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20p12.3 microdeletion predisposes to Wolff-Parkinson-White syndrome with variable neurocognitive deficits

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Abstract

Background—Wolff-Parkinson-White syndrome (WPW) is a bypass reentrant tachycardia that results from an abnormal connection between the atria and ventricles. Mutations in *PRKAG2* have been described in patients with familial WPW syndrome and hypertrophic cardiomyopathy. Based on the role of bone morphogenetic protein (BMP) signaling in the development of annulus fibrosus in mice, it has been proposed that BMP signaling through the type 1a receptor and other downstream components may play a role in preexcitation.

Methods and Results—Using the array comparative genomic hybridization (CGH), we identified five individuals with non-recurrent deletions of 20p12.3. Four of these individuals had WPW syndrome with variable dysmorphisms and neurocognitive delay. With the exception of one maternally inherited deletion, all occurred *de novo*, and the smallest of these, harbored a single gene,

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Patient consents All images of patients in this manuscript have been submitted with written consents from the patient or his/her legal guardian for publication in print and online.

BMP2. In two individuals with additional features of Alagille syndrome, deletion of both *JAG1* and *BMP2* were identified. Deletion of this region has not been described as a copy-number variant in the Database of Genomic Variants and has not been identified in 13,321 individuals from other cohort examined by array CGH in our laboratory.

Conclusions—Our findings demonstrate a novel genomic disorder characterized by deletion of *BMP2* with variable cognitive deficits and dysmorphic features and show that individuals bearing microdeletions in 20p12.3 often present with WPW syndrome.

Keywords

Wolff-Parkinson-White syndrome; *BMP2*; 20p12.3; TGF-beta signaling; *JAG1*

Key points

- We describe a novel genomic disorder characterized by Wolff-Parkinson-White (WPW) syndrome, dysmorphic features, and variable cognitive deficits associated with microdeletion of the *BMP2* region within 20p12.3.
- Although WPW is frequently observed with the deletion of 20p12.3, there is some evidence of incomplete penetrance of this phenotype, in contrast to the musculoskeletal and neurocognitive characteristics, which appear to be fully penetrant in this disorder.
- A contiguous gene deletion syndrome characterized by deletion of both *BMP2* and *JAG1* can result in preexcitation with features of Alagille syndrome.

Wolff-Parkinson-White syndrome (WPW) is a preexcitation disorder that is associated with specific EKG findings, including a shortened for age PR interval and a slurred upstroke to the QRS complex (i.e. delta wave). While some individuals may remain asymptomatic throughout their lives, others are prone to tachyarrhythmias that may be life threatening. The prevalence of this condition based on the EKG criteria is estimated to be 1-3/1000 individuals.[1] A genetic basis for this condition was initially inferred by Vidaillet *et al*[2] who found familial occurrence of accessory AV pathways in 3.4% of WPW probands. Typically, WPW syndrome occurs sporadically; however, in a minority of cases it is inherited either as an autosomal dominant simple preexcitation trait,[3] or as part of a more complex phenotype, such as Ebstein's anomaly,[4] mitochondrial disease,[5] or hypertrophic cardiomyopathy (HCM).[6] The first description of a molecular basis of WPW syndrome was provided by Blair *et al*[7] and Gollob *et al*[8] who reported mutations in the gamma-2 regulatory subunit of AMP-activated protein kinase (*PRKAG2*) in patients with familial HCM and WPW syndrome.

Here we describe four patients with electrocardiographic features of WPW syndrome, cognitive delay and dysmorphic features, who were found to have variable chromosomal deletions involving *BMP2*.

Patients and Methods

Written informed consents were obtained from the patients and the study was performed in accordance with the institutional guidelines for human research with approval by the Institutional Review Board at Baylor College of Medicine. Our patients consisted of two groups; group 1 included five individuals with variable 20p12 deletions and group 2 was comprised of 20 individuals with either isolated WPW syndrome or WPW with associated HCM. Ventricular preexcitation was diagnosed in four out of five individuals in group 1 and all patients in group 2, based on standard 12-lead electrocardiography, on the basis of a short PR interval (<120 msec), with a prolonged QRS complex (>110 msec) and an abnormal initial QRS vector (a delta wave).

