Report

Characterization of a New Syndrome That Associates Craniosynostosis, Delayed Fontanel Closure, Parietal Foramina, Imperforate Anus, and Skin Eruption: CDAGS

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We describe the clinical characterization, molecular analyses, and genetic mapping of a distinct genetic condition characterized by craniosynostosis, delayed closure of the fontanel, cranial defects, clavicular hypoplasia, anal and genitourinary malformations, and skin eruption. We have identified seven patients with this phenotype in four families from different geographic regions and ethnic backgrounds. This is an autosomal recessive condition that brings together apparently opposing pathophysiologic and developmental processes, including accelerated suture closure and delayed ossification. Selected candidate genes—including *RUNX2, CBFB, MSX2, ALX4, TWIST1,* and *RECQL4*—were screened for mutations, by direct sequencing of their coding regions, and for microdeletions, by fluorescent in situ hybridization. No mutations or microdeletions were detected in any of the genes analyzed. A genomewide screen yielded the maximum estimated LOD score of +2.38 for markers *D22S283* and *D22S274* on chromosome 22q12-q13. We hypothesize that the gene defect in this condition causes novel context-dependent dysregulation of multiple signaling pathways, including RUNX2, during osteoblast differentiation and craniofacial morphogenesis.

In this study, we present the clinical characterization of a distinct genetic condition that associates a severe form of craniofacial dysplasia with genitourinary and skin manifestations. We have used the acronym "CDAGS" because it summarizes the most prominent features of this disorder: "C" stands for craniosynostosis and clavicular hypoplasia; "D," for delayed closure of the fontanel, cranial defects, and, in some patients, deafness; "A," for anal anomalies, including anterior placement of the anus and imperforate anus; "G," for genitourinary malformations; and "S," for skin eruption, which, in some

of the patients, has been classified as porokeratosis. To date, we have identified seven patients, in four families, with the CDAGS phenotype.

The craniofacial component of CDAGS overlaps with some of the features seen in cleidocranial dysplasia (CCD [MIM 119600]), an autosomal dominant disorder characterized by hypoplastic or absent clavicles, delayed closure of the cranial sutures and fontanels, dental anomalies, and delayed skeletal development (Cooper et al. 2001). Most cases of CCD are caused by mutations in *RUNX2*, located on chromosome 6p21 (Lee et al. 1997; Mundlos et al. 1997). *RUNX2* encodes a runt domain transcriptional factor RUNX2, which is required for both osteoblast differentiation and chondrocyte maturation (Zhou et al. 1999, Zheng et al. 2003). Other conditions, such as Yunis-Varon syndrome (MIM 216340) and Crane-Heise syndrome (MIM 218090) share some phenotypic characteristics with CCD, such as cranial dysostosis and

Figure 1   *A*, Craniofacial features of CDAGS syndrome in the index patient at age 8 mo. Note the brachycephaly, frontal bossing, and sparse eyelashes and eyebrows. The skin lesions on the patient's face became markedly hyperpigmented at age 2 mo. *B*, Skin lesions on arms and legs, which occur in a psoriasis-like distribution and affect the margins of the earlobes. Color pictures are included in appendix A (online only). *C*, Posterolateral and anterolateral views of the 3D reconstruction of the CT of the head of the index patient at age 1 mo. Notice the wide metopic suture extending to the nasal bridge, wide-open anterior and posterior fontanels, bilateral PF, and coronal synostosis (*arrows*).

clavicular hypoplasia; the fact that similar skeletal elements are affected in some of these conditions suggests that they may result from mutations in genes that affect the context-dependent action of RUNX2 on its downstream targets. Moreover, phenocopies of this clinical feature may help to identify in vivo regulators of the RUNX2 transcriptional network.

Our index patient is an African American female who was referred for evaluation because of imperforate anus and dysmorphic features. Initial assessment on day 1 of life demonstrated midface hypoplasia, frontal bossing,

large anterior and posterior fontanels, open metopic suture extending down to the nasal bridge, and wide-open sagittal suture. Her clavicles were hypoplastic, and her shoulders could be brought together at the midline. Her genitalia appeared normal, but she had an imperforate anus with rectoperineal fistula. Skin examination revealed multiple papules over the brow and posterior hairline as well as hyperkeratotic areas on her scalp. She had very sparse hair, eyelashes, and eyebrows, which did not improve with age (fig. 1*A*). Her prenatal history was negative for complications, maternal illness, and toxic ex-

posure. The family history is negative for consanguinity, malformations, and fetal demise.

