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Review

Cleidocranial dysplasia and RUNX2-clinical phenotype–genotype correlation

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Runt-related transcription factor 2 (*RUNX2/Cbfa1*) is the main regulatory gene controlling skeletal development and morphogenesis in vertebrates. It is located on chromosome 6p21 and has two functional isoforms (type I and type II) under control of two alternate promoters (P1 and P2). Mutations within *RUNX2* are linked to Cleidocranial dysplasia syndrome (CCD) in humans. CCD is an autosomal skeletal disorder characterized by several features such as delayed closure of fontanels, dental abnormalities and hypoplastic clavicles. Here, we summarize recent knowledge about *RUNX2* function, mutations and their phenotypic consequences in patients.

Conflict of interest

All authors declare no conflict of interest.

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RUNX2 (CBFA1/AML3/PEBP2αA) is one of three members of RUNX family of Runt-related transcription factors (*RUNX1-3*). RUNX1 participates in hematopoietic stem cell differentiation (1) and mutations in this gene result in myeloid leukemia (2). *RUNX2* is necessary for osteoblast differentiation, skeletal morphogenesis (3, 4), and mutations in this gene lead to Cleidocranial dysplasia (CCD) in humans (OMIM #119600). RUNX3 acts as a tumor suppressor gene and was found to be linked to gastric cancer (5). This review will summarize the recent progress in studies of various mutations in RUNX2 and their phenotypic consequences in CCD.

RUNX2 structure

The human and murine *RUNX2* genes are localized on chromosome 6p21 (6) and 17 (7), respectively. Human *RUNX2* gene comprises a region of 223 kb in size (Chr6:45328317-45551082) (6) and consists of eight exons. Nevertheless, several authors have numbered exons differently depending on the use of alternate promoters and/or alternative splicing yielding 12 transcript variants encoding protein isoforms of RUNX2. For instance, Terry et al. (8) identified two alternate promotors and reported differential utilization of 3′ terminal exons in mouse and human. They also distinguished nine alternatively spliced exons among which exist three variants of exon 5 (5, 5.1, 5.2) and two alternatives of exon 6 (6, 6.1). Moreover, Makita et al. (9) distinguished three alternatively spliced isoforms that arises from omission of exon 5 and/or exon 7 (figures (8, 9)).

RUNX2 presents two in-frame ATG codons, both of which can serve as potential translation start sites.

Two promoters drive expression of both isoforms with different spatiotemporal pattern which suggests specialized functions of each (10). The gene expression from the proximal promoter (P2) generates type I RUNX2 mRNA differing at the 5′ end from the type II RUNX2 mRNA being under control of P1 distal promoter. P1 promoter is termed the 'bone-related' because of driving expression of the isoform widely associated with bones (11–16).

The translation start codon for the type I *RUNX2* is situated within exon 2 and encodes 507-amino acid protein starting with the MRIPV N-terminal sequence (MRIPV isoform; UniProt: Q13950-2) (17). The alternative start codon of type II localizes within exon 1, thus protein begins with the MASNS sequence and is 521-amino acid long (MASNS isoform; UniProt: Q13950-1) (18) (Fig. 1).

In spite of abundance of information, the consistent exon numbering has not been established. Specifically, the exon 1 (9, 12, 14, 19, 20) is termed by some authors 'exon 0' (21–24). Nonetheless, MASNS isoform is indicated as 'canonical' for osteogenesis (13). More importantly, recently NCBI annotation team removed the 507-aa MRIPV annotation (isoform c; NM_004348.3) due to insufficient support for existence of that isoform. Thus, two main remaining isoforms are 521-aa MASNS (isoform 1 or a; NM_001024630.3; NP_001019801.3) and 485-aa MRIPV without seventh exon (isoform 4 or d; NM_001278478.1; NP_001265407) (Fig. 1). Furthermore, NCBI and ENSEMBL nomenclature differs in transcript lengths and there is no clear distinction between type I (MRIPV) and type II (MASNS) RUNX2.

