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Deletions of the RUNX2 Gene are present in about 10% of Individuals with Cleidocranial Dysplasia



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For Peer Review

Deletions of the *RUNX2* Gene are present in about 10% of Individuals with Cleidocranial Dysplasia



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ABSTRACT: Cleidocranial Dysplasia (CCD) is an autosomal dominant skeletal disorder characterized by hypoplastic or absent clavicles, increased head circumference, large fontanels, dental anomalies, and short stature. Hand malformations are also common. Mutations in *RUNX2* cause CCD, but are not identified in all CCD patients. In this study we screened 135 unrelated patients with the clinical diagnosis of CCD for *RUNX2* mutations by sequencing analysis and demonstrated 82 mutations 48 of which were novel. By quantitative PCR we screened the remaining 53 unrelated patients for copy number variations in the *RUNX2* gene. Heterozygous deletions of different size were identified in 13 patients, and a duplication of the exons 1 to 4 of the *RUNX2* gene in one patient. Thus, heterozygous deletions or duplications affecting the *RUNX2* gene may be present in about 10% of all patients with a clinical diagnosis of CCD which corresponds to 26% of individuals with normal results on sequencing analysis. We therefore suggest that screening for intragenic deletions and duplications by qPCR or MLPA should be considered for patients with CCD phenotype in whom DNA sequencing does not reveal a causative *RUNX2* mutation. ©2010 Wiley-Liss, Inc.

KEY WORDS: cleidocranial dysplasia. deletion. duplication. mutation. *RUNX2*.

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2 < Ott C.E. et al.>

INTRODUCTION

Cleidocranial dysplasia (CCD, MIM #119600) is an autosomal dominant disorder characterized by hypoplastic or aplastic clavicles, multiple dental abnormalities, and delayed closure of the cranial sutures. The latter results in abnormally large, wide-open fontanels at birth that may remain open throughout life. The most prominent clinical findings are abnormal clavicles with narrow, sloping shoulders that can be apposed at the midline. In addition, mid-face hypoplasia, short stature as well as dental and skeletal anomalies are common (Mundlos, 1999). Abnormal dentition includes delayed eruption of secondary dentition, failure to shed the primary teeth, supernumerary teeth with dental crowding, and malocclusion (Golan et al., 2003). Further skeletal abnormalities include brachydactyly, tapering fingers, and short, broad thumbs, as well as other skeletal/orthopaedic problems such as pes planus, genua valga, and scoliosis. In many cases manifestations vary among individuals within the same family (Otto et al., 2002).

The molecular cause of CCD is linked to mutations in the *RUNX2* gene (Runt-related Transcription Factor 2, MIM *600211), also known as *CBFA1* (Core Binding Factor A1), on chromosome 6p21 (Mundlos, 1999). The osteoblast-specific transcription factor *RUNX2* belongs to the runt-domain gene family and promotes the differentiation of mesenchymal cells into osteoblasts in the cartilage anlagen (Ducy et al., 1997). The molecular pathogenesis of CCD is related to loss of function or haploinsufficiency of *RUNX2* (Mundlos, 1999). In this work, we screened 135 unrelated individuals with CCD and identified 82 mutations by standard DNA sequencing analysis. We then screened the remaining 53 persons for deletions or duplications by quantitative PCR analysis and identified 13 deletions and one duplication affecting the *RUNX2* gene. Thus, our results suggest that deletions/duplications may be present in around 10% of individuals presenting with CCD phenotype for mutations analysis in *RUNX2*.

MATERIALS AND METHODS

DNA samples:

We screened 135 unrelated individuals with CCD by standard DNA sequencing analysis of the *RUNX2* gene. These subjects were unselected and consecutively referred with the suspected diagnosis of CCD. All samples were obtained following informed consent for DNA testing. Genomic DNA was extracted from peripheral blood using standard methods. Mutations are given referring to GenBank entry NM_001024630.3 according to the Human Gene Mutation Database (<http://www.hgmd.org>, entry: *RUNX2*) and the approved nomenclature (Otto et al., 2002) numbering the exons corresponding to GenBank: AH005498.1 which is equivalent to the Ensembl transcript ENST00000371438. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence. The initiation codon is codon 1.