The patient's skin lesions progressed, and, at age 4 mo, they were present on the face and extremities as papules and ruptured vesicles, with an erythematous indurated border in a psoriasis-like distribution (fig. 1B). A skin biopsy of a lesion on the right forearm was performed. Gross inspection revealed heavily pigmented skin with a surface plaque. Microscopic examination showed hyperkeratosis, acanthosis, and an intraepithelial pustule containing mixed inflammatory cells. There was focal disruption of the dermal-epidermal junction, and some of the keratinocytes had cytoplasmatic granules containing melanin. Skin biopsy of a lesion on the left thigh showed a lichenoid infiltrate composed of lymphocytes, neutrophils, and occasional eosinophils, with numerous colloid bodies within the epithelium.

A chest X-ray showed absence of the medial aspects of the clavicles, kyphosis of the thoracic spine, and short ribs. The scapula and vertebral bodies appeared normal. A skeletal survey also revealed poor ossification of the parietal bones and apparent hypoplasia of the sphenoid bone and of the midfacial bones involving the nasion and the maxillary bones. The remainder of the skeleton was within normal limits. A CT scan of the head showed a brachycephalic skull with bilateral coronal synostosis, wide-open metopic suture, and bilateral parietal foramina (PF) (fig. 1C). A magnetic resonance image (MRI) of the brain showed no structural abnormalities and a myelination pattern normal for age. Spinal and renal ultrasound results were normal. A hearing screen identified unilateral sensorineural hearing loss. Chromosome analysis by G-banding revealed a normal 46,XX karyotype.

We have identified and examined six additional patients from a total of four pedigrees, with similar findings. The families come from different geographic regions and ethnic backgrounds (fig. 2). Three of the families have two affected individuals, and two of those families have both male and female affected siblings. For all individuals evaluated, the family history was negative for similar defects. The pattern of transmission observed is consistent with an autosomal recessive Mendelian trait.

Family 2 is of Mexican American descent. The clinical and radiological findings for the two affected individuals in this family show striking similarities to those of the index patient, with severe skin rash, imperforate anus, and cranial defects, including bilateral PF and coronal synostosis (fig. 3). Biopsy of skin lesions on the arms showed histological findings of porokeratosis. In addition to these findings, the older affected individual in this family has profound bilateral sensorineural hearing loss and severe developmental delay. For detailed clinical information about the patients, see appendix A (online only).
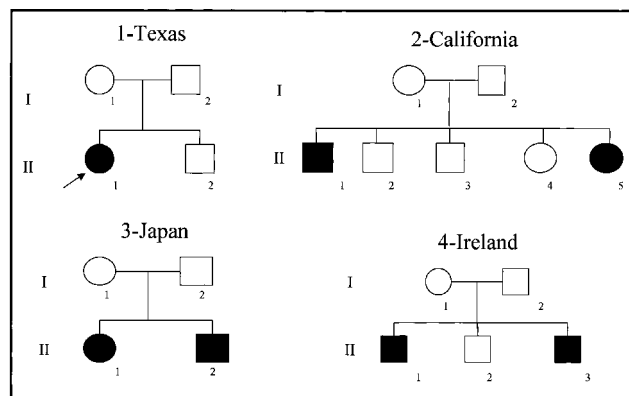
Figure 2   Pedigrees of the four families with CDAGS that have been identified to date. Three families have more than one affected individual, and two have an affected male and female. In all families, there was no history of previous affected individuals, and all families deny consanguinity.

Family 3 is of Japanese descent and comprises two affected siblings, a boy and a girl. These patients were originally described by Fukuda et al. (1981) as having an atypical form of CCD, characterized by clavicular hypoplasia, wide-open sutures and fontanel, anal atresia, and skin lesions described as erythematous plaques with erosion. Watanabe et al. (1996) published another article about these patients and described the skin lesions as a form of psoriasiform erythrokeratodermia.

Family 4 is white and is of Irish descent. Judge et al. (1990) published a study describing the older individual as having porokeratosis and craniosynostosis. Flanagan et al. (1998) described this patient and his brother as having familial craniosynostosis, anal anomalies, and porokeratosis and used the acronym "CAP" (MIM 603116) for the constellation of findings. Both individuals in this family presented with brachycephaly, wide-open anterior and posterior fontanels, and complete coronal synostosis. Flanagan et al. (1998) tested the patients of this family for mutations associated with craniosynostosis in the fibroblast growth-factor receptors 1, 2, and 3 (FGFR1, FGFR2, and FGFR3) and in the TWIST1 genes, and no abnormalities were found (Flanagan et al. 1998).

