Published in final edited form as:

Metabolism. 2018 March ; 80: 48–56. doi:10.1016/j.metabol.2017.08.002.

CLINICAL AND EXPERIMENTAL ASPECTS OF NOTCH RECEPTOR SIGNALING: HAJDU-CHENEY SYNDROME AND RELATED DISORDERS

Ernesto Canalis^{a,b,*}

^aDepartment of Orthopaedic Surgery, UConn Health, Farmington, CT, 06030

^bDepartment of Medicine, and the UConn Musculoskeletal Institute, UConn Health, Farmington, CT, 06030

Abstract

There are four Notch transmembrane receptors that determine the fate and function of cells. Notch is activated following its interactions with ligands of the Jagged and Delta-like families that lead to the cleavage and release of the Notch intracellular domain (NICD); this translocates to the nucleus to induce the transcription of Notch target genes. Genetic disorders of loss- and gain-of-NOTCH function present with severe clinical manifestations. Hajdu Cheney Syndrome (HCS) is a rare genetic disorder characterized by acroosteolysis, fractures, short stature, neurological manifestations, craniofacial developmental abnormalities, cardiovascular defects and polycystic kidneys. HCS is associated with *NOTCH2* gain-of-function mutations. An experimental mouse model of the disease revealed that the bone loss is secondary to increased osteoclastogenesis and bone resorption due to enhanced expression of receptor activator of nuclear factor kappa B ligand (Rankl). This would suggest that inhibitors of bone resorption might prove to be beneficial in the treatment of the bone loss associated with HCS. Notch2 is a determinant of B-cell allocation in the marginal zone of the spleen and “somatic” mutations analogous to those found in HCS are associated with B-cell lymphomas of the marginal zone, but there are no reports of lymphomas associated with HCS. In conclusion, HCS is a serious genetic disorder associated with *NOTCH2* mutations. New experimental models have offered insight on mechanisms responsible for the manifestations of HCS.

Keywords

Hajdu Cheney Syndrome; genetic disorders; Notch; osteoporosis; acroosteolysis

*Corresponding author: Ernesto Canalis, M.D., Department of Orthopaedic Surgery and Medicine, UConn Health, Farmington, CT, 06030-5456, USA, Tel: (860) 679-7978, Fax: (860) 679-1474, canalis@uchc.edu.

DISCLOSURE STATEMENT

The author has nothing to disclose.

AUTHOR'S CONTRIBUTIONS

EC conceived, wrote and edited the manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Notch Receptors

Notch has emerged as a novel signal that plays a key role in cell fate decisions and in the differentiation and function of cells of multiple lineages. There are four Notch receptors (Notch1 to 4) and five classic Notch ligands termed Jagged (Jag)1 and Jag2, and Delta-like (Dll)1, Dll3 and Dll4 [1]. Notch and its ligands are transmembrane proteins that retain structural similarity and mediate communication between neighboring cells. The extracellular domain of Notch is the site of interaction with its ligands, and consists of multiple epidermal growth factor (EGF) repeats. At the junction of the extracellular and the transmembrane domain of Notch rests the negative regulatory region (NRR). This is the site of cleavage required for the initial activation of Notch following its interactions with Jag or Dll ligands [2,3]. The intracellular domain of Notch (NICD) consists of an Rbpj κ -association module (RAM) domain, ankyrin repeats and nuclear localization sequences. These domains are required to induce the transcription of Notch target genes [4,5]. The C-terminus of Notch contains a proline (P)-, glutamic acid (E)-, serine (S)-, threonine (T) (PEST) domain, which is targeted by ubiquitin ligases. This domain is required for the proteasomal degradation of Notch (Figure 1). Notch ligand interactions lead to its proteolytic cleavage and the release of the NICD, and its translocation to the nucleus, where it forms a complex with recombination signal-binding protein for Ig of κ region (Rbpj κ) and mastermind-like (Maml) to regulate transcription [6–8]. The human ortholog of Rbpj κ is termed CBF1, Suppressor of Hairless, Lag 1 (CSL) [9]. The interaction of the NICD, Rbpj κ and Maml leads to the displacement of transcriptional repressors and recruitment of activators of transcription by the NICD to induce the expression of Notch target genes (Figure 2). This has been termed the Notch canonical signaling pathway, and results in the induction of hairy and enhancer of split (Hes)1, Hes5, Hes 6 and Hes7, and HES-related with YRPW motif (Hey)1, Hey2 and HeyL [10–12]. Importantly Rbpj κ , and not the NICD, binds to DNA. Although Rbpj κ acts as a transcriptional activator in Notch signaling, in the absence of Notch-ligand interactions Rbpj κ acts as a transcriptional repressor due to its ability to recruit transcriptional co-repressors and histone deacetylases. This complex is disrupted by the NICD, leading to the recruitment of co-activators of transcription, histone acetylation and enhanced transcription of target genes [13,14]. Cyclin-dependent kinase 8 phosphorylates the PEST domain of the NICD, causing the disassembly of the ternary NICD, Rbpj κ , Maml complex followed by the ubiquitination of the NICD by E3 ubiquitin-ligases and the degradation of Notch [7]. This is necessary to avoid the persistence of Notch signaling which would lead to a gain-of-Notch function.