Quantitative PCR:

To evaluate genomic DNA for *RUNX2* copy number changes we used quantitative PCR (qPCR) with a set of primer pairs located within the coding region and flanking untranslated regions (UTR). qPCR was performed in a total volume of 12 μ l containing 6 μ l of SYBR Green PCR Master Mix (Applied Biosystems), 5 μ l of genomic DNA (2 ng/ μ l), and 1 μ l of primers (0.2 μ mol each). Samples were run on the Applied Biosystems 7900HT Real-Time PCR System in triplicate in separate reactions to permit the quantification of the target sequences normalized to the gene for albumin (*ALB*). PCR conditions were as follows: 10 min initial denaturation step at 95°C followed by 40 cycles at 95°C for 15sec and 60°C for 1min. By use of calibrator samples of normal control DNA, the gene copy number was estimated on the basis of the comparative $\Delta\Delta$ Ct method. As quality control, we performed a sex determination for the individuals, calculating the copy number of the gene for Factor VIII (*F8*) relative to the autosomal (i.e., two-copy) control *ALB* (Klopocki et al., 2008). Primer sequences are available upon request.

Array-CGH & interphase FISH:

For Array-CGH a whole human genome tiling path bacterial artificial chromosome (BAC) array was used as described previously (Klopocki et al., 2006). Genomic positions in the text are according to human genome version hg19. We performed FISH on interphase nuclei using the BAC clone RP11-342L7 labelled in

SpectrumOrange as probe for the *RUNX2* locus. CEP6 (Vysis) was used as control probe. In total 232 nuclei were analyzed.

RESULTS

Heterozygous *RUNX2* mutations can be detected by sequencing analysis in about two thirds of all cases with the clinical diagnosis of CCD and include nonsense mutations and frame shifts which can be expected to result in RNA degradation, and missense mutations in the DNA binding region which interfere with DNA binding (Mundlos et al., 1997; Quack et al., 1999; Otto et al., 2002; Baumert et al., 2005). In the current study, we identified mutations by DNA sequencing analysis in 82 of 135 index patients, i.e. 61%. The spectrum of these mutations is summarized in Table 1. Heterozygous mutations identified in this study which have to our knowledge not been described previously are given in Table 3. These mutations were classified as disease causing if an amino acid exchange affected the Runt (p.101-229) or PST domain, if a known disease causing mutation affected the same codon or if the identified mutation segregated with the CCD phenotype. Additionally, pathogenic potential of the mutations was analysed using the MutationTaster prediction tool (<http://www.mutationtaster.org/>). In 13 out of the 25 cases (52%) for which parental DNA samples were available, the mutation occurred as a *de novo* event. Transmission occurred from an affected father in seven cases and from an affected mother in five cases.

Table 1. Spectrum of mutations identified in our lab by sequencing analysis

| Mutation type | Frequency |
|--------------------|-----------|
| missense | 43 (52%) |
| frameshift | 22 (27%) |
| nonsense | 13 (16%) |
| splice-site | 3 (4%) |
| in-frame insertion | 1 (1%) |
| Total | 82 |

In about one third of cases the molecular cause remained unknown. Because of a recent case report on intragenic deletions in patients with CCD (Lee et al., 2008), we screened the remaining 53 patients for intragenic deletions by qPCR. A deletion was identified in 13 patients, and a duplication in one patient (Table 2).

Table 2. Heterozygous deletions or duplications within the *RUNX2* gene identified by qPCR

| Sample # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9* | 10 | 11 | 12 | 13 | 14 |
|------------|---|---|---|---|---|---|---|---|----|----|----|----|----|----|
| 5'-UTR | | | | | | | | | | | | | | |
| Intron 1-2 | | | | | | | | | | | | | | |
| Exon 2 | | | | | | | | | | | | | | |
| Exon 3 | | | | | | | | | | | | | | |
| Exon 4 | | | | | | | | | | | | | | |
| Exon 5 | | | | | | | | | | | | | | |
| Exon 6 | | | | | | | | | | | | | | |
| Exon 7 | | | | | | | | | | | | | | |
| Exon 8 | | | | | | | | | | | | | | |
| 3'-UTR | | | | | | | | | | | | | | |

Columns represent the DNA samples and lines the results for the amplicons within the *RUNX2* gene. Numbering of exons corresponds to GenBank: AH005498.1. * This case has been clinically described by Unger et al. (Unger et al., 2002). Deletions in the *RUNX2* gene detected by qPCR are shown in light grey. The localization of the duplication is shown in dark grey.

