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Microdeletion 9q22.3 Syndrome Includes Metopic Craniosynostosis, Hydrocephalus, Macrosomia, and Developmental Delay

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Basal cell nevus syndrome (BCNS), also known as Gorlin syndrome (OMIM #109400) is a well-described rare autosomal dominant condition due to haploinsufficiency of *PTCH1*. With the availability of comparative genomic hybridization arrays, increasing numbers of individuals with microdeletions involving this locus are being identified. We present 10 previously unreported individuals with 9q22.3 deletions that include *PTCH1*. While 7 of the 10 patients (7 females, 3 males) did not meet strict clinical criteria for BCNS at the time of molecular diagnosis, almost all of the patients were too young to exhibit many of the diagnostic features. A number of the patients exhibited metopic craniosynostosis, severe obstructive hydrocephalus, and macrosomia, which are not typically observed in BCNS. All individuals older than a few months of age also had developmental delays and/or intellectual disability. Only facial features typical of BCNS, except in those with prominent midforeheads secondary to metopic craniosynostosis, were shared among the 10 patients. The deletions in these individuals ranged from 352 kb to 20.5 Mb in size, the largest spanning 9q21.33 through 9q31.2. There was significant overlap of the deleted segments among most of the patients. The smallest common regions shared among the deletions were identified in order to localize putative candidate genes that are potentially responsible for each of the non-BCNS features. These were a 929 kb region for metopic craniosynostosis, a 1.08 Mb region for obstructive hydrocephalus, and a 1.84 Mb region for macrosomia. Additional studies are needed to further characterize the candidate genes within these regions. © 2011 Wiley Periodicals, Inc.

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INTRODUCTION

Basal cell nevus syndrome (BCNS) is a well described cancer predisposition and multiple malformation syndrome, manifested by a constellation of findings that include lamellar calcification of the falx cerebri at less than 20 years of age, palmar and/or plantar pits, jaw keratocysts, basal cell carcinomas at less than 30 years of age (or more than four in a lifetime), cleft lip and/or cleft palate, vertebral and rib anomalies, pre- or postaxial polydactyly, ocular anomalies, occipitofrontal circumference of greater than 97th centile, and an increased risk of childhood medulloblastomas (primitive neuroectoderm tumor, PNET), lymphomesenteric or pleural cysts, and ovarian or cardiac fibromas [Kimonis et al., 2004]. Various dysmorphic facial features have been described, including a broad forehead with bossing, hypertelorism, epicanthal folds, midfacial hypoplasia, short, high-set upturned nose, a long and tented philtrum, and coarsened facies. This condition is known to result from autosomal dominant loss of function mutations in the *PTCH1* gene and from haploinsufficiency of the human homolog to the Drosophila *patched* protein [Hahn et al., 1996; Wicking et al., 1997]. While the average age of clinical diagnosis of BCNS is 25 years of age, with the increasing use of array-based comparative genomic hybridization technology (CGH), individuals with contiguous gene deletions that include *PTCH1* are being identified at earlier ages, often before manifestation of the characteristic age-dependent BCNS features [Slater et al., 2000]. To date, 27 patients have been reported with 9q22 deletions that either met clinical criteria for diagnosis of BCNS, or include *PTCH1* within the deleted interval, excluding intragenic deletions [Sekhon et al., 1982; Ying et al., 1982; Farrell et al., 1991; Robb et al., 1991; Evans et al., 1993; Kroes et al., 1994; Shimkets et al., 1996; Paoloni-Giacobino et al., 2000; L'Hermine et al., 2002; Olivieri et al., 2003; Haniffa et al., 2004; Midro et al., 2004; Boonen et al., 2005; Cajaiba et al., 2006; Chen et al., 2006; Redon et al., 2006; Fujii et al., 2007; Nowakowska et al., 2007; de Ravel et al., 2009; Musani et al., 2009; Shimojima et al., 2009; Yamamoto et al., 2009].

Recently, several authors have reported an emerging phenotype separate from that of BCNS resulting from microdeletions of 9q22.3 that include *PTCH1* [Redon et al., 2006; Shimojima et al., 2009; Yamamoto et al., 2009]. Macrosomia in these individuals has been hypothesized to result from de novo deletions of the paternal allele specifically, possibly due to the loss of one or more as-of-yet unidentified imprinted genes, or possibly *PTCH1* itself [Redon et al., 2006; Yamamoto et al., 2009].