Table 1 summarizes the clinical findings in the seven patients identified to date. The most consistent features of CDAGS syndrome are coronal synostosis, wide-open anterior and posterior fontanel with PF, underdeveloped eyebrows and eyelashes, imperforate anus, and skin lesions. In two of the families, the histological findings of the skin biopsy were compatible with a diagnosis of porokeratosis. Additional features present in some but not all of the patients include hypoplastic clavicles, sensorineural hearing loss, developmental delay, cleft palate, and hypospadias. The four patients with developmental

Figure 3   Clinical and radiological findings in family 2, showing striking similarities with the index patient. Notice the characteristic skin rash on the 3-year-old female's face (*A*) and extremities (*B*), whereas, in her 11-year-old brother, although there is persistence of hyperkeratotic plaques on the elbows and knees (*C*), facial skin lesions have improved significantly (*D*). Both patients have dysmorphic features and sparse eyebrows and eyelashes. Color pictures are included in appendix A (online only). Anteroposterior and lateral views of the 3D reconstruction of the CT of the head in the female (*panels E and F*) and male (*panels G and H*) at age 1 mo demonstrate the characteristic cranial defects.

delay have some degree of hearing impairment, which may account for some of the deficits. However, these patients have problems with speech and motor skills that do not improve with therapy.

To identify the molecular basis of the disorder, we initially undertook a candidate-gene approach. Figure 4 summarizes the genes that were considered on the basis of the phenotypic characteristics. Because the phenotype shares multiple characteristics with CCD, *RUNX2* and its coactivator, the core binding factor beta (*CBFB*) were attractive candidates (Kundu et al. 2002; Yoshida et al. 2002). PF are symmetric defects of parietal bone ossification that can be seen as an isolated defect or as part of different clinical syndromes. When the defects are large, they may be the main manifestation of the condition known as "parietal foramina" (PFM [MIM 168500]). PF have been described in association with CCD in two families whose affected members have hypoplastic clavicles and delayed closure of the fontanel but no additional findings (PF with CCD [MIM 168550]) (Golabi et al. 1984). Heterozygous loss-of-function mutations in *MSX2* have been described in three unrelated families with PF

(Wilkie et al. 2000). Moreover, a mouse model with a targeted disruption of *Msx2* showed ossification defects in the frontal bone as well as developmental defects in three ectodermal organs, including teeth, hair follicles, and mammary glands (Satokata et al. 2000). PF are also a feature of Potocki-Shaffer syndrome (MIM 601224), which is caused by a proximal 11p deletion that includes the *ALX4* gene. Isolated mutations in *ALX4* have also been described in families segregating PF (Wuyts et al. 2000; Mavrogiannis et al. 2001). Both MSX2 and ALX4 have been shown to interact with RUNX2, and, together, these proteins participate in skull-vault differentiation (Hermanns and Lee 2001; Shirakabe et al. 2001; Antonopoulou et al. 2004).

In contrast to these developmental defects that result from loss of normal function, the premature fusion of the sutures of the cranium has been shown to result from molecular mechanisms that lead to an excess of signal. Activating mutations in *FGFR1, FGFR2,* and *FGFR3* cause seven different clinical syndromes that are characterized by craniosynostosis and variable degrees of skeletal abnormalities. In addition, a missense gain-of-func-

tion mutation in *MSX2* (P148H), which increases the DNA-binding ability of the protein, has been found to cause Boston-type craniosynostosis (Ma et al. 1996). Transgenic mice overexpressing wild-type *Msx2* in the skull develop craniosynostosis and ectopic bone formation, suggesting a dominant positive effect of this protein in suture closure (Liu et al. 1995). Another important player in craniofacial development and craniosynostosis is *TWIST1*. Deletions and point mutations that result in loss of function of *TWIST1* have been found in patients with Saethre-Chotzen syndrome (MIM 101400), which is characterized by craniosynostosis, delayed closure of fontanels, and hand anomalies (Johnson et al. 1998). TWIST1 has been shown to interact with RUNX2 and to repress its function (Yousfi et al. 2002; Bialek et al. 2004).