4 < Ott C.E. et al.>

Since patient 1 presented with congenital hypothyroidism in addition to the classical CCD phenotype, high resolution array CGH was used to further characterize the deletion size shown initially by qPCR. This analysis revealed a microdeletion of about 2.1 Mb [arr 6p21.1(43,552,921-45,654,196)x1] which encompasses *RUNX2* and about 20 additional genes. The aberration sizes in patients 2-5 and 9-14 were not investigated further since these patients showed no clinical features except for CCD features.

More than one exon was deleted (or duplicated) in all samples except for two. For these samples (# 6 and 8), additional primer pairs flanking the distinct exons were used to confirm the deletion of single exons. Due to their size these two intragenic deletions would not have been detected by standard mutation detection methods such as DNA sequencing, fluorescence in situ hybridization (FISH) analysis or array comparative genomic hybridization (array CGH).

By qPCR we confirmed that the duplication was inherited from the affected mother. In three of the index patients with heterozygous deletions the parents were available and also investigated by qPCR. *De novo* occurrence could be confirmed in two of these families. However, in the mildly affected father of patient 7 qPCR results led us to suspect a mosaicism for the deletion of exons 3 to 6. We therefore performed FISH analysis of the corresponding BAC clone (RP11-342L7) in interphase nuclei and could identify diminished signal intensity in comparison to the other allele in 36 of 232 nuclei, i.e. about 15%. The deletion seemed to be smaller than the BAC clone, as there was no complete loss of the second signal detectable in any nucleus.

Table 3. Newly-identified heterozygous mutations in *RUNX2*

| nucleotide change | predicted protein or RNA change | type of mutation | similar mutations described by others |
|-------------------|---------------------------------|------------------|--|
| c.193C>T | p.Gln65X | nonsense | p.Gln64X, p.Gln66X (Otto et al., 2002) |
| c.207_208ins13 | p.Glu72GlnfsX93 | frameshift | |
| c.230_276del | p.Ala77ValfsX68 | frameshift | |
| c.274delC | p.Arg92GlyfsX52 | frameshift | |
| c.276_277ins17 | p.Leu93X | nonsense | |
| c.278T>A | p.Leu93X | nonsense | |
| c.282delG | p.Pro95ArgfsX49 | frameshift | |
| c.329delC | p.Pro110ArgfsX34 | frameshift | |
| c.353G>A | p.Ser118Asn | missense | p.Ser118Arg (Quack et al., 1999) |
| c.383C>A | p.Ser128X | nonsense | |
| c.391C>A | p.Arg131Ser | missense | p.Arg131Gly (Kim et al., 2006) |
| c.391C>T | p.Arg131Cys | missense | p.Arg131Gly (Kim et al., 2006) |
| c.396C>A | p.Cys132X | nonsense | |
| c.407T>C | p.Leu136Pro | missense | |
| c.467T>A | p.Val156Asp | missense | p.Val156Gly (Otto et al., 2002) |
| c.469dupA | p.Met157AsnfsX4 | frameshift | |
| c.484G>T | p.Glu162X | nonsense | |
| c.523A>G | p.Met175Val | missense | p.Met175Arg (Lee et al., 1997) |
| c.524T>A | p.Met175Lys | missense | p.Met175Arg (Lee et al., 1997) |
| c.560T>C | p.Phe187Ser | missense | |
| c.578G>A | p.Arg193Gln | missense | p.Arg193X (Zhou et al., 1999) |
| c.599C>T | p.Thr200Ile | missense | p.Thr200Ala (Zhou et al., 1999) |
| c.624_625dup | p.Gln209ProfsX3 | frameshift | |
| c.627A>T | p.Gln209His | missense | p.Gln209Arg (Zhou et al., 1999), p.Gln209X (Yamachika et al., 2001) |