Here we report on 10 new patients with 9q22.3 deletions that include *PTCH1*, of varying ethnic backgrounds and age at diagnosis, whose deletions were analyzed by array CGH after referral for evaluation by a medical geneticist. We characterize new phenotypic features not consistent with BCNS (metopic craniosyntosis, obstructive hydrocephalus, macrosomia, and developmental delay), and map the common areas of overlap among these deletions.

MATERIALS AND METHODS

Study Design

Patient 7 was ascertained by the lead and corresponding authors at an outpatient referral for consultation by a medical geneticist. The other authors were then contacted directly for solicitation of similar patients for inclusion in a retrospective case review series study, after approval of the study and consent forms by the Stanford Institutional Review Board (IRB). The other nine affected individuals had been previously evaluated by one or more of the other authors after referral from a medical geneticist evaluation. Clinical and molecular diagnosis of BCNS was confirmed by detection of an interstitial 9q22.3 deletion that included *PTCH1* by array-based CGH, including BAC array, 44K oligonucleotide array, 105K oligonucleotide array, and/or SNP array; some individuals had more than one of these analyses. The only study inclusion criterion was a confirmed molecular diagnosis by one of the described methods in a previously unreported individual.

Medical records regarding the affected individual's phenotype, including clinic notes by the authors, cytogenetic study reports, and facial photographs if explicitly authorized, were sent by each physician for compilation and review by the lead author. In compiling the phenotypic features for each patient, macrosomia was defined as both height or length and weight of greater than 95th centile.

Analysis

Each individual's reported deletion breakpoints were put into the UCSC Genome Browser (http://genome.ucsc.edu/) NCBI36/hg18 March 2006 build for analysis, in accordance to the human genome assembly used in the cytogenetic report for each patient [Kent et al., 2002]. The isolated regions were then converted to the current GRCh37/hg19 February 2009 human genome assembly to obtain the revised breakpoints, using tools available at the Genome Browser site. The revised, current build breakpoints were then input into the NCBI MapViewer website for gene annotation using human genome build 37.2 (http://www.ncbi.nlm.nih.gov/mapview/). Basepairs described in this report are for the NCBI36/hg18 March 2006 build.

RESULTS

The 10 affected individuals included 7 females and 3 males, who ranged in age at time of initial geneticist evaluation from newborn to 7 years. Their ages at the time of confirmed array-based molecular diagnosis spanned from less than 1 month to 13 years. Gestational ages at birth ranged from 37 to 41 weeks. Each of these individuals was the only affected member in his or her family, and the deletions were confirmed as de novo in the nine for whom parental samples were obtained. The ethnic background was European Caucasian (8), one of whom was Mennonite, Mexican (1), and Chinese (1). These findings are summarized in Table I.

Using the previously proposed diagnostic criteria of two major features, or one major plus two minor features, only three individuals (Patients 4, 5, and 6) met a clinical diagnosis of BCNS [Evans et al., 1993; Slater et al., 2000]. Each person exhibited some of the characteristic features of BCNS, which included absolute macrocephaly (6/10), rib and/or vertebral anomalies (5/8), ocular malformations (3/10), numerous basal cell nevi (3/10), mandibular odontogenic keratocysts (3/10), Sprengel deformity (3/10), cardiac fibromas (2/8), polydactyly (2/10), palmoplantar pits (2/10), and