The dermatological manifestations of CDAGS syndrome are a striking and consistent finding in the families studied. The histological findings on biopsy of two families were consistent with a diagnosis of porokeratosis, a clinically heterogeneous disorder of keratinization (Hivnor et al. 2004). The genetics of this group of disorders is still poorly understood, although there are reports of multiple pedigrees that show autosomal dominant transmission, and several loci have been suggested through linkage analysis. Mutations in the gene *SSH1* (MIM 606778) on chromosome 12q24 were recently described in Chinese families with disseminated superficial actinic porokeratosis (Zhang et al. 2004). A common mecha-
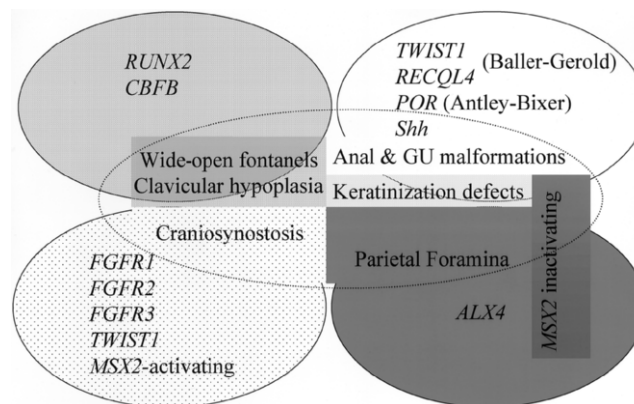
Figure 4 Venn diagram summarizing the features that characterize CDAGS syndrome and its potential molecular mechanism. GU = genitourinary.

nism linking the skin findings and coronal synostosis in our patients could be the deregulation of *MSX2* expression. In addition to its expression during cranial development, *MSX2* is expressed in the matrix epithelial cells of hair follicles and the nail bed (Reginelli et al. 1995). In addition to craniosynostosis, transgenic mice overexpressing *Msx2* showed thickened skin with hyperkeratosis, desquamation, and flakiness, with fewer hairs (Jiang et al. 1999).

Genitourinary and anal abnormalities are another con-

Table 1

Clinical Summary of the Most Prominent Features of CDAGS Syndrome in the Four Families Analyzed

	FINDING IN PATIENT						
CHARACTERISTIC	1-II-1	2-II-1	2-II-5	3-II-1	3-II-2	4-II-1	4-II-3
Sex	F	M	F	F	M	M	M
Coronal craniosynostosis	+	+	+	ND	ND	+	+
Other craniosynostosis	−	Lambdoid[a]	Lambdoid	ND	ND	Sagittal	Sagittal
Wide-open fontanels	++	++	++	++	++	ND	+
PF	++	++	++	++	++	ND	ND
Hearing loss	Unilateral	Sensorineural	−	ND	Mixed	−[b]	−[b]
Developmental delay[c]	−	Severe	Mild	Severe	Moderate[d]	−	−
Hypoplastic clavicles	+	+	+	+	+	−	ND
Imperforate anus	+	+	+	+	+	+/−[e]	+
Hypospadias	−	+	−	−	+	+	+/−[f]
Urethrorectal fistula	−	−	+	+	+	−	−
Skin lesions[g]	+	+	+	+	+	+[h]	+[h]
Porokeratosis on biopsy	−	+	ND	−	−	+	ND

NOTE.—Plus sign (+) = present; double plus sign (++) = severe; minus sign (−) = absent; +/− = partial related finding; ND = not documented.

[a] Squamosal and lambdoid synostosis and cleft palate.

[b] Normal hearing per report, but not formally tested.

[c] The developmental delay present in some individuals is variable and affects both motor and language development.

[d] IQ < 40.

[e] Anteriorly placed anus.

[f] Bifid scrotum.

[g] The eruption is composed of hyperkeratotic papules and plaques and has a reticulate pattern on the face.

[h] Patient exhibited a Koebner phenomenon.