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| nucleotide change | predicted protein or RNA change | type of mutation | similar mutations described by others |
|----------------------------|---------------------------------|------------------|--|
| c.631G>C | p.Ala211Pro | missense | |
| c.631_633dup | p.Ala211dup | duplication | |
| c.652A>C | p.Lys218Gln | missense | p.Lys218Asn (Yoshida et al., 2002) |
| c.652A>G | p.Lys218Glu | missense | p.Lys218Asn (Yoshida et al., 2002) |
| c.667G>A | p.Gly223Arg | missense | |
| c.674G>T | p.Arg225Leu | missense | p.Arg225Gln, p.Arg225Trp (Quack et al., 1999) |
| c.682A>G | p.Arg228Gly | missense | |
| c.694C>T | p.Gln232X | nonsense | |
| c.718_721del | p.Ser240CysfsX9 | frameshift | |
| c.831delT | p.Phe277LeufsX31 | frameshift | |
| c.859G>A | p.Asp287Asn | missense | |
| c.879_885del | p.Ser294ArgfsX12 | frameshift | |
| c.977delG | p.Gly326AlafsX158 | frameshift | |
| c.1056G>A | p.Trp352X | nonsense | |
| c.1153_1154del | p.Ser385ProfsX104 | frameshift | |
| c.1169delC | p.Pro390HisfsX94 | frameshift | |
| c.1205delC | p.Pro402ArgfsX82 | frameshift | |
| c.1250_1251del | p.His417LeufsX72 | frameshift | |
| c.1254C>A | p.Tyr418X | nonsense | |
| c.1259C>A | p.Thr420Asn | missense | p.Thr420Ile (Wang et al., 2010) |
| c.1335_1336del | p.Tyr446LeufsX43 | frameshift | |
| c.1385dupG | p.Asp463ArgfsX27 | frameshift | |
| IVS3+1G>T (c.580+1G>T) | Loss of splice donor site | splice site | |
| IVS7-1G>C (c.1088-1G>C) | Loss of splice acceptor site | splice site | |

Mutations are given referring to GenBank entries NM_001024630.3 and NP_001019801.3. Numbering of exons corresponds to GenBank: AH005498.1 which is equivalent to the Ensembl transcript ENST00000371438. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence. The initiation codon is codon 1.

DISCUSSION

Screening of the *RUNX2* gene in individuals presenting with CCD phenotype generally reveals a *RUNX2* mutation in about two thirds of the patients (Mundlos et al., 1997; Baumert et al., 2005). There are a number of reasons for this observation. Some other hereditary disorders resemble CCD to a greater or lesser degree, including the parietal foramina with cleidocranial dysplasia due to mutations in *MSX2* (Garcia-Minaur et al., 2003). Additionally, abnormalities in Core-binding factor beta (CBFB), which interacts with *RUNX2*, cause skeletal anomalies such as large fontanels and delayed ossification (Kundu et al., 2002; Goto et al., 2004; Khan et al., 2006). Mutations and SNPs within the promoter of *RUNX2* have been described. However, population studies and functional analyses of these promoter variants did not support causality for the CCD phenotype (Napierala et al., 2005). Larger deletions encompassing the *RUNX2* gene (Izumi et al., 2006; El-Gharbawy et al., 2010) and chromosomal rearrangements with a breakpoint upstream of the *RUNX2* gene (Purandare et al., 2008) have also occasionally been reported to cause CCD. In accordance with the report by El-Gharbawy and colleagues (El-Gharbawy et al., 2010) two patients with C-terminal deletions showed a more severe phenotype (11, 12) as well as the patient (9) clinically described in more detail by Unger and colleagues (Unger et al., 2002). However, we could not observe a distinct genotype-phenotype correlation in patients with copy number aberrations.

6 < Ott C.E. et al.>

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3 In the current study, we identified CCD mutations in 82 of 135 by DNA sequencing, which is comparable to the
4 results of previous studies. We additionally performed an exhaustive analysis for intragenic deletions or
5 duplications by qPCR and identified aberrations in 14 persons, or 10% of all patients investigated in our study.
6 Mendoza-Londono and Lee (Mendoza-Londono and Lee, 1997-2010) reported that microdeletions may be found
7 by FISH in up to 13% of individuals with normal results on sequencing analysis based on a study population of 40
8 individuals. Due to the higher resolution of the methods applied in this study the frequency of microdeletions/
9 duplications is 26% and thus, two-times higher in individuals with normal results on sequencing analysis.
10