TABLE I. Summary of Patient Data

	Patient									
Features	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Gender	M	F	F	M	F	F	M	F	F	F
Presenting age	Birth	10 Days	2 Weeks	9 y	Birth	9 mo	7 mo	Birth	5 y	7 y
Race/Ethnicity	Caucasian	Caucasian	Caucasian	Mennonite	Caucasian	Chinese	Mexican	Caucasian	Caucasian	Caucasian
Diagnosis age	2 weeks	1 y	1 mo	9 y	13 y	2 y	2 y	2 mo	11 y	7 y
Last exam age	4 mo	5 y	9.5 mo	22 y	16 y	3 y	3 y 7 mo	13 mo	N/A	N/A
Deletion (Mb)	20.5	10.85	9.85	8.28	8.07	4.5	2.03	1.84	1.08	0.352
Metopic fusion	+	−	−	−	+	−	+	+	−	−
Hydrocephalus	−	+	+	+	+	−	−	−	+	−
Macrosomia	−	−	−	−	−	+	+/−	+	−	−
Delay	?	Global	Motor[a]	Global	Global	Global	Global	Motor	Motor	Motor
Intellect impaired	?	+	?	+	+	?	?	?	−	−
Molecular array	BAC	BAC, 105K	105K	SNP	105K	BAC, 105K	BAC, 105K	105K	105K, MLPA	44K
BCNS FEATURES										
Craniofacial										
Bossing	−	+	+	+	−	+	+	−	−	+
Coarse face	+	+	+	−	+	−	−	−	−	−
Synophrys	+	−	−	−	−	−	−	−	−	−
Epicanthal folds	+	+	−	−	+	+[b]	+	+	−	−
Palpebral slanting	−	Down	−	−	Up	Down	−	−	Down	Up
Cleft	Palate	−	−	Uvula	−	−	−	−	−	−
Short nose	+	+	−	+	+	+	−	+	−	−
Philtrum:										
Long	+	+	−	+	+	−	−	+	−	−
Tented	+	+	−	−	+	−	−	+	−	−
Ears: low, angled	+	−	+	+	+	−	−	+	+	+
Abnormal dentition	?	−	−	−	−	−	−	+	+	−
Jaw keratocysts	−	−	−	+	+	−	−	−	+	−
Macrocephaly	−	+	−	+	+	+	+	+	−	+
Ocular anomalies	−	−	Nerve	−	−	−	−	Micro	Retinal	−
Skeletal										
Rib anomaly	+	?	−	+	−	+	+	?	+	−
Vertebral anomaly	+	?	−	−	−	+	−	?	+	−
Sprengel deformity	−	−	−	+	−	+	−	−	+	−
Pectus deformity	−	−	+	+	−	−	−	−	−	−
(Kypho)scoliosis	+	+	−	−	−	+	−	−	+	−
Polydactyly	−	−	−	−	−	+	−	+	−	−
Syndactyly	−	−	−	−	−	−	−	+	−	−
Oligodactyly	−	−	−	+	+[c]	−	−	−	−	−
Cardiac tumor	RV	−	−	−	RA	−	−	−	?	?
Dermatological										
Basal cell Nevi	−	−	−	+	−	+	+	−	−	−
Palmoplantar Pits	−	−	−	−	−	+	−	−	−	+
Neurological										
Hypotonia	+	+	+	−	+	+	−	−	−	−

+, present; −, absent; ?, unknown or not assessed; 44K, 44K-oligonucleotide array; 105K, 105K-oligonucleotide array; BAC, bacterial artificial chromosome array; micro, microphthalmia and optic nerve hypoplasia; MLPA, multiplex ligation-dependent probe amplification; N/A, not applicable; nerve, optic nerve anomaly; retinal, abnormal retinal myelination; RA, right atrial; RV, right ventricular; SNP, single nucleotide polymorphism array.

[a]Not assessed for speech or other delays.
[b]Epicanthal folds were present, but were also consistent with patient's ethnicity.
[c]Bilateral thumb hypoplasia was present.

palatal clefting (2/10), among other features. Features not characteristic of BCNS were metopic craniosynostosis, hydrocephalus, macrosomia, and developmental delay with or without intellectual disability. Trigonocephaly, resulting from metopic craniosynostosis, was present in four individuals (Patients 1, 5, 7, and 8), each of whom underwent corrective surgery. Obstructive hydrocephalus was apparent in five individuals (Patients 2–5, 9), each of whom required ventriculo-peritoneal shunting. One child, Patient 6, had

mild ventricular enlargement that did not require shunting. Macrosomia was present in two individuals (6, 8), each of whom also had macrocephaly. One child (Patient 7) had a height between the 90th and 95th centiles, and a weight at the 98th centile. As this did not meet our strict definition of macrosomia, his deletion was excluded from the overlap region analysis for macrosomia. In all eight individuals who were older than a few months of age and who could be fully assessed, developmental delays were apparent. Only motor delays were seen in the children with the smallest deletions, which in some cases resolved over time. Global motor, speech/language, and social/behavioral impairments were observed in those with the larger deletions. Intellectual disability was apparent in the three globally delayed patients who were followed to school age and older. Hypotonia was present in half of the individuals (Table I).

Various dysmorphic facial features were present in a number of patients, and included a broad forehead with bossing, vertical forehead creases, epicanthal folds, angulated palpebral fissures, micrognathia, and low-set and posteriorly rotated ears. Noted among many of the patients were a short nose with the appearance of being "high set," and a long and tented philtrum (Table I; Fig. 1). The facial features also coarsened over time in two individuals (Patients 2, 8; Fig. 2).