sistent finding in patients with CDAGS. Imperforate anus can be found as an isolated defect or can be part of a syndrome or a sequence. Multiple genes have been studied as candidates in anorectal malformation syndromes, and there are several reports of families segregating this feature as autosomal recessive and X-linked traits (Villavicencio et al. 2000; Mo et al. 2001). The strongest evidence for genes responsible for imperforate anus is derived from animal models and points toward genes involved in the sonic hedgehog pathway (Kim et al. 2001; Mo et al. 2001). Some syndromes, such as Baller-Gerold syndrome (MIM 218600) and Antley-Bixler syndrome (MIM 207410), demonstrate an association between craniosynostosis and genitourinary/anal malformations. Although the etiology of these syndromes has not been completely elucidated, some patients with Antley-Bixler syndrome carry mutations in the P450 oxidoreductase gene (*POR* [MIM 124015]) that result in abnormal steroidogenesis (Adachi et al. 2004; Fukami et al. 2005). Patients with features of Baller-Gerold syndrome have been found to have mutations in *TWIST1* (Seto et al. 2001) as well as in the DNA helicase *RECQL4* (Van Maldergem et al. 1992, 2004). Interestingly, mutations in *RECQL4* result in a spectrum of autosomal recessive disorders that also includes Rothmund-Thomson syndrome (RTS [MIM 268400]) and RAPADILINO syndrome (MIM 266280) (Siitonen et al. 2003). RTS is characterized by short stature, cataracts, a predisposition to tumors, and a poikilodermatous rash that partly resembles the one seen in our patients (Megarbane et al. 2000; Wang et al. 2001).

All seven affected individuals and their first-degree relatives were enrolled in a research program approved by the Baylor College of Medicine institutional review board, and appropriate informed consent was obtained. We isolated genomic DNA of the two patients who were first ascertained, individuals 1-II-1 and 3-II-2, for candidate-gene studies. Sequence analysis of all eight exons and the promoter of *RUNX2* was performed as described elsewhere (Zhou et al. 1999). Similarly, the coding regions of the genes *CBFB, MSX2, TWIST1,* and *RECQL4* were amplified by PCR and were analyzed by direct sequencing. No mutations were found in the coding regions of any of these genes. Because of the possibility that this syndrome may be caused by a contiguous gene deletion, we performed FISH with probes specific for *RUNX2, CBFB,* and *ALX4.* All FISH analyses showed a normal hybridization pattern, consistent with the presence of both alleles in each case (data not shown).

On the basis of the pattern of inheritance, we assumed an autosomal recessive mode of transmission and performed simulation analysis to estimate the power to detect linkage. Assuming full penetrance, an allele frequency of 0.001, and no locus heterogeneity, we estimated the

maximum LOD score to be +2.38 with a mean (±SE) of 1.66 (±0.03). We isolated DNA from all affected patients and first-degree relatives of the four families. Using Linkage Mapping Set v2.5-MD10 (Applied Biosystems), we performed a genomewide screen with 400 markers at an average resolution of 10 cM. We performed parametric two-point linkage analysis, using the Fastlink software (National Center for Biotechnology Information), to locate the genomic region harboring the gene responsible for the phenotype, and we used Allegro for multipoint analysis and fine mapping. Analysis of the genotype data showed maximum estimated LOD score of +2.38 for markers *D22S283* and *D22S274* on chromosome 22q12-q13 (fig. 5 and table B1 [online only]). Haplotype analysis allowed us to narrow the region of interest to a 34-cM interval between *D22S1163* and *D22S1170.* These results suggest that the disease gene lies in this region on 22q12-q13.

Chromosome region 22q12-q13 spans 20 Mb and contains >100 genes. There are several reports of patients with structural abnormalities involving this region, but none of them provides clues as to the possible gene responsible for CDAGS syndrome. Deletions spanning the segment 22q12-qter may result in developmental delay but only minor anomalies (Watt et al. 1985; Kirshenbaum et al. 1988). Isolated deletions of 22q13 have common features that include accelerated growth, developmental delay, hearing loss, delayed myelination, hypotonia, and minor dysmorphisms (Lindquist et al. 2005). Duplication of 22q12-qter has been associated with intrauterine growth restriction (IUGR), cleft palate, mild hearing loss, imperforate anus, and talipes (Mirza et al. 2000), and an isolated duplication of 22q13 resulted in true hermaphroditism in a patient with IUGR and am-
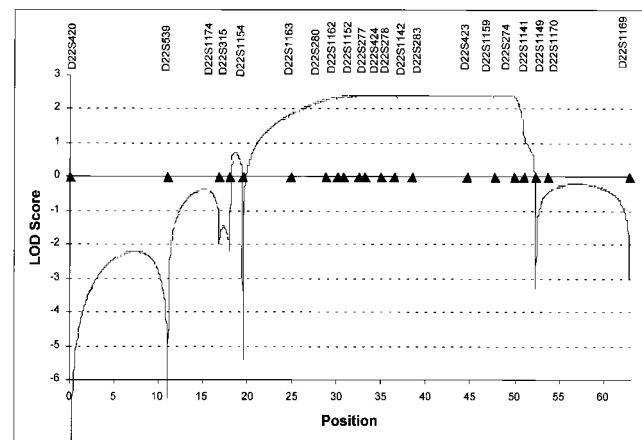
Figure 5 Multipoint linkage analysis with polymorphic markers throughout the genome demonstrated the maximum expected LOD score of +2.38 for markers *D22S283* and *D22S274* on the long arm of chromosome 22 (22q12-q13).

biguous genitalia that is due to an unbalanced translocation (Aleck et al. 1999).