Patient 1

In the first group, patient 1 was ascertained when array CGH was performed at age 2.5 months because of dysmorphisms, mild hypotonia, and WPW syndrome. He was born after a full term pregnancy; his birth weight was 3,140g (25^{th} centile), his length was 47cm (10^{th} centile) and his head circumference was 34cm (25^{th} centile). He was incidentally found to have WPW during the evaluation for suspected seizures and mild respiratory difficulties in the newborn period. Echocardiogram showed an atrial septal defect. Brain imaging studies, EEG, and Brainstem Auditory Evoked Response (BAER) testing were normal. He began walking at 14 months of age. Language delay was suspected and formal testing at 20 months using the Preschool Language Scales-Fourth Edition (PLS-4), determined a score of 79, below the average range of abilities for comprehension and use of spoken language. Using Stark Assessment of Early Vocal Development (SAEVD), he was placed at Level 5, the “Canonical Syllables” stage of vocal development, typically mastered between 6 to 10 months of age. His physical examination at 20 months of age showed both weight and height 2 SD below the mean and head circumference at 25^{th} centile. Dysmorphic features included downslanting palpebral fissures, bilateral epicanthal folds, broad nasal root and bridge, malar hypoplasia, full cheeks with microstomia, pectus deformity with a carinatum appearance superiorly and excavatum appearance inferiorly, and persistent fetal pads (fig. 1A, B). He continues to have asymptomatic WPW.

Patients 2 and 3

Patient 2 in group 1 was born full term with birth weight of 8 lb (75^{th} centile) and length of 19 inches (25^{th} centile) to a mother with a history of learning difficulties. Four hours after birth, he developed supraventricular tachycardia and was diagnosed with WPW syndrome. On echocardiogram, there was no evidence of a structural heart defect. Brain MRI showed ventriculomegaly with a prominent subarachnoid space consistent with benign external hydrocephalus. BAER testing was normal. He had moderate cognitive, speech and gross motor delays. He started walking independently at 17 months of age. Using the Bayley Scales of Infant Development at 5 years of age, he tested at 34-month, 42-month and 39-month levels for cognition, receptive communication, and expressive communication, respectively. His physical examination at the age of 5 years showed weight at 75^{th} centile, height at 25^{th} centile and head circumference 2.5 SD above the mean. His dysmorphic features included macrocephaly, a frontal upsweep, downslanting palpebral fissures, epicanthal folds, hypertelorism, long philtrum, microstomia, small ears with thickened helices, and broad thumbs and toes with persistent fetal pads (fig. 1C, D). Supraventricular tachycardia in this patient was controlled with Sotalol.

Patient 3, the mother of patient 2, exhibited mild cognitive delay and required special education from elementary through high school years. On Wechsler Adult Intelligence Scale-Third Edition (WAIS-III), her overall IQ was determined to be 90, with below average score of 84 (14th percentile) for working memory and processing speed. Using the Behavior Rating Inventory of Executive Function, Parent Form, she scored in the clinically significant range with metacognition index T score of 67 (93%), indicating difficulty to initiate, plan, organize, and sustain future-oriented problem solving. Using Conners' ADHD Index, she scored in the clinically significant range for inattention and memory problems. Her dysmorphisms, when evaluated at 37 years of age, included macrocephaly, with head circumference 2.25 SD above the mean, hypertelorism, malar hypoplasia and persistent fetal pads (fig. 1E, F). Although she had a previous history of palpitations and suspected SVT, she did not have features consistent with WPW by EKG and Holter monitoring at the time of evaluation.

Patients 4 and 5

The clinical features and cytogenetic findings of patients 4 and 5 in group 1 have been described previously[9, 10] and are summarized in table 1.

Array CGH

Peripheral blood samples from patients 1, 2, and 3 in group 1 were submitted for clinical testing to the Baylor Medical Genetics Laboratories (http://www.bcm.edu/cma/) for BAC array CGH analyses. Reciprocal dye reversal experiments were performed as previously described.[11] Hybridized arrays were scanned into 16-bit TIFF image files using an Axon two-color microarray scanner 4000B and quantified by using GenePix Pro 6.0 software (GenePix 4000B from Axon Instruments, Union City, CA). Data analysis was performed by a web-based software platform with the capability of displaying the raw, normalized, and integrated data of all clones to detect genomic copy-number changes for each patient relative to a gender-matched control as described.[12] A copy-number loss was determined by a combined $\log_2$ ratio of test/control <-0.2 and a p-value <0.05. FISH experiments on metaphase were performed to confirm deletion of the region corresponding to BAC clone, RP11-116E13 in three patients identified by the clinical array-CGH. Miniprep BAC DNA (100 ng) was labeled with Spectrum Orange-dUTP or Spectrum Green-dUTP (Vysis, Downers Grove, IL), according to the manufacturer's instructions, and used as probes for FISH analysis using standard protocols.[13] To precisely map the deletion breakpoints, genome-wide analysis for DNA copy-number alterations was performed in patient 1 using Illumina HumanHap300 genotyping BeadChip array (317K, Illumina, San Diego, CA) and in patients 2, 3 and 5 using the Agilent Human Genome Microarray Kit 244A (Agilent Technologies, Santa Clara, CA) according to the manufacturer's specifications, as described previously.[14, 15]