Features present in some members of our population were synophrys, hypertelorism, partial corpus callosum dysgenesis, Chiari I malformation, seizures, joint hypermobility, camptodactyly, ataxia, short stature, broad/webbed neck, and inguinal hernias (data not shown). One child (6) had unilateral renal cysts, mild ipsilateral ureterectasis that resolved spontaneously, and contralateral mild renal pelviectasis. None of the other nine individuals had any documented renal anomalies.

All of our reported individuals except Patient 9 had G-banded chromosomal analysis. The deletions of Patients 1, 3, and 4 were visible on high-resolution analysis. The others had lower-resolution chromosomal analysis, and their deletions were not visible microscopically (data not shown). Overall, the deletion sizes ranged from 352 kb, containing only *PTCH1* and part of *FANCC*, to 20.5 Mb, spanning 273 genes, as illustrated (Fig. 3). Individual 3 also had a second 44 Kb deletion located at 9q21.3, which contained no genes (data not shown). The smallest region of deletion overlap for the five affected individuals with metopic craniosynostosis was 929 kb, between bases 96,771,450 and 97,700,488, and which contained

FIG. 1. Five individuals with interstitial 9q22.3 deletions. Please note the short nose and long philtrum that is best observed in patients 1, 3, and 8, but is also present in patient 4, and the lowset and posteriorly rotated ears in all of the patients. Tenting of the philtrum is also present in patients 1 and 8.

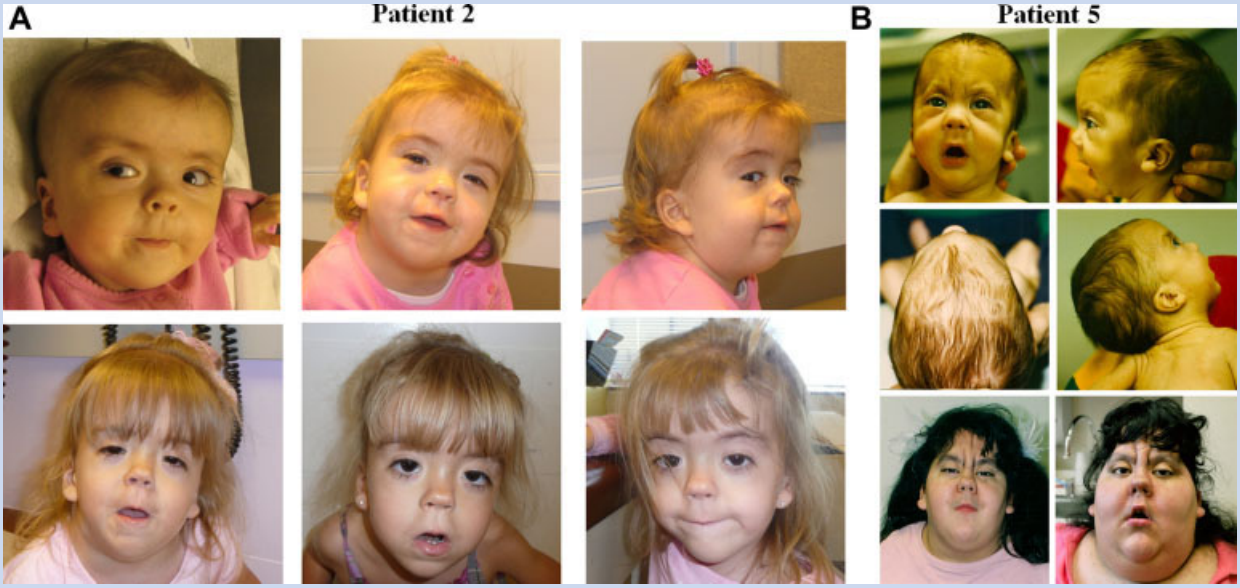

FIG. 2. Age progression in individuals with interstitial 9q22.3 deletions. A. Patient 2 at age 6 months and 19 months (top row, left-to-right), and at 34 months, 4 years, and 5 years (bottom row, left-to-right). Age progression in individuals with interstitial 9q22.3 deletions. B. Patient 5 at age 5 months (top and middle rows), 11 years (bottom left), and 16 years (bottom right). Again note the short nose and long, tented philtrum in both patients.