In this study, we present a complete clinical characterization of CDAGS syndrome, a distinct genetic condition that associates craniofacial anomalies, clavicular hypoplasia, anal and genital abnormalities, and severe skin eruption. The clinical features of these patients point to the apparently opposing biological processes of delayed ossification and accelerated suture closure. It is well known that the function of the transcription factor RUNX2 is context dependent and is regulated by multiple coactivators and repressors. Whereas this transcription factor is essential for osteoblast cell-fate commitment, it is also a major determinant of chondrocyte maturation. Hence, it serves critical functions during both endochondral and intramembranous ossification. Interestingly, genes that have been implicated in craniosynostosis, such as *TWIST* and *MSX2,* have been reported to directly regulate *RUNX2* activity both in cell studies and in vivo. We hypothesize that the gene defect responsible for CDAGS syndrome causes novel context-dependent dysregulation of multiple signaling pathways, including RUNX2, during osteoblast differentiation and craniofacial morphogenesis. It is possible that patients with this constellation of findings have mutations in a central regulatory factor that specifies the balance of *RUNX2* activity in these diverse tissues. At the same time, this factor must also serve a function independent of *RUNX2* in tissues such as skin and in the genito-urinary system, where RUNX2 is not expressed.

Web Resources

The URLs for data presented herein are as follows:

National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/CBBresearch/Schaffer/fastlink.html (for genetic analysis software)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for CCD, Yunis-Varon syndrome, Crane-Heise syndrome, CAP, PFM, PF with CCD, Potocki-Shaffer syndrome, Saethre-Chotzen syndrome, *SSH1,* Baller-Gerold syndrome, Antley-Bixler syndrome, *POR,* RTS, and RAPADILINO syndrome)

References

Adachi M, Asakura Y, Tachibana K, Shackleton C (2004) Abnormal steroidogenesis in three patients with Antley-Bixler syndrome: apparent decreased activity of 17α-hydroxylase, 17,20-lyase and 21-hydroxylase. Pediatr Int 46:583–589

Aleck KA, Argueso L, Stone J, Hackel JG, Erickson RP (1999) True hermaphroditism with partial duplication of chromosome 22 and without SRY. Am J Med Genet 85:2–4

Antonopoulou I, Mavrogiannis LA, Wilkie AO, Morriss-Kay GM (2004) Alx4 and Msx2 play phenotypically similar and additive roles in skull vault differentiation. J Anat 204:487–499

Bialek P, Kern B, Yang X, Schrock M, Sosic D, Hong N, Wu H, Yu K, Ornitz DM, Olson EN, Justice MJ, Karsenty G (2004) A twist code determines the onset of osteoblast differentiation. Dev Cell 6:423–435

Cooper SC, Flaitz CM, Johnston DA, Lee B, Hecht JT (2001) A natural history of cleidocranial dysplasia. Am J Med Genet 104:1–6

Flanagan N, Boyadjiev SA, Harper J, Kyne L, Earley M, Watson R, Jabs EW, Geraghty MT (1998) Familial craniosynostosis, anal anomalies, and porokeratosis: CAP syndrome. J Med Genet 35:763–766

Fukami M, Horikawa R, Nagai T, Tanaka T, Naiki Y, Sato N, Okuya T, Nakai H, Soneda S, Tachibana K, Matsuo N, Sato S, Hom K, Nishimura G, Hasegawa T, Ogata T (2005) POR (P450 oxidoreductase) mutations and Antley-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients. J Clin Endocrinol Metab 90:414–426

Fukuda K, Miyanomae T, Nakata E, Tanaka M, Tanaka Y, Usui T (1981) Two siblings with cleidocranial dysplasia associated with atresia ani and psoriasis-like lesions: a new syndrome? Eur J Pediatr 136:109–111

Golabi M, Carey J, Hall BD (1984) Parietal foramina clavicular hypoplasia: an autosomal dominant syndrome. Am J Dis Child 138:596–599

Hermanns P, Lee B (2001) Transcriptional dysregulation in skeletal malformation syndromes. Am J Med Genet 106:258–271