Southern analysis

Deletion mapping of patient 4 was performed by semiquantitative Southern blotting using 9 genic and RFLP probes, spanning loci from 20p11.23 to 20p13. Measurements were performed with most probes on three independent Southern blots. DNA probes used were pBMP for *BMP*, lambdaHDGI for *PDYN*, HuPrPcDNA for *PRNP*, hSGI for *SCGI*, pR12.21 for *D20S5*, pD3H12 for *D20S6*, pFMS76 for *D20S23* and Mfd25 for *D20S27*.

BMP2 gene copy-number analysis and sequencing

Group 2 patients were studied with a customized high definition CGH microarray (Agilent Technologies) in a 44K format that included 45 predesigned, in silico-validated oligonucleotide probes (~60 bp) spanning exonic, intronic, and 5′ and 3′ regions of *BMP2*. Agilent data were analyzed using Feature Extraction and CGH Analytics softwares from the manufacturer. Mutation analysis of *BMP2* was also performed in this group after amplifying the protein-encoding sequences of *BMP2* through the polymerase chain reaction (PCR) from genomic DNA (sequence of primers and amplification conditions available on request). The PCR products were purified using ExoSAP-IT (USB Corporation, Cleveland, OH) and sequenced with a dye terminator cycle-sequencing system (Applied Biosystems 3730xl).

Generating *Bmp2* null allele

To generate the *Bmp2LacZ* knock-in allele, a targeting vector was constructed that introduced SalI and Xho sites into exon3 that encodes the Bmp2 mature ligand. A LacZ-cassette followed by a loxp-flanked PGKneomycin resistance gene was cloned into the SalI and Xho sites resulting in removal of the majority of the coding sequence in exon3 to generate a *Bmp2* null allele. The mice for this study were on a mixed genetic background B6/129/ICR.

Cardiac electrophysiology

Fourteen *Bmp2*+/- mice of different age groups (9: 3 wks old; 3: 9 wks old and 2: 18 mo old) were analyzed for preexcitation. The *Bmp2*+/- mice and 4 WT were anesthetized with isoflurane (1.5% with 100% O2). In vivo cardiac electrophysiology was performed using a computer-based data acquisition system. An 8 electrode lumen catheter (1.1F, model EPR-800, Millar instruments) was inserted through the jugular vein and advanced into the right atrium and ventricle for pacing and recording. Surface and intracardiac electrophysiology parameters were assessed at baseline, as described.[16] Right atrial pacing was performed using 2-ms current pulses delivered by an external stimulator (STG-3008, MultiChannel Systems). After a period of baseline rhythm, decremental atrial pacing was applied whereby the cycle length was repeatedly shortened by 2ms, starting from a pacing cycle length of 20ms less than the intrinsic sinus cycle length, until continuous atrial capture could not be maintained. Sinus node recovery time (SNRT), measured after a 15 s pacing train with a basic cycle length (BCL) of 100 ms, was defined as the interval between the last stimulus in the pacing train and the onset of first sinus return beat. Atrial effective refractory period (AERP), measured at a BCL of 100 ms, was defined as the longest S1-S2 coupling interval for atria that failed to capture. Atrioventricular nodal ERP (AVERP) was defined as the maximal S1S2 coupling interval that failed to propagate to the ventricle. To assess the presence of accessory pathway, adenosine (2mg/kg intravenously) was administered and the recordings were evaluated for AV block. Inducibility of atrial fibrillation was tested twice, according to the protocol described by Verheule *et al*.[17]

Results

Array CGH

The diagnostic array CGH analysis in patient 1 identified genomic loss of copy-number involving 20p12.3, detected by one BAC clone RP11-116E13 (fig. 2A). In patients 2 and 3, in addition to this genomic region, a loss of the genomic region corresponding to the BAC clone RP11-973N22 and located ~1 Mb centromeric to RP11-116E13 was found (fig. 2D). These findings were subsequently verified by FISH analyses (fig. 2C, E). Using genome-wide CGH array, patient 1 was found to have a *de novo* copy-number loss of 1.1 Mb, involving only one gene, *BMP2* (fig. 2B). Patients 2 and 3, the proband and his mother were both found to have a larger 2.3 Mb deletion involving *BMP2* and seven other genes (fig. 2F, G). This deletion was not identified in the unaffected parents of patient 3 (supplementary figure 1, available online). To address whether the identified deletion was a benign copy-number variant (CNV), we analyzed the hybridization data from these two clones in 13,321 individuals subjected to the clinical array CGH at Baylor Medical Genetics Laboratory. None of the other individuals analyzed demonstrated loss of copy-number detected by these clones with combined $\log_2$ ratio of test/control <−0.2 as seen in patients 1, 2 and 3. In addition, no CNVs in the deleted region were identified in the Database of Genomic Variants (http://projects.tcag.ca/variation/), indicating that there are no large common copy-number polymorphisms in this genomic region.