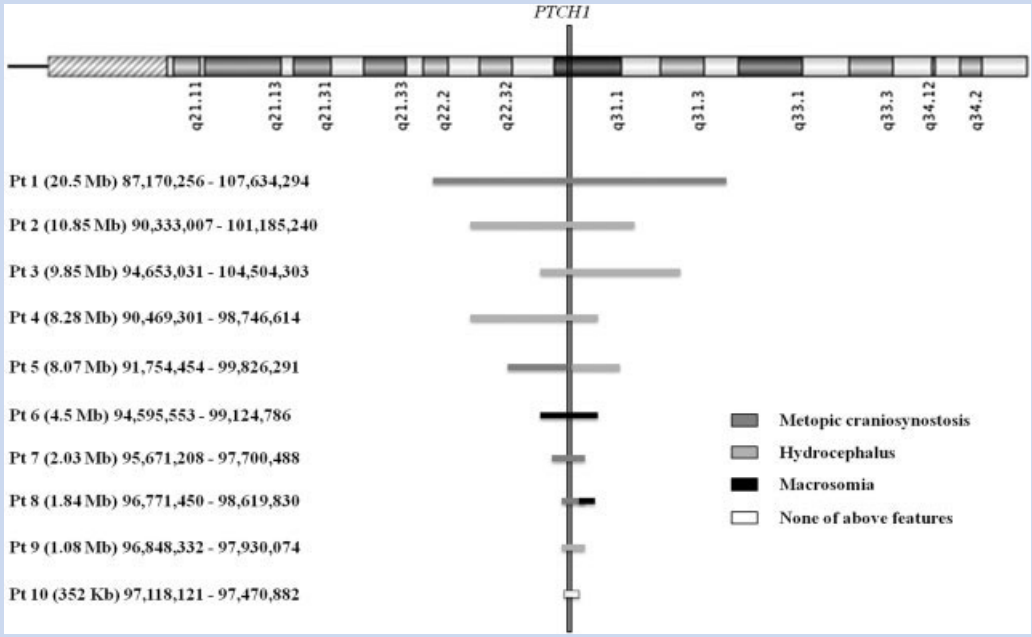

FIG. 3. Schematic representation of the deletions in affected individuals. The deletions for each affected individual, listed on the left, are represented to scale by horizontal bars of various colors, in comparison to a chromosome 9q ideogram along the top. Bars composed of two colors indicate the presence of both features. The relative position and size of PTCH1 is represented by a vertical bar. Listed deletion breakpoints are according to the NCBI 36/hg18 build.

15 annotated genes other than *PTCH1*. There was a common area of deletion overlap of 1.08 Mb among patients with obstructive hydrocephalus that contained 17 genes other than *PTCH1*. This region localized to bases 96,848,332 through 97,930,074. The smallest region of deletion overlap for the two patients with macrosomia was 1.8 Mb, located between bases 96,771,450 and 98,619,830. This region contained 30 genes other than *PTCH1*.

DISCUSSION

We have expanded the phenotype of individuals with interstitial 9q22 deletions that contain *PTCH1* to include metopic craniosynostosis, obstructive hydrocephalus, and macrosomia, which are separate from the typical features of BCNS. This would suggest that there are genes other than *PTCH1* in the deleted region that when deleted contribute to these conditions.

The common area of overlap among the patients with metopic craniosynostosis, resulting in trigonocephaly, was between basepairs 96,771,450 and 97,700,488. There are 15 genes other than *PTCH1* within this region. These are *FANCC*, encoding the Fanconi anemia complementation group C protein, open reading frames that encode a zinc aminopeptidase (*c9orf3*), a putative repair/recombination helicase (*c9orf102*), and a nasopharyngeal carcinoma-associated protein (*c9orf130*), four pseudogenes, two hypothetical RNA-encoding genes (LOC100507319, LOC100507346), one gene that is expressed in human heart and brain and encodes a hypothetical protein of unknown function (LOC100506667), and four microRNA genes. While there are no obvious candidate genes among those listed, it is possible that the deleted region may include one gene whose haploinsufficiency is but one of many factors that influence the development of metopic craniosynostosis in affected individuals, perhaps explaining why every individual who shared our mapped minimal region for metopic craniosynostosis did not demonstrate that finding. The presence of metopic craniosynostosis in a substantial percentage of all reported individuals with 9q22.3 microdeletion is interesting. We do suggest that 9q22.3 microdeletion be included in the differential diagnosis for individuals with metopic craniosynostosis in the absence of multiply affected sutures, and as such, CGH studies may be indicated.