Hivnor C, Williams N, Singh F, VanVoorhees A, Dzubow L, Baldwin D, Seykora J (2004) Gene expression profiling of porokeratosis demonstrates similarities with psoriasis. J Cutan Pathol 31:657–664

Jiang TX, Liu YH, Widelitz RB, Kundu RK, Maxson RE, Chuong CM (1999) Epidermal dysplasia and abnormal hair follicles in transgenic mice overexpressing homeobox gene MSX-2. J Invest Dermatol 113:230–237

Johnson D, Horsley SW, Moloney DM, Oldridge M, Twigg SRF, Walsh S, Barrow M, Njølstad PR, Kunz J, Ashworth GJ, Wall SA, Kearney L, Wilkie AOM (1998) A comprehensive screen for *TWIST* mutations in patients with craniosynostosis identifies a new microdeletion syndrome of chromosome band 7p21.1. Am J Hum Genet 63:1282–1293

Judge MR, Michaels M, Sams VR, David TJ, Harper JI (1990) Disseminated porokeratosis in an infant with craniosynostosis. Br J Dermatol 123:249–254

Kim J, Kim P, Hui CC (2001) The VACTERL association: lessons from the sonic hedgehog pathway. Clin Genet 59:306–315

Kirshenbaum G, Chmura M, Rhone DP (1988) Long arm deletion of chromosome 22. J Med Genet 25:780

Kundu M, Javed A, Jeon JP, Horner A, Shum L, Eckhaus M, Muenke M, Lian JB, Yang Y, Nuckolls GH, Stein GS, Liu PP (2002) Cbfβ interacts with Runx2 and has a critical role in bone development. Nat Genet 32:639–644

Lee B, Thirunavukkarasu K, Zhou L, Pastore L, Baldini A, Hecht J, Geoffroy V, Ducy P, Karsenty G (1997) Missense mutations abolishing DNA binding of the osteoblast-specific transcription factor OSF2/CBFA1 in cleidocranial dysplasia. Nat Genet 16:307–310

Lindquist SG, Kirchhoff M, Lundsteen C, Pedersen W, Erichsen G, Kristensen K, Lillquist K, Smedegaard HH, Skov L, Tommerup N, Brondum-Nielsen K (2005) Further delineation of the 22q13 deletion syndrome. Clin Dysmorphol 14:55–60

Liu YH, Kundu R, Wu L, Luo W, Ignelzi MA Jr, Snead ML, Maxson RE Jr (1995) Premature suture closure and ectopic cranial bone in mice expressing Msx2 transgenes in the developing skull. Proc Natl Acad Sci USA 92:6137–6141

Ma L, Golden S, Wu L, Maxson R (1996) The molecular basis of Boston-type craniosynostosis: the Pro148→His mutation in the N-terminal arm of the MSX2 homeodomain stabilizes DNA binding without altering nucleotide sequence preferences. Hum Mol Genet 5:1915–1920

Mavrogiannis LA, Antonopoulou I, Baxova A, Kutilek S, Kim CA, Sugayama SM, Salamanca A, Wall SA, Morriss-Kay GM, Wilkie AO (2001) Haploinsufficiency of the human homeobox gene ALX4 causes skull ossification defects. Nat Genet 27:17–18

Megarbane A, Melki I, Souraty N, Gerbaka J, El Ghouzzi V, Bonaventure J, Mornand A, Loiselet J (2000) Overlap between Baller-Gerold and Rothmund-Thomson syndrome. Clin Dysmorphol 9:303–305

Mirza G, Imaizumi K, Ragoussis J (2000) Partial trisomy 22 in a liveborn resulting from a rearrangement between chromosomes 6 and 22. J Med Genet 37:E22

Mo R, Kim JH, Zhang J, Chiang C, Hui CC, Kim PC (2001) Anorectal malformations caused by defects in sonic hedgehog signaling. Am J Pathol 159:765–774

Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, Lindhout D, Cole WG, Henn W, Knoll JH, Owen MJ, Mertelsmann R, Zabel BU, Olsen BR (1997) Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. Cell 89:773–779

Reginelli AD, Wang YQ, Sassoon D, Muneoka K (1995) Digit tip regeneration correlates with regions of Msx1 (Hox 7) expression in fetal and newborn mice. Development 121:1065–1076

Satokata I, Ma L, Ohshima H, Bei M, Woo I, Nishizawa K, Maeda T, Takano Y, Uchiyama M, Heaney S, Peters H, Tang Z, Maxson R, Maas R (2000) Msx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation. Nat Genet 24:391–395