11 We therefore suggest that screening for intragenic deletions and duplications by qPCR or MLPA should be
12 considered for patients referred for suspected CCD in whom DNA sequencing does not reveal a causative *RUNX2*
13 mutation, especially since these methods are comparatively cheap and easy to perform. Alternatively, array CGH
14 or FISH analysis could be used, but depending on the platform array CGH would potentially miss some of the
15 smaller aberrations. FISH analysis would require an additional lithium-blood sample and would also potentially
16 miss smaller copy number changes.
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20 REFERENCES

- 21
22 Baumert U, Golan I, Redlich M, Akinin JJ, Muessig D. 2005. Cleidocranial dysplasia: molecular genetic analysis and
23 phenotypic-based description of a Middle European patient group. *Am J Med Genet A* 139A(2):78-85.
24
25 Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. 1997. *Osf2/Cbfa1*: a transcriptional activator of osteoblast
26 differentiation. *Cell* 89(5):747-54.
27
28 El-Gharbawy AH, Peeden JN, Jr., Lachman RS, Graham JM, Jr., Moore SR, Rimoin DL. 2010. Severe cleidocranial dysplasia
29 and hypophosphatasia in a child with microdeletion of the C-terminal region of *RUNX2*. *Am J Med Genet A* 152A(1):169-
30 74.
31
32 Garcia-Minaur S, Mavrogiannis LA, Rannan-Eliya SV, Hendry MA, Liston WA, Porteous ME, Wilkie AO. 2003. Parietal
33 foramina with cleidocranial dysplasia is caused by mutation in *MSX2*. *Eur J Hum Genet* 11(11):892-5.
34
35 Golan I, Baumert U, Hrala BP, Muessig D. 2003. Dentomaxillofacial variability of cleidocranial dysplasia: clinicoradiological
36 presentation and systematic review. *Dentomaxillofac Radiol* 32(6):347-54.
37
38 Goto T, Aramaki M, Yoshihashi H, Nishimura G, Hasegawa Y, Takahashi T, Ishii T, Fukushima Y, Kosaki K. 2004. Large
39 fontanelles are a shared feature of haploinsufficiency of *RUNX2* and its co-activator *CBFB*. *Congenit Anom (Kyoto)*
40 44(4):225-9.
41
42 Izumi K, Yahagi N, Fujii Y, Higuchi M, Kosaki R, Naito Y, Nishimura G, Hosokai N, Takahashi T, Kosaki K. 2006.
43 Cleidocranial dysplasia plus vascular anomalies with 6p21.2 microdeletion spanning *RUNX2* and *VEGF*. *Am J Med Genet*
44 A 140(4):398-401.
45
46 Khan A, Hyde RK, Dutra A, Mohide P, Liu P. 2006. Core binding factor beta (*CBFB*) haploinsufficiency due to an interstitial
47 deletion at 16q21q22 resulting in delayed cranial ossification, cleft palate, congenital heart anomalies, and feeding
48 difficulties but favorable outcome. *Am J Med Genet A* 140(21):2349-54.
49
50 Kim HJ, Nam SH, Park HS, Ryoo HM, Kim SY, Cho TJ, Kim SG, Bae SC, Kim IS, Stein JL, van Wijnen AJ, Stein GS, Lian
51 JB, Choi JY. 2006. Four novel *RUNX2* mutations including a splice donor site result in the cleidocranial dysplasia
52 phenotype. *J Cell Physiol* 207(1):114-22.
53
54 Klopocki E, Fiebig B, Robinson P, Tonnies H, Erdogan F, Ropers HH, Mundlos S, Ullmann R. 2006. A novel 8 Mb interstitial
55 deletion of chromosome 8p12-p21.2. *Am J Med Genet A* 140(8):873-7.
56
57 Klopocki E, Ott CE, Benatar N, Ullmann R, Mundlos S, Lehmann K. 2008. A microduplication of the long range *SHH* limb
58 regulator (*ZRS*) is associated with triphalangeal thumb-polysyndactyly syndrome. *J Med Genet* 45(6):370-5.
59
60 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. *Cbfbeta* interacts with *Runx2* and has a critical role in bone development. *Nat Genet* 32(4):639-44.