Since haploinsufficiency for *JAG1*, located in close proximity to *BMP2* on 20p12.2, causes Alagille syndrome (MIM 118450),[18] we investigated whether WPW syndrome was reported in previously described patients with Alagille syndrome having larger deletions involving 20p12. We identified two such cases, patient 4 with del(20)(p11.23)[9] and patient 5 with del (20)(p12p13).[10] Genotyping of patient 4 by semiquantitative Southern blotting mapped the distal breakpoint between *CHGB* and *PRNP* and the proximal breakpoint between *D20S6* and *D20S23*. Thus the deletion of this patient spanned a segment of at least 4.2 Mb harboring the *BMP2* gene (fig. 3). Using genome-wide CGH array analysis, patient 5 was found to have a genomic copy-number loss of 10.7 Mb, including *BMP2* (fig. 3). We then assessed whether point mutations in *BMP2* account for WPW, both in the isolated cases as well as in those with

WPW associated with hypertrophic cardiomyopathy. Analysis of 20 such individuals did not reveal mutations in the coding region of this gene. Additionally, a customized high resolution array-CGH analysis did not detect significant copy-number alterations involving the *BMP2* gene in these patients.

Phenotype characterization of *Bmp2*+/- mice by electrophysiology

There was no significant difference in the PR interval (46 ± 1.7 versus 43 ± 0.8 ms; ns) or QRS duration (9.8 ± 1.9 versus 8.8 ± 0.2 ms; ns) comparing WT and *Bmp2*+/- mice. The atrioventricular refractory period, atrial effective refractory period and the sinus node recovery times were not altered in the WT and *Bmp2*+/- mice (data not shown). There was no evidence of delta wave in any of the 14 *Bmp2*+/- mice studied. Evidence of accessory pathway in *Bmp2* +/- was evaluated using atrial programmed electrical stimulation. Decremental atrial pacing resulted in prolonged AV conduction in both WT and *Bmp2*+/- mice. Intravenous adenosine injection during atrial pacing resulted in AV block in all the mice (4 WT and 14 *Bmp2*+/-) studied, indicating the absence of an accessory pathway. None of the mice showed evidence of atrial arrythmias.

Discussion

Over the last decade, it has become increasingly evident that the genetic mechanisms for many disease traits involve genomic rearrangements rather than point mutations in single genes.[19] Locus-specific mutation rates for genomic rearrangements have been found to be 2-4 orders of magnitude greater than nucleotide-specific rates for base substitutions.[20] Here, we report the first clinical description of a genomic disorder causing ventricular preexcitation and cognitive delay associated with heterozygous deletion within 20p12.3. The description of the smallest *de novo* deletion involving only the *BMP2* gene in patient 1 and others with variable 20p12 deletions, all including *BMP2*, suggests that the clinical phenotype of WPW in these patients may be a consequence of hemizygosity of *BMP2*. *JAG1*, the gene responsible for Alagille syndrome, maps approximately 3.8 Mb centromeric to *BMP2*. Alagille syndrome is a complex multisystem disorder with clinical manifestations including intrahepatic bile duct paucity and right ventricular outflow tract defects.[21] While WPW is not a feature of Alagille syndrome, we hypothesized that rare individuals with deletions involving both *JAG1* and *BMP2* would have WPW syndrome. This was indeed demonstrated in both patients 4 and 5, who were described previously with cytogenetically visible deletions of chromosome 20p. Furthermore, we studied an additional patient with Alagille syndrome with a large (~6 Mb) 20p deletion who did not manifest WPW and found that while the deletion encompassed *JAG1*, the distal breakpoint of the deletion mapped approximately 400 kb centromeric to *BMP2*, involving the most adjacent proximal gene, *HAO1* and leaving the *BMP2* gene intact (supplementary figure 2, available online).