RUNX2 is a multidomain protein which in this review will be described in N to C terminal order. The deletion analysis identified the first 19 amino acid fragment as the first activation domain. Next, QA domain composed of glutamine–alanine repeats (23 Q and 17 A repeats) is located within N-terminus with a transactivation activity as well. Additionally, it has been shown that this domain can prevent heterodimerization of type II RUNX2 with CBFβ (core-binding factor subunit beta) (25). This transcriptional partner of RUNX proteins improves its DNA-binding affinity by promoting structural changes that reveal RUNX2-DNA interaction surface (26). CBFβ also protects and stabilizes RUNX proteins against proteolytic degradation (27, 28). CBFβ does not bind directly to DNA but heterodimerizes with Runt homologous domain (RHD). All members of RUNX family (RUNX1-3) are characterized by the presence of that highly conserved motif, homologous to pair-rule gene *runt* involved in body segmentation of *Drosophila melanogaster* (6). RHD consists of 128 amino acids and is responsible for DNA binding (17) to a DNA motif TGXGGTX (X is pyrimidine) (29). A 9-amino acid sequence (PRRHRQKLD) following RHD acts as nuclear localization signal (NLS). This domain is related to the NLS of c-Myc, and is conserved within all RUNX proteins (25).

The NLS domain is followed by the proline/serine/threonine rich region (PST) necessary for transcriptional activation of target genes (third transactivation domain) and containing the phosphorylation sites recognized by Akt kinase (30, 31). Also ERK/MAPK and PKA kinases phosphorylate RUNX2 protein but in different regions [for review (32)]. Next domain is the nuclear matrix targeting sequence (NMTS). This 38 amino acids motif determines RUNX2 subnuclear localization and displays binding affinity to other proteins like histone deacetylase (30, 33). At the very C-terminus

