critical regions in which to search for phenotype-contributing genes.

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