In comparison, the commonly deleted region among individuals with obstructive hydrocephalus was located between 96,848,332 and 97,930,074. Within this region were the same three open reading frames, four microRNA genes, *FANCC*, hypothetical protein gene, and one of the two hypothetical non-protein encoding RNA genes which were also present in the deletions of individuals with metopic craniosynostosis. Unique to this region was another pseudogene, a non-protein coding RNA gene, and an additional hypothetical RNA-encoding gene. Again, there are no obvious candidate genes among them.

We note that asymmetric ventricles, dilated ventricles, and dilation due to cerebral atrophy have been reported in the literature on patients with the clinical diagnosis of BCNS [Chen et al., 2006]. We cannot determine whether all of these individuals have interstitial deletions that contain *PTCH1*, as the diagnosis of BCNS was purely clinical before the genetic etiology was identified [Hahn et al., 1996]. Similarly, some of the 27 previously described individuals with known interstitial 9q22 deletions that contain *PTCH1* by mapping or by BCNS diagnosis have had ventricular dilation of varying degrees [Robb et al., 1991; Kroes et al., 1994; Midro et al., 2004; Boonen et al., 2005; Cajaiba et al., 2006; Chen et al., 2006; Redon et al., 2006; Fujii et al., 2007], or like many of our newly reported patients, hydrocephalus requiring ventriculo-peritoneal shunting [Farrell et al., 1991; Shimkets et al., 1996]. Without the higher resolution mapping available with current technology, we cannot refine the critical regions for those patients beyond those large regions achieved by linkage analysis or BAC array-based methods utilized in those studies. We do expect the critical region to overlap with our proposed minimal intervals, however, due to the large size of the reported deletions in the older case reports. In two of the most recently described patients, mild dilation of one or more ventricles was present, and linked to the presence of a space-occupying lesion [Shimojima et al., 2009; Yamamoto et al., 2009]. We do not consider this to be obstructive hydrocephalus of a nature similar to that in our described patients who required shunting, though one of our patients (6) did have mild enlargement of the ventricle and cerebral sulci (Table II). Overall, a higher proportion of our newly reported individuals had more severe forms of ventricular dilation requiring intervention than those reported previously, whereas the earlier reports described a higher frequency of less severe forms. This may be the result of the more severely affected individuals being brought to medical attention at younger ages in response to rapidly increasing head size, and/or seizures, prompting additional evaluations for other abnormal phenotypic features, and thus allowing diagnosis of 9q22.3 microdeletion earlier than would otherwise be expected. Alternatively, milder forms may only be ascertained incidentally as part of a brain imaging evaluation for developmental delay or as part of the neuroimaging for BCNS, may be generally underreported, or may be missed altogether because such imaging is sometimes difficult in developmentally delayed or impaired children who are behaviorally noncompliant during the study. We suggest that brain imaging be performed as part of the initial evaluation for 9q22.3 microdeletion, and surveillance monitoring of head circumference be performed at all clinical visits (Table III).

We described two individuals with macrosomia, as pre-defined by height or length and weight each at the 95th centile or greater. The deletions of these children contained the common region of basepairs 96,771,450 through 98,619,830. In addition to the previous 18 genes (other than *PTCH1*) described for the regions common among affected individuals with metopic craniosynostosis and among those with hydrocephalus, there were 12 genes that were unique to this region. These included three pseudogenes, a hypothetical RNA-encoding gene, and an open reading frame (*c9orf21*) that encodes a protein with peroxiredoxin-like domains. Also among the genes unique to this common deletion were the genes *HSD17B3*, which encodes a 17-hydroxy-steroid dehydrogenase; *SLC35D2*, which encodes an endoplasmic reticulum/Golgi apparatus nucleotide sugar transporter; and, *HABP4*, encoding an intracellular hyaluronan binding protein. Perhaps most interestingly, also located within the region are *CDC14B*, encoding the human homolog of a yeast cell division cycle protein, and *ZNF510* and *ZNF782*, which encode novel zinc finger-containing proteins that have been implicated in association studies with regard to modulation of stature specifically within the Chinese population