The role of bone morphogenic proteins has been extensively studied in heart, neural, bone, and cartilage development. The signaling of these proteins belonging to the class of transforming growth factor, TGF-beta superfamily of proteins is known to be mediated through specific type I and II serine/threonine kinase receptors. BMP2 binds to the type II receptors, BMPRII and ActRIIA, and the type I receptors, ALK3 and ALK6.[22] The interaction of intracellular Smad signaling molecules with these receptor complexes and other transcription factors defines the range of biological responses associated with the BMPs. Although homozygous null mutation of *BMP2* in mouse results in embryonic lethality,[23] conditional ablation of *Bmp2* has shown that *Bmp2* deficient endothelium is unable to undergo epithelial to mesenchymal transformation (EMT), thus impairing the development of endocardial cushion.[24] Gaussin *et al*[25] showed that Alk3/Bmpr1a receptor is required for development of the atrioventricular canal (AVC) into valves as well as the formation of annulus fibrosus, which insulates the

ventricles from inappropriate excitation by the atria. By selectively deleting *Alk3* in cardiac myocytes of the AVC in mice, conduction defects compatible with preexcitation were observed. In these mice, the disruption of the posterior annulus fibrosus created a myocytic connection between the left atrium and ventricle connected by gap junctions, Cx43 to conduct the electrical impulse. In WPW syndrome, it is well-recognized that the muscular connections outside of the specialized conduction system are composed of myocardial fibers expressing Cx43.[26, 27] Indeed, disruption of annulus fibrosus by glycogen-filled myocytes has been shown to be caused by mutations in *PRKAG2*.[28] These functional analyses facilitate our understanding of the disease pathogenesis as it relates to *BMP2* signaling.

There are about seven genes telomeric to *BMP2*, that are deleted in patients 2, 3, 4 and 5 including *KIND1* (associated with autosomal recessive Kindler disease), *LRRN4*, *CRLS1*, *MCM8*, *TRMT6*, *CHGB*, and *PROKR2*. While it is possible that the expression of any of these genes could potentially be affected by the smallest deletion seen in patient 1, *BMP2* remains an important candidate gene for WPW based on the common deletion in all affected cases and the phenotype observed in *Alk3* conditional knockout mice. Since *Bmp2*-/- mice are embryonic lethal, we wanted to assess if the WPW phenotype could be recapitulated in *Bmp2*+/- mice. Detailed electrophysiology study of 14 *Bmp2*+/- mice did not reveal any evidence of preexcitation. Apart from the physiological differences between mice and humans, and incomplete penetrance of the cardiac phenotype in *Bmp2* heterozygous mice, these findings possibly reflect redundancy among multiple BMP ligands, as promiscuity in the interaction of vertebrate TGFβ ligands and receptors is well characterized and is also evident among protosomal organisms.[29]

In this study, we have shown the presence of WPW by EKG in four out of five individuals with deletion of this genomic region. It is important to realize that while anterograde conduction through an accessory pathway produces full preexcitation resulting in the formation of a typical delta wave, in individuals with concealed accessory pathways, preexcitation may not be readily visible on a standard 12-lead EKG, as conduction through the pathway is primarily retrograde (ventriculo-atrial). This can be true even in patients who have documented typical episodes of atrioventricular tachycardia.[30] This is indeed a consideration in patient 3 with history of palpitations and a normal EKG. It is also possible that this individual did at one time have more prominent antegrade conduction through an accessory pathway and the conduction characteristics have since changed such that preexcitation is currently not easily detected. It is well described that antegrade conduction through an accessory pathway can be variable or intermittent. Without prior EKGs or documentation of arrythmias in this patient, the only definitive recourse would be an invasive electrophysiologic evaluation. While concealed accessory pathway is one explanation for the lack of WPW phenotype on EKG in patient 3, incomplete penetrance of the cardiac phenotype is also possible. Although the penetrance of preexcitation in patients with deletion of this region on 20p12.3 will be better evaluated when more individuals are ascertained, the neurocognitive deficits and dysmorphisms appear to be fully penetrant in this disorder.

Based on its embryonic pattern of expression, it is likely that disruption of *BMP2* signaling causes diverse phenotypes in humans. Whether the *BMP2* deletion alone is causally related to dysmorphisms and developmental problems seen in patients 1, 2, and 3 is not yet certain; however, this may reflect pleiotropic effects of *BMP2* on musculoskeletal and language development.