Seto ML, Lee SJ, Sze RW, Cunningham ML (2001) Another TWIST on Baller-Gerold syndrome. Am J Med Genet 104:323–330

Shirakabe K, Terasawa K, Miyama K, Shibuya H, Nishida E (2001) Regulation of the activity of the transcription factor Runx2 by two homeobox proteins, Msx2 and Dlx5. Genes Cells 6:851–856

Siitonen HA, Kopra O, Kaariainen H, Haravuori H, Winter RM, Saamanen AM, Peltonen L, Kestila M (2003) Molecular defect of RAPADILINO syndrome expands the phenotype spectrum of RECQL diseases. Hum Mol Genet 12:2837–2844

Van Maldergem L, Megarbane A, Siitonen HA, De Roy M, Fourneau C, Jalkh N, Chouery E, Gillerot Y, Verloes A (2004) Revisiting the craniosynostosis-radial ray hypoplasia association: Baller-Gerold syndrome caused by mutations in RECQL4 helicase gene. American Society of Human Genetics, 54th Annual Meeting abstract book, p 55

Van Maldergem L, Verloes A, Lejeune L, Gillerot Y (1992) The Baller-Gerold syndrome. J Med Genet 29:266–268

Villavicencio EH, Walterhouse DO, Iannaccone PM (2000) The sonic hedgehog–patched–Gli pathway in human development and disease. Am J Hum Genet 67:1047–1054

Wang LL, Levy ML, Lewis RA, Chintagumpala MM, Lev D, Rogers M, Plon SE (2001) Clinical manifestations in a cohort of 41 Rothmund-Thomson syndrome patients. Am J Med Genet 102:11–17

Watanabe K, Hatamochi A, Arakawa M, Ueki H, Nomura S, Osawa G, Hata T (1996) Congenital psoriasiform erythrokeratodermia with cleidocranial dysplasia, urogenital anomalies and atresia ani. Dermatology 192:368–372

Watt JL, Olson IA, Johnston AW, Ross HS, Couzin DA, Stephen GS (1985) A familial pericentric inversion of chromosome 22 with a recombinant subject illustrating a "pure" partial monosomy syndrome. J Med Genet 22:283–287

Wilkie AO, Tang Z, Elanko N, Walsh S, Twigg SR, Hurst JA, Wall SA, Chrzanowska KH, Maxson RE Jr (2000) Functional haploinsufficiency of the human homeobox gene MSX2 causes defects in skull ossification. Nat Genet 24:387–390

Wuyts W, Cleiren E, Homfray T, Rasore-Quartino A, Vanhoenacker F, Van Hul W (2000) The ALX4 homeobox gene is mutated in patients with ossification defects of the skull (foramina parietalia permagna, OMIM 168500). J Med Genet 37:916–920

Yoshida CA, Furuichi T, Fujita T, Fukuyama R, Kanatani N, Kobayashi S, Satake M, Takada K, Komori T (2002) Corebinding factor β interacts with Runx2 and is required for skeletal development. Nat Genet 32:633–638

Yousfi M, Lasmoles F, Marie PJ (2002) TWIST inactivation reduces CBFA1/RUNX2 expression and DNA binding to the osteocalcin promoter in osteoblasts. Biochem Biophys Res Commun 297:641–644

Zhang Z, Niu Z, Yuan W, Liu W, Xiang L, Zhang J, Chu X, Zhao J, Jiang F, Chai B, Cui F, Wang Y, Zhang K, Wang Y, Xu S, Xia L, Gu J, Zhang S, Meng X, Wang S, Gao S, Fan M, Nie L, Zheng Z, Huang W (2004) Fine mapping and identification of a candidate gene SSH1 in disseminated superficial actinic porokeratosis. Hum Mutat 24:434–438

Zheng Q, Zhou G, Morello R, Chen Y, Garcia-Rojas X, Lee B (2003) Type X collagen gene regulation by Runx2 contributes directly to its hypertrophic chondrocyte-specific expression in vivo. J Cell Biol 162:833–842

Zhou G, Chen Y, Zhou L, Thirunavukkarasu K, Hecht J, Chitayat D, Gelb BD, Pirinen S, Berry SA, Greenberg CR, Karsenty G, Lee B (1999) CBFA1 mutation analysis and functional correlation with phenotypic variability in cleidocranial dysplasia. Hum Mol Genet 8:2311–2316