TABLE II. Comparison of Patients With Interstitial 9q22.3 Deletions

	Affected individuals					
	This study		Previous studies		Total cumulative	
Non-BCNS features	**Number**	**Percent (%)**	**Number**	**Percent (%)**	**Number**	**Percent (%)**
Metopic craniosynostosis	4/10	40	4/27	14.8	8/37	21.6
Ventricular dilation[a]						
Hydrocephalus	5/10	50	2/27	7.4	7/37	18.9
Mild dilation	1/10	10	8/25[b]	32	9/35	25.7
Macrosomia[c]	2/10	20	6/27	22	8/37	21.6
Developmental delay						
Motor only	3/8[d]	37.5	7/22[e]	31.8	10/30	33.3
Speech only	0/8	0	0/22	0	0/30	0
Global	5/8	62.5	14/23[e]	60.8	19/31	61.3
Intellectual disability	3/5	60	14/23[e]	60.8	17/28	60.7

[a]Hydrocephalus refers to severe and obstructive ventricular dilation that required ventriculo-peritoneal shunting, whereas mild dilation did not, and may have been asymmetric, or of only one ventricle.
[b]Excludes one case caused by cerebral atrophy and two cases caused by space-occupying masses.
[c]Defined as both height (length) and weight >95 percentile.
[d]Excludes patients with motor delay, but who were not old enough to assess speech and for other impairments.
[e]Excludes patients without clear description of delays and/or impairment.

[Lei et al., 2009]. Of note, one of our two reported individuals with macrosomia was Chinese.

Previous case reports have focused on correlating overgrowth in individuals with 9q22.3 deletions that include *PTCH1* with loss of the paternal allele [Redon et al., 2006; Shimojima et al., 2009]. The inference is that loss of one or more imprinted genes within the region, perhaps even *PTCH1*, is a cause of macrosomia. In our review of the 27 previously reported affected individuals, six had macrosomia [Cajaiba et al., 2006; Chen et al., 2006; Redon et al., 2006; Shimojima et al., 2009; Yamamoto et al., 2009]. Notably, this number differs from those reviewed and reported by Shimojima et al., as we included an individual with hemihyperplasia, and we excluded the third patient of Farrell et al. [Farrell et al., 1991; Cajaiba et al., 2006]. This latter child had a birth length and weight of 90th and 97th centiles, respectively, and her length had decreased to 75th centile by 7 months of age. Of the six individuals with macrosomia,

TABLE III. Suggested Evaluations After a Diagnosis of 9q22.3 Microdeletion

Clinical concern	**Evaluation strategy**
Hydrocephalus, ventricular dilation	Brain imaging
	Complete neurological evaluation
	Surveillance monitoring of head circumference
Cerebral tumors[a]	Surveillance monitoring of growth parameters
Macrosomia, hemihyperplasia	Complete craniofacial assessment
Metopic craniosynostosis	Carefully consider cost versus benefit of radiographic imaging
Developmental delay	Microarray/CGH of high resolution to confirm extent of deletion
	Formal developmental assessment
Intellectual impairment	Childhood intervention services referrals
Renal anomalies	Renal ultrasound
Ovarian fibroma[a]	Abdominal/pelvic ultrasound
Basal cell carcinoma[a]	Periodic complete dermatological assessment
	Avoidance of ionizing and UV radiation exposure
Cardiac anomalies, fibroma[a]	Echocardiogram
Ocular anomalies[a]	Ophthalmological evaluation.
Skeletal anomalies[a]	Carefully consider cost versus benefit of radiographic imaging
Odontogenic keratocysts[a]	

CGH, comparative genomic hybridization.
[a]Indicates a feature of BCNS.

five had lost the paternal allele, though Yamamoto et al. did not report the parent of origin for their patient's allele [Farrell et al., 1991; Cajaiba et al., 2006; Chen et al., 2006; Redon et al., 2006; Shimojima et al., 2009; Yamamoto et al., 2009]. Three other individuals with loss of the paternal allele had normal or unreported growth parameters [Shimkets et al., 1996; Olivieri et al., 2003; Fujii et al., 2007]. In contrast, of the four people who were known to have lost the maternal allele, one was reported as having normal growth, one was reported as having small stature, and the other two did not have reported growth descriptions [Ying et al., 1982; Shimkets et al., 1996; Midro et al., 2004; Fujii et al., 2007; Shimojima et al., 2009; Yamamoto et al., 2009]. We did not determine the parental origin for the deleted allele in our reported individuals, as these studies were not originally performed for any of the individuals on whom parental samples were available, and were outside of the scope permitted by our institutional IRB-approved protocol and consent for this retrospective study. In all of the previously reported individuals with macrosomia for whom we could identify the breakpoints from the published report, each shared the smallest commonly deleted region identified in our two children with macrosomia, though again, comparison with the earlier studies before the use of array CGH technology is difficult [Chen et al., 2006; Redon et al., 2006; Shimojima et al., 2009]. Based on all of the reported patients to date, there is no consistent evidence for imprinted genes located around or including *PTCH1*, as there do not seem to be two distinct (opposite) phenotypes, each segregating with loss of a particular parental allele, especially with respect to growth. While we cannot definitively exclude the possibility of candidate genes within the commonly deleted interval, or their downstream effectors, being imprinted at this time, there is no uniparental disomy 9 phenotype described the in the medical literature [Wilkinson et al., 1996; Slater et al., 2000]. We do feel that additional study with regard to parent of origin for the deleted alleles is warranted, and also suggest consideration of SNP or array CGH as part of the evaluation of individuals with macrosomia or generalized overgrowth. There is no evidence at this time to determine whether routine surveillance for abdominal tumors in individuals with 9q22.3 microdeletion and macrosomia or hemihyperplasia is warranted. It is expected that individuals with 9q22.3 microdeletion have the same or similar risk for tumor types that are seen with increased frequency in individuals with BCNS, though there is limited information available, given how few affected individuals have been reported to date.