Our findings demonstrate a novel genomic disorder characterized by WPW syndrome and cognitive deficits and associated with microdeletion of the *BMP2* region within 20p12.3. Additionally, we describe a new contiguous gene deletion syndrome with *BMP2* and *JAG1* deletions resulting in both WPW and features of Alagille syndrome. We have shown that

patients bearing microdeletions in 20p12.3 often present with WPW. These individuals also exhibit variable cognitive deficits and dysmorphic features. If haploinsufficiency of *BMP2* is indeed the mechanism involved in the conduction abnormalities seen in these patients, other defects of TGFβ signaling may be important in causing this relatively common developmental defect.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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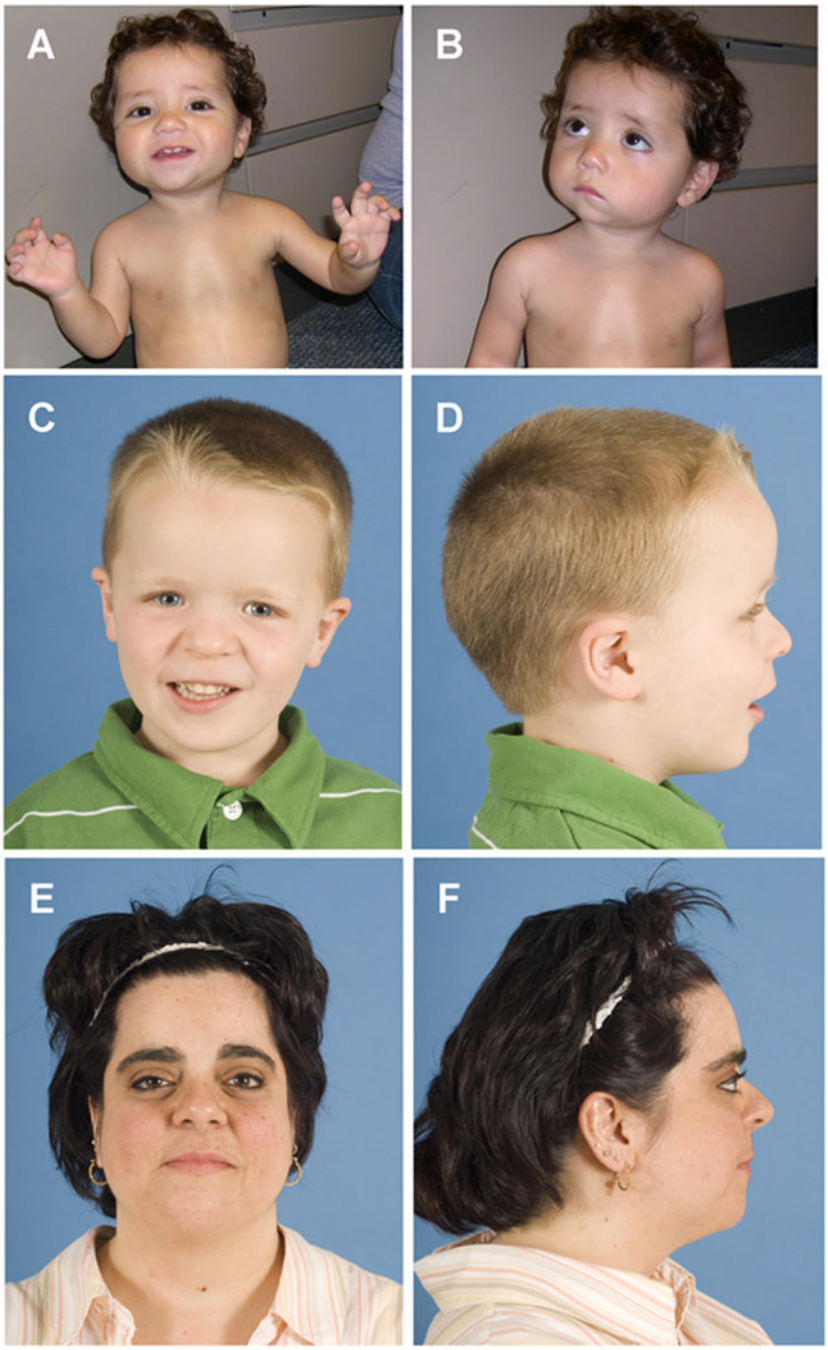

Figure 1.
Photographs of individuals with submicroscopic *BMP2* deletion (submitted with written consents from the patient or his/her legal guardian for publication in print and online). Patient 1 (A, B) at the age of 12 months, with downslanting palpebral fissures, malar hypoplasia, long philtrum, microstomia, and pectus deformity. Patient 2 (C, D) at the age of 5 years, with macrocephaly, a frontal upsweep, downslanting palpebral fissures, hypertelorism, long philtrum, microstomia, and small ears with thickened helices. Patient 3 (E, F) at the age of 37 years, with hypertelorism, malar hypoplasia and macrocephaly.