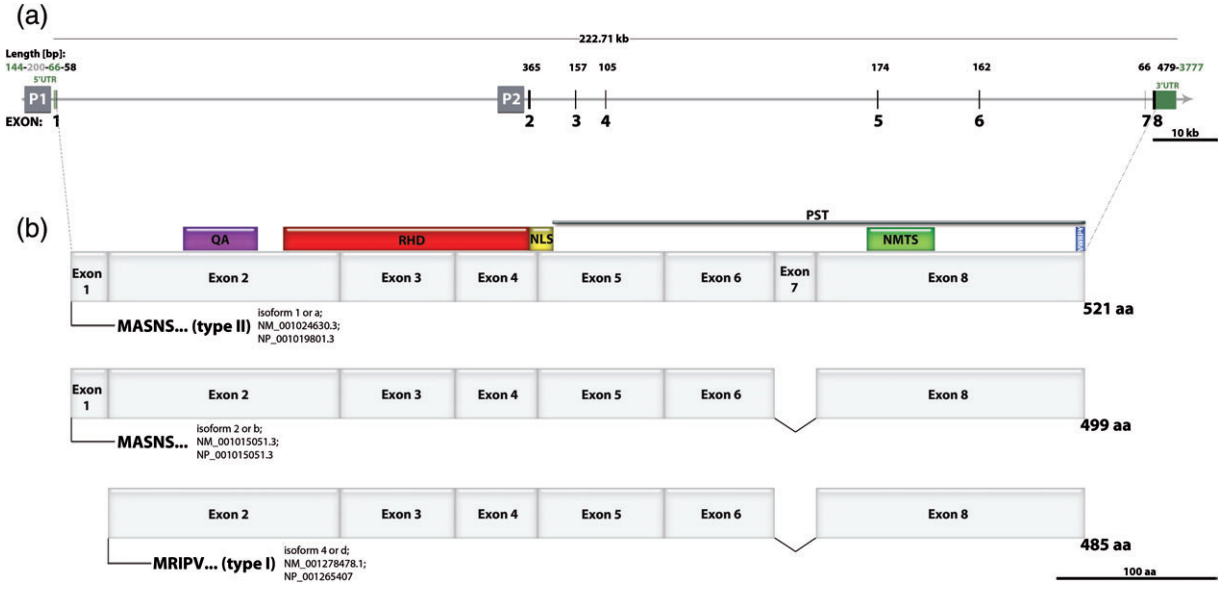

Fig. 1. Schematic representation of RUNX2. (**a**): *RUNX2* gene spans 222.71 kb and is under the control of two promoters: P1 and P2. Coding sequence are marked as black boxes and length of coding sequences is in block; green boxes indicate 5′UTR and 3′UTR, and UTR length is in green; grey line shows introns. (**b**) Representation of two types of RUNX2: type I and type II starting with MRIPV or MASNS, respectively. QA, polyglutamine and polyalanine domain, RHD, runt homologous domain; NLS, nuclear localization signal; PST, proline/serine/threonine rich region; NMTS, nuclear matrix targeting signal; VWRPY, carboxyterminal pentapetide.

is present the VWRPY pentapeptide sequence which is recognized as a repression domain. This motif is also conserved among all runt proteins and interacts with transcriptional corepressor TLE2 (transducing-like enhancer of split 2) (25, 34) (Fig. 1).

Biological function of RUNX2

Bones are formed through one of two ossification processes: (i) intramembranous based on direct conversion and differentiation of mesenchymal progenitors into osteoblasts occurring in parts of the craniofacial skeleton as well as the clavicle (35) or (ii) endochondral ossification. In this process, mesenchymal cells differentiate into chondrocytes, which undergo hypertrophy leading among others to the secretion of vascular endothelial growth factor (VEGF) that attracts blood vessels, and permits the formation of bone marrow cavity concomitant with osteoblast differentiation.

RUNX2 is essential for differentiation of osteoblast during both intramembranous and endochondral ossification. Hypertrophy of chondrocytes is positively regulated by RUNX2 which activates the expression of type X collagen (36). Other factors such as SOX9, IHH (Indian hedgehog), PTHRP (parathyroid hormone-related protein) and BMPs (bone morphogenetic proteins) are also responsible for tight regulation of endochondral bone formation (37–40). The initial osteoblast differentiation is triggered through IHH induced by RUNX2 (41–43). RUNX2 activates the IHH promoter and thus stimulates IHH expression in prehypertrophic chondrocytes, which regulates chondrocyte proliferation and maturation through IHH/PTHRP loop (Fig. 2) (44, 45). Additionally, IHH induces expression of RUNX2 in neighboring perichondrium but full commitment to osteoblasts requires Osterix (Osx) transcription factor for both types of ossification (46). Also RUNX2, as a transcription factor involved in bone formation, upregulates the expression of bone matrix genes including collagen I, osteocalcin, bone sialoprotein and osteopontin.

Normal bone development requires precise spatiotemporal regulation of *RUNX2* expression and several factors have been associated with this process, and it has been a subject of a recent review (47). Some factors such as Msh homeobox 2 (Msx2), twist or promyelocytic leukemia zinc-finger protein (PLZF), Osx participate in the induction of RUNX2 expression. Others like sex-determining region Y-box 9 (Sox9) acts as a repressor due to interaction with runt domain. Additionally, STAT family transcription factor (Stat1) inhibits nuclear localization of RUNX2. It is worth mentioning that extending recent knowledge about complex regulation of bone formation by transcription factors is essential for further studies in field of TF-targeted gene therapy (48).

RUNX2 type I and type II regulate expression of bone-related genes, however it has been suggested that they might have different functions in skeletogenesis (10). Type I, is expressed in T cells, osteoblasts and chondrocytes (49) and contributes to the intramembranous bone development (50). The expression of Type II increases during osteoblast differentiation (14, 51), and it