In all eight of our affected individuals who were of sufficient age and could be adequately assessed, developmental delays and/or intellectual disability was present. The smallest deletion, containing only *PTCH1* and a portion of *FANCC*, was present in individual 10, who exhibited motor milestone delays. It is likely that her delays are related to *PTCH1* haploinsufficiency alone. In our reported individuals with deletions of approximately 2 Mb and larger, global impairment, including impairment of speech and language, and in the older individuals, intellectual disability, were present in addition to motor developmental delay. This phenomenon is not unique to the specific genes within the deleted intervals, as numerous contiguous gene deletion syndromes show more severe impairment with increasing deletion sizes [Slavotinek, 2008; Musani et al., 2009]. Our most severely affected person overall, with an approximate 20.5 Mb deletion, was too young to be fully assessed, but was noted to have hypotonia and failure to thrive, and died in infancy of complications of a respiratory illness. Had he lived, it is likely that he would have had significant developmental and intellectual disabilities in the future.

The majority of our reported individuals were female. While equal numbers of affected males and females are expected, based on the autosomal nature of the chromosome deletion, we cannot explain this finding by any systemic ascertainment bias. Of the 27 previously described people with 9q22 deletions that cause BCNS or include *PTCH1*, there were 14 males, 12 females, and 1 sex unreported, showing no sex-related skewing.

Overall, as summarized in Table II, 40% of our population demonstrated metopic craniosynostosis, compared to approximately 15% of the previously reported 27 individuals with interstitial 9q22 deletions. In our reported affected individuals, half had obstructive hydrocephalus, compared to approximately 7% of those who have been previously reported. In contrast, almost a third of the previously reported individuals presented with milder forms, whereas only one child (10%) in our study did. Similar to the previous reports, 20% of our population demonstrated macrosomia of greater than 95th centile for height (or length) and for weight. Developmental delay and/or irreversible intellectual impairment was present to a similar degree both in our affected individuals, and in those in the previous case reports, though direct comparison is difficult because of limited data and the widely variable ages of all of the patients.

It is unclear as to the mechanism which predisposes the 9q22.3 region to deletion. In silico analysis using the RepMask 3.27 tool available on the UCSC Genome Browser (http://genome.ucsc.edu/) identified numerous SINEs, large LINEs, and LTRs flanking *PTCH1* and adjacent genes in the region, which potentially could result in recombinatory deletion and duplication events. Perhaps reflective of a reciprocal recombination event to the 9q22.3 microdeletion, a mother and child with the shared phenotype of microcephaly and mild developmental delay was each reported to have a 360 kb duplication of the 9q22.3 region that included *PTCH1* and exon 1 of *FANCC* [Derwinska et al., 2009].

In summary, we describe 10 additional individuals with deletions involving 9q22 that include the *PTCH1* gene. The findings in these 10 people broaden the phenotype to include metopic craniosynostosis, hydrocephalus, macrosomia, and developmental delay with intellectual disability. While many of the affected individuals shared similar dysmorphic features that have been previously described in patients with BCNS, no other unifying characteristic facial phenotype was recognized, except with respect to that resulting from metopic craniosynostosis. These findings have implications for clinical care in individuals with 9q22.3 microdeletion (summarized in Table III), as well as for identifying genes that contribute to the expanded phenotype. Further studies are necessary to further refine the smallest areas of overlap in order to identify the genes implicated in these findings.

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