Although the four Notch receptors retain structural similarities, their function is not the same. Functional differences between the four Notch receptors are related to individual structural properties, to their temporal and cellular expression, to variations in the affinity of the Notch extracellular domain for its ligands and to differential interactions of the NICD with Rbpj κ [15,16]. For example, Notch1, 2 and 3 are expressed in bone, whereas Notch4 is not detectable in skeletal cells [17]. Notch3 is predominantly expressed in vascular cells, and as a consequence plays a key role in vascular development, and Notch4 is expressed in endothelial cells and plays a role in the development of vertebrate endothelium [18]. *Notch4* mRNA is detected in the endothelium of highly vascularized adult tissues, such as lungs,

heart and kidney. Notch needs to interact with ligands present in neighboring cells of the same or different lineages for its activation, and Jagged1 is the prevalent ligand expressed by skeletal cells [19]. Notch1 and Notch2 are structurally similar, but either the *Notch1* or *Notch2* inactivation results in embryonic lethality, indicating that their functions are not redundant [20–22]. The structure of Notch3 diverges, and the amino acid identity of the Notch3 NICD is significantly different from that of Notch1 and 2 [23]. This, as well as a distinct cellular pattern of expression, confers to Notch3 a unique physiological role. The actions of Notch are cell-context dependent and cellular responses are linked to the stage of cell maturation at the time of Notch activation. For example, when Notch1 is activated in osteoblast precursors it inhibits osteoblast differentiation, whereas when activated in mature osteoblasts and osteocytes Notch1 inhibits bone resorption and causes an osteopetrotic phenotype [24,25]. Depending on the cell environment, Notch 2 can lead to progenitor cell depletion or to mature cell reallocation [26,27]

2. Hajdu Cheney Syndrome – Clinical Aspects

In 1948, Hajdu and Kauntze reported a young accountant suffering from severe osteoporosis, acroosteolysis and neurological complications that years later took his life [28]. The clinical condition was reported as a syndrome by Cheney in 1965 [29]. Hajdu Cheney Syndrome (HCS) (OMIM 102500) is a rare genetic disorder, and under 100 cases have been reported although its prevalence is probably higher. HCS is characterized by craniofacial developmental abnormalities that appear at a young age and evolve as the child matures (Table 1). There is high clinical variability and a phenotypical evolution of the disease. Subjects with HCS present with facial dysmorphism, synophrys and epicanthal folds. The distance between the eyes is short, and thick eyebrows extend toward the midline; there is malar hypoplasia, a long and smooth philtrum and micrognathia [30]. The nasal bridge is flattened and becomes broad, and facial features are coarse and the neck is short.

The craniofacial developmental defects include wormian bones, open sutures, platybasia and basilar invagination. These are serious manifestations of the disease since they can cause severe neurological complications, including central respiratory arrest and sudden death. Abnormal dental eruptions, and tooth decay with premature loss of teeth are common [31]. Short stature, generalized and local joint hypermobility are reported frequently. Vertebral abnormalities include fractures, kyphosis and scoliosis and long bone deformities. A limited number of subjects undergoing bone biopsies have been reported, but the results have not been conclusive and findings included normal, increased and decreased bone remodeling [32–34]. In selected individuals, an increased number