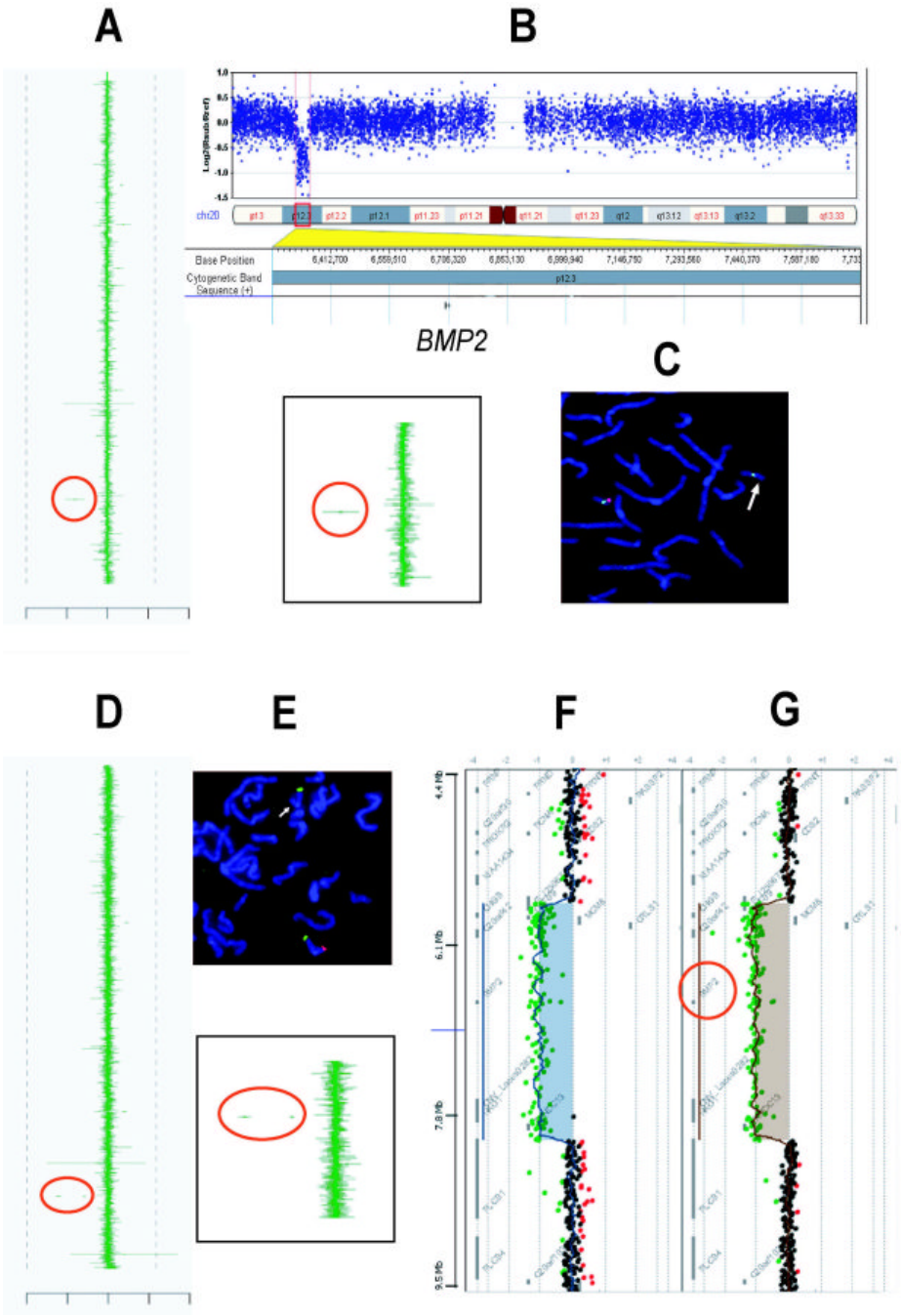

Figure 2.

Summary of the results using targeted array CGH, FISH and genome wide array CGH analyses in patients 1, 2, and 3. Note the genomic loss of copy-number detected by RP11-116E13 in patients 1, 2, 3 with additional loss of copy-number detected by RP11-973N22 in patients 2, 3 (A, D, circled), identified on the clinical array CGH. FISH confirmed the deletion detected by RP11-116E13 in these patients by the loss of hybridization signal on one copy of chromosome 20 (C, E, deletion represented by the highlighted arrow). Fine mapping of the breakpoints using Illumina HumanHap300 genotyping BeadChip array (317K) in patient 1 (B), showed a *de novo* 1.1 Mb deletion involving a single gene, *BMP2*. The 244k Human Genome

Agilent microarray showed 2.3 Mb deletion involving multiple genes, including *BMP2* in patients 2 and 3 (F, G respectively).