< Deletions of *RUNX2* in Cleidocranial Dysplasia > 7

- 1
2
3 Lee B, Thirunavukkarasu K, Zhou L, Pastore L, Baldini A, Hecht J, Geoffroy V, Ducy P, Karsenty G. 1997. Missense
4 mutations abolishing DNA binding of the osteoblast-specific transcription factor OSF2/CBFA1 in cleidocranial dysplasia.
5 Nat Genet 16(3):307-10.
6
7 Lee MT, Tsai AC, Chou CH, Sun FM, Huang LC, Yen P, Lin CC, Liu CY, Wu JY, Chen YT, Tsai FJ. 2008. Intragenic
8 microdeletion of *RUNX2* is a novel mechanism for cleidocranial dysplasia. Genomic Med 2(1-2):45-9.
9
10 Mendoza-Londono R, Lee B. 1997-2010. Cleidocranial dysplasia. In: Washington Uo, editor. Gene Reviews. Seattle:
11 GeneTests: Medical Genetics Information Resource (database online).
12
13 Mundlos S. 1999. Cleidocranial dysplasia: clinical and molecular genetics. J Med Genet 36(3):177-82.
14
15 Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, Lindhout D, Cole WG, Henn W, Knoll JH, Owen MJ,
16 Mertelsmann R, Zabel BU, Olsen BR. 1997. Mutations involving the transcription factor CBFA1 cause cleidocranial
17 dysplasia. Cell 89(5):773-9.
18
19 Napierala D, Garcia-Rojas X, Sam K, Wakui K, Chen C, Mendoza-Londono R, Zhou G, Zheng Q, Lee B. 2005. Mutations and
20 promoter SNPs in *RUNX2*, a transcriptional regulator of bone formation. Mol Genet Metab 86(1-2):257-68.
21
22 Otto F, Kanegane H, Mundlos S. 2002. Mutations in the *RUNX2* gene in patients with cleidocranial dysplasia. Hum Mutat
23 19(3):209-16.
24
25 Purandare SM, Mendoza-Londono R, Yatsenko SA, Napierala D, Scott DA, Sibai T, Casas K, Wilson P, Lee J, Muneer R,
26 Leonard JC, Ramji FG, Lachman R, Li S, Stankiewicz P, Lee B, Mulvihill JJ. 2008. De novo three-way chromosome
27 translocation 46,XY,t(4;6;21)(p16;p21.1;q21) in a male with cleidocranial dysplasia. Am J Med Genet A 146A(4):453-8.
28
29 Quack I, Vonderstrass B, Stock M, Aylsworth AS, Becker A, Brueton L, Lee PJ, Majewski F, Mulliken JB, Suri M, Zenker M,
30 Mundlos S, Otto F. 1999. Mutation analysis of core binding factor A1 in patients with cleidocranial dysplasia. Am J Hum
31 Genet 65(5):1268-78.
32
33 Unger S, Mornet E, Mundlos S, Blaser S, Cole DE. 2002. Severe cleidocranial dysplasia can mimic hypophosphatasia. Eur J
34 Pediatr 161(11):623-6.
35
36 Wang GX, Sun RP, Song FL. 2010. A novel *RUNX2* mutation (T420I) in Chinese patients with cleidocranial dysplasia. Genet
37 Mol Res 9(1):41-7.
38
39 Yamachika E, Tsujigiwa H, Ishiwari Y, Mizukawa N, Nagai N, Sugahara T. 2001. Identification of a stop codon mutation in
40 the CBFA1 runt domain from a patient with cleidocranial dysplasia and cleft lip. J Oral Pathol Med 30(6):381-3.
41
42 Yoshida T, Kanegane H, Osato M, Yanagida M, Miyawaki T, Ito Y, Shigesada K. 2002. Functional analysis of *RUNX2*
43 mutations in Japanese patients with cleidocranial dysplasia demonstrates novel genotype-phenotype correlations. Am J Hum
44 Genet 71(4):724-38.
45
46 Zhou G, Chen Y, Zhou L, Thirunavukkarasu K, Hecht J, Chitayat D, Gelb BD, Pirinen S, Berry SA, Greenberg CR, Karsenty
47 G, Lee B. 1999. CBFA1 mutation analysis and functional correlation with phenotypic variability in cleidocranial dysplasia.
48 Hum Mol Genet 8(12):2311-6.
49
50
51
52
53
54
55
56
57
58
59
60