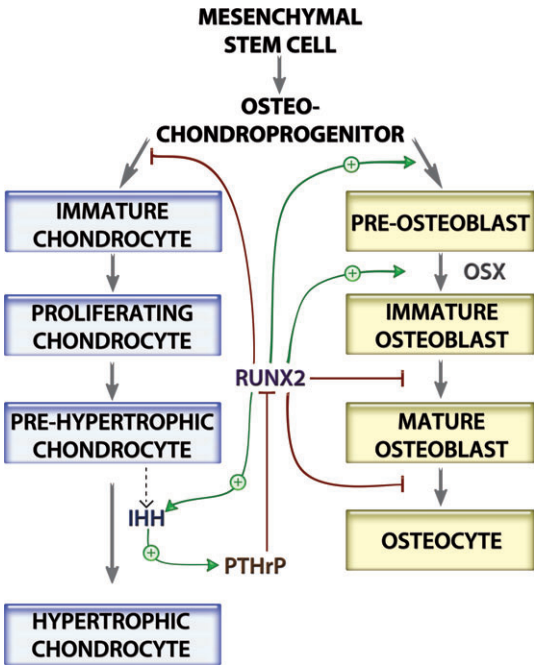

Fig. 2. Regulation of osteoblast and chondrocyte maturation by RUNX2. IHH, Indian hedgehog; PTHrP, parathyroid hormone-related protein; OSX, osterix; green lines indicate induction, red, inhibition and dashed line shows the expression of IHH.

has been shown to be responsible for endochondral bone formation (10). Furthermore, it was reported that these isoforms vary in their dependence on the Cbfβ cofactor (52). Moreover, *RUNX2* is also responsible for regulating expression of genes that, when deregulated, cause craniosynostosis like NEL-like 1 (NELL1). It also plays a role in hormonal regulation of bone formation by taking part in glucocorticoid signaling pathway necessary for normal osteogenesis (48).

Besides its osteogenic function, *RUNX2* has also been linked to breast and prostate cancer and specifically with more aggressive phenotype of these diseases (31, 53–55). Additionally, high expression of RUNX2 is observed in cancers that have high tendency to metastasize to skeleton (56).

Animal models to study Runx2 function

Targeted inactivation of the gene in mouse results in the phenotype similar to CCD in humans. Specifically, mice were displaying skeletal, CCD-like, abnormalities and had decreased body weight. In heterozygous mice ($RUNX2^{+/-}$) formation of the tooth primodorium was delayed but otherwise normal. Since mice present only one dentition, development of supernumerary teeth does not occur like it does in humans with CCD (18). Homozygous ($RUNX2^{-/-}$) mice died soon after birth, and during postmortal examination, failure in bone ossification was detected (48).

Number of experiments have been performed to establish the role of RUNX2 mutations in CCD pathology. Lou et al. (57) generated a hypomorphic *RUNX2* mutant

allele (*RUNX2*^{neo7}) where only part of the transcript was processed to full length. This model allowed them to determine the minimal expression level of *RUNX2* required for normal skeletal development. Specifically, PGK (cGMP-dependent protein kinase)-driven neomycine resistance cassette (PGK*neo*) was inserted into intron 7 interfering with splicing and reducing the expression of wild-type mRNA. The insertion of PGKneo into exon 7 resulted in alternative splicing between exon 7 of *RUNX2* and neo cassette. This led to expression of mutant protein lacking C-terminus (thus still including NMTS and a repression domain) (57). It is not excluded that observed phenotype can be a dominant negative activity resulting in the production of partial protein. Nonetheless, their findings demonstrate that less than 70% of wild-type RUNX2 level resulted in CCD phenotype, whereas mice expressing RUNX2 at higher level showed no skeletal abnormalities. Interestingly, it was also reported that RUNX2 overexpression can also cause dysplasia. Experiment resulted in mice suffering from osteopenia along with bone fractures (58).

Bone unrelated function of RUNX2 has been described in zebrafish (Danio rerio) where the gene is an essential determinant for ventral zygotic genes. Importantly, two additional orthologues of mammalian gene called *RUNX2a* and *RUNX2b* sharing sequence and structure homology have been identified in zebrafish but with divergent expression patterns explaining the retention of duplicate genes through evolution (59). Further studies in zebrafish revealed that RUNX2b appears to be the only known regulator of Vent family of transcription factors (60). Members of this family, VOX, VENT and VED, play a role as repressors in dorsoventral axis establishment (61). Depletion of RUNX2b results in loss of expression of VOX, VENT and VED and consequently in embryo dorsalization. Silencing experiments showed that RUNX2b2 regulation of VENT transcription factors is direct, by interacting with promoter. Additionally, rescue experiments in zebrafish using murine RUNX2 type 2 shown that it can substitute for zebrafish orthologues, suggesting that RUNX2 role in axis formation could be evolutionarily conserved (60).

Recently, it has been shown that Runx2 regulates fate of epithelial cells of mammary glands during breast cancer development. Deletion of Runx2 during pregnancy in mice decreased differentiation by disrupting population of alveolar progenitor cells while exogenous overexpression of Runx2 in mammary epithelial cells impaired milk production. These findings suggest that decreased amount of endogenous Runx2 is required for full differentiation. Additionally, it was observed that overexpression of RUNX2 led to transition changes in normal mammary epithelial cells while its deletion in developing breast cancer inhibited cancerous cellular phenotype (62).

Runx2 in CCD

CCD was initially described as cleidocranial dysostosis since it was believed to affect only membranous-derived bones. Further clinical studies have shown that the disorder affected skeleton in general, not only skull and clavicles, so it should be considered as dysplasia rather than dysostosis (63). Spectrum of phenotypes is wide, ranging from mildly affected patients with dental and clavicles anomalies only to individuals with severe defects in skeletal development. Intriguingly, these phenotypic variations occur even within one family (64). The most common features of CCD are delayed closure of fontanels and cranial sutures, brachycephaly, depressed nasal bridge, supernumerary teeth, delayed eruption of permanent teeth, Wormian bones and hypoplasia of clavicles (65). Since it was suggested that RUNX2 regulates not only skeleton development but also the expression of mesenchymal tissue, controlling differentiation o dental epithelium, it could partially explain dental abnormalities. Mutations associated with severe dental abnormalities (supernumerary teeth, eruption failure) affect Runt-domain. In contrast, mild dental problems are correlated with mutations outside the Runt-domain (66–68).

Mutations in human RUNX2

Majority of RUNX2 mutations in individuals suffering from classic CCD occur in the *runt* domain and the most common DNA disruptions are missense mutations that prevent RUNX2 binding to DNA or nonsense mutations that result in biosynthesis of truncated protein. While missense mutations are uniquely found within runt-domain region, nonsense and frame-shift types were described throughout the gene (22, 63, 66, 69–80) (Fig. 3, Table 1).

A screening analysis within a group of Japanese patients was carried out to investigate the link between a mutation and its phenotypic manifestation (67). It described 15 heterozygous mutations among 24 families and 1 polymorphism. Most of discovered mutations were missense and all of them located within runt-domain which confirmed results reported previously (81). Besides six missense, seven nonsense and frame-shift mutations, two exon-skipping mutations between third exon and intron were also identified. One of them, IVS3+3delAAGT, occurred within splice-donor signal leading to in-frame skipping of the complete third exon. This resulted in decreased functionality of donor site where GT motif was followed by segment rich in pyrimidine (AAGTACTC) (67). Second exon-skipping mutation, cIVS3+2T>C, was present at the same donor site. It is worth mentioning that different study also described skipping of exon 5 although in this case runt domain and NLS remained intact (81). Extensive genetic analysis of patient groups with CCD led to discovery of several polymorphisms. D170, synonymous mutation, caused by c510T>C was identified within second exon on one allele in one patient. Mother of the patients was a homozygous carrier of this mutation as well (67). In the second case, polymorphism – A80 (c240A>G) – was found on one allele of patient and the mother. The causative silent transition was located within Q/A region (65). Besides the missense mutations (Table 1), several other mutations have been reported such as a mutation in runt-domain, T200A that does

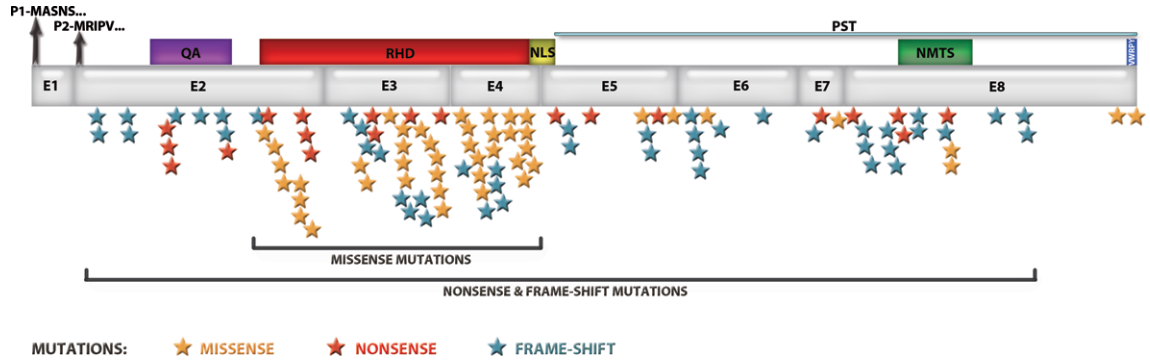

Fig. 3. Schematic representation of mutation types within *RUNX2* gene. QA, polyglutamine and polyalanine domain; RHD, runt homologous domain; NLS, nuclear localization signal; PST, proline/serine/threonine rich region; NMTS, nuclear matrix targeting signal VWRPY-carboxyterminal pentapetide.

not affect binding to DNA and is correlated with mild CCD phenotype manifested only by delayed eruption of permanent teeth (81). Electrophoretic mobility shift assay (EMSA) of wild-type and mutant protein revealed that T200A mutation did not affect neither the binding to OSE2 nor transactivation activity. *In vivo* effect of T200A mutation remains thus unknown. One could speculate that the mutation may be hypomorphic but *in vitro* studies are not sufficiently sensitive. Alternatively, the mutation may have neomorphic nature and affect different functions of runt domain (81).

Besides point mutations, intragenic microdeletions have been also reported for RUNX2 and linked to CCD. A family has been identified having intragenic deletion covering region between exons 2 and 6, including runt domain and NLS. Mutation was detected within three generations of this family and all patients presented short stature and small hands (82, 83). Another case of microdeletion involved not only RUNX2 but also other gene, VEGF, located 4.6 Mb away. Phenotype reflected the defects for both genes: characteristic features of CCD and degenerated pattering of pharyngeal arch-derived arteries, characteristic for VEGF, were observed (84).

Besides microdeletion, the genomic region containing RUNX2 has also been microduplicated. Recently, large duplication downstream of RUNX2 has been discovered in CCD patients who suggested that regulatory sequences of RUNX2 may be disrupted in this case. Family did not participate in further analysis, so segregation analysis and additional gene diagnostics of a possible pathogenic effect of the duplicated region were not carried out (85).

Moreover, a case of family where three children, one of them a half-brother, presented all features of classic CCD phenotype while the mother examination showed no abnormalities (86). Sequencing analysis revealed 864insC mutation within exon 6 in affected family members and a single nucleotide polymorphism IVS6+46G>A in the same exon in the mother. High resolution melting analysis was performed in order to test for maternal mosaicism. Results, showing different melting profiles in each patient, pointed to a possibility of additional sequence variation in mother. Further, subcloning and resequencing identified 864insC mutation in several clones isolated from blood or buccal swab samples. It was found in *cis* to IVS6+46G allele therefore confirming low-level mosaicism in mother (86).

Recently, a novel duplication 181_189dupGCGGGC GGCT was reported resulting in expansion of polyalanine residues within Q/A domain and, in consequence, in CCD phenotype. Further experiments confirmed that increasing number of alanine residues has a significant impact on RUNX2 transcriptional activity. Expansion by 3 Ala did not impair the normal activity of RUNX2 while 10 Ala expansion significantly decreased it. The Ala expansion caused rapid intracellular aggregation of RUNX2 resulting in the protein exclusion from the nuclei (87). Recently, the plausible connection between the number of alanine repeats within QA domain and the absence of clavicles in patients with CCD has been revealed. The analysis of polyalaninie tracts in warm blooded mammals (including human) revealed that species having 17 or 18 alanine tracts have clavicles, while the absence of repeats or its increased number is associated with the reduction or lack of clavicles (88). Polyalanine repeats however are not the only ones linked with CCD phenotype since a case of polyglutamine tract insertion was reported (213_214insCAGCAGCAGCAG), also in Q/A domain, resulting in reduced transactivation of RUNX2 (89).

Genotype–phenotype correlation

It has been notoriously difficult to establish direct genotype–phenotype correlation for RUNX2 because of very variable phenotypic penetrance of the mutations (4). Most mutations occurring within runt-domain result in a classical CCD phenotype. There are however some intriguing exceptions like heterozygous T200A mutation. It was found in father who suffered only from dental abnormalities, but his two children showed all the features of CCD (81). Another example of an unusual genotype–phenotype correlation is an insertion mutation localized at the beginning of the transcript, between two translational start sites, 90insC. It was described to be associated with mild CCD phenotype and abnormal dentition (81). Another study investigated phenotype–genotype correlation in a large group of patients. Patients from one group had mutations within

Table 1. Precise location of mutations within *RUNX2* gene[a]

	Mutation type		
Exon number	Nonsense	Missense	Frame-shift
1 (1–19 aa)			
2 (20–141 aa)	c.190 C>T [Q64X] c.193 C>T [Q65X]] c.196 C>T [Q66X] c.273T>A [L93X] c. 276_277ins17 [L93X] c.334G>T [E112X] c.383 C>A [S128X] c.389G>A [W130X] c.396 C>A [C132X]	c.338T>G [L113R] c.354 C>T [S118R] c.362T>G [F121C] c.366_367delGTinsTC [C123R] c.391 C>G [R131G] c.391 C>T [R131C] c.391 C>A [R131S] c.407T>C [L136P]	c.90_91insC c.92_93insCGGT c.134_135insGTCC c.136delC c.207_208ins13 c.230-276del c.274delC c.282delG c.329delC
3 (142–194 aa)	c.481-482delGA [D161X] c.484G>T [E162X] c.535 C>T [R179X] c.577 C>T [R193X]	c.467T>A [V156D] c.475G>C [G159R] c.506G>C [R169P] c.523 A>G [M175V] c.524T>A [M175L] c.524T>G [M175R] c.526 C>T [R176W] c.548T>C [F183S] c.560T>C [F187S] c.568C>T [R190W] c.569G>A [R190Q] c.572G>A [S191N] c.578G>A [R193Q]	c.453delT c.469insA c.476delG c.495delT c.522insA c.532delC c.549delC c.553_554delCT
4 (194–229 aa)		c.590T>C [F197S] c.599C>T [T200I] c.602T>A [I201K] c.612A>T [K204N] c.614C>G [T205R] c.617 C>T [T206I] c.627 A>T [Q209H] c.631G>C [A211P] c.632G>A [R211Q] c. 635- 638delCCTA [T212I] c.652 A>C [L218Q] c.652 A>G [L218E] c.662T>G [V221G] c.667G>A [G223R] c.673 C>T [R225W] c.674G>A [R225Q] c.674G>T [R225L] c.682 A>G [R228G]	c.592delA c.624_625insCC c.635_638delCCTA c.636delC c.644delG
5 (229–287 aa)	c.694 C>T [Q232X] c.793 C>T [Q266X] c.838 C>T [Q280X]	c.817G>A [D273N] c.859G>A [D287N]	c.718_721del c.722delT c.824delG c.831delT
6 (287–341 aa)		c.896 C>T [P299L]	c.873_874delCA c.879_885del c.884delC c.887delC c.915delC c.977delG
7 (341–363 aa)	c.1056G>A [W352X]	c.1085 C>T [A362V]	c.1043 C>T (splice region variant)
8 (363–521 aa)	c.1096G>T [E366X] c.1171 C>T [R391X] c.1182 T>A [Y394X] c.1254 C>A [Y418X]	c.1259 C>A [T420N] c.1259 C>T [T420I] c.1531G>A [G511S] 1565G>C [X522S] (stop lost)	c.1111_1129del19 c.1119delC c.1127_1128insT c.1153_1154delAG c.1157delG c.1169delC c.1205delC c.1205_1206insC c.1250_1251delAC c.1335_1336delCT c.1379_1380insC c.1385insG

[a]The table contains the data as available in public databases on April 2016.

Table 2. Examples of genotype–phenotype correlation

Family	Mutation	Type	Delayed closure of sutures	Hypoplastic clavicles	Supernumerary teeth	Height	Other phenotype anomalies
(87) Patient	181_189dupGC GGCGGCT	Leading to polyalanine tract expansion	+	+	Multiple	Short	Scoliosis Midface hypoplasia Sloping shoulders Brachydactyly (mild)
(89) Patient	213_214insCAGC AGCAGCAG	Leading to polyglutamine tract expansion	+	Absence of clav.	Multiple	Short	Defected occipital bones Sclerosis of cranial base Multiple wormian bones Frontal bossing Midface hypoplasia
(92)							
Patient 1	674 G→A	Missense	NA	+	Multiple + eruption failure	NA	NA
Patient 2	1119delC	Framshift	NA	+	Multiple	NA	NA
Patient 3	1171 C→T	Missense	NA	Absence of clav.	2 + eruption failure	NA	NA
(90)							
Family 1 (boy)	273 T→A	Nonsense	NA	+	9	Normal	NA
Family 2 (girl)	633 C→T	Missense	+	Absence of clav.	1	Short	NA
(74) Patient 1 (+10 family members)	407 T→C	Missense	–	Aplastic Aplastic or normal	Teeth maloclusion	Short	Asymmetric face Bossing of forehead Hyperthelorism Sloping shoulders Hyperplastic fingernails and toenails Wormian bones -hypoplastic pubic bones
(63) Patient	1182 T→A	Nonsense	+	+	Multiple	Short	Hypoplastic distal phalanges High weight Coxa vara Delayed ossification of pubic bone Flattened face Broad forehead Hypertelorism Reduced length and increased width of nose
(86)							
Patient 1			+	+	+	Short	NA
Patient 2	864insC	Nonsense	+	+	+	Short	NA
Patient 3	IVS6+46G>A	SNP	+	+	+	Short	NA
Patient 4	IVS6+46G>A	SNP	–	–	–	Normal	Normal

NA, not applicable; SNP, single nucleotide polymorphism.

runt-domain. Individuals from the second group had runt-domain unaffected while did have all the features of CCD. This suggests that phenotype may be caused by mutation in a regulatory region on RUNX2 or in other gene interacting with RUNX2. Besides the range of CCD phenotypes present in these patients, most common difference was the height score, significantly lower in group with impaired runt-domain. Findings suggested that growth of the skeleton deteriorates when one allele activity is lower by around 50% and it progressively worsens with further loss of gene activity (67).

There is also a weak genotype–phenotype correlation in case of dental aspect of CCD phenotypes, especially with respect to teeth development (90). Among several hypotheses trying to explain phenotype diversity, one suggests that intramembranous bone formation may require more RUNX2 in comparison to endochondral-mediated skeletal bones development (67). Additionally, recent report demonstrates that there are more dental-related phenotypes correlated with CCD describing a sporadic case of 14 supernumerary teeth in 20-year old individual. Patient was found with, previously unreported, fused primary teeth due to c.578G>A (R193Q) mutation (68). There are many similarities between osteo- and odontogenesis. This, together with the role that osteoblasts play in the formation of mandible and maxilla, explains the severity of craniofacial phenotypes in CCD patients (91).

In summary, RUNX2 is a master regulatory gene for skeletal development and morphogenesis. Mutations within the gene or its regulatory regions are commonly found in CCD patients. Further studies are however necessary to establish in more detail the genotype–phenotype correlation (Table 2) (63, 74, 86, 87, 89, 90, 92) and to fully understand mechanism of underlying pathogenesis of CCD.

Acknowledgements

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