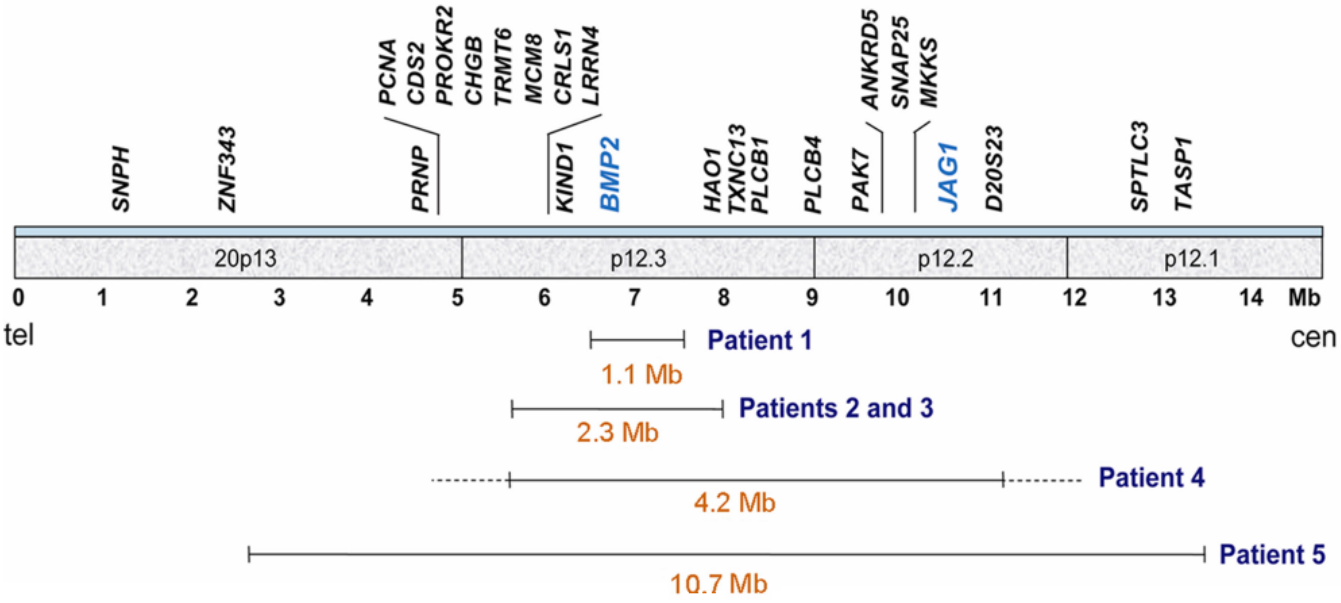

Figure 3.
Schematic illustration of the identified deletions of distal chromosome 20p in patients 1-5, with contiguous genes represented between the 4.7-10.6 Mb interval.

Table 1

Clinical features of five patients with variable deletions involving *BMP2*

	Clinical Features	Patient 1	Patient 2	Patient 3	Patient 4[9]	Patient 5[10]
Karyotype		46,XY	46,XY	46,XX	46,XX,del(20)(p11.23)	46,XX,del(20)(p12p13)
20p deletion		1.1 Mb	2.3 Mb	2.3 Mb	~4.2 Mb	10.7 Mb
Gender		M	M	F	F	F
Inheritance		*de novo*	Maternal	*de novo*	*de novo*	*de novo*
Craniofacial	Macrocephaly	-	+	+	-	-
	Microcephaly	-	-	-	+	U
	Hypertelorism	+	+	+	+	+
	Down slanting palpebral fissures	+	+	-	U	+
	Iris colobomata	-	-	-	+	-
	Maxillary hypoplasia	+	-	+	+	U
	Cleft lip	-	-	-	-	+
	Microstomia	+	+	-	-	U
Liver	Jaundice	-	-	-	-	+
Heart	WPW syndrome	+	+	-	+	+
	ASD	+	-	-	-	-
	PDA	-	-	-	+	-
	Pulmonic stenosis	-	-	-	+	-
	Peripheral pulmonary artery stenosis	-	-	-	+	+
	Right ventricular hypertrophy	-	-	-	-	+
Skeletal	Vertebral abnormalities	-	-	-	+	U
	Pectus deformity	+	-	-	-	+
	Short stature	+	-	+	+	+
	Persistent fetal pads	+	+	+	U	U
Hearing	Hearing loss	-	-	-	+	+
Development	Motor delay	-	+	+	+	+
	Neurocognitive delay	+	+	+	+	+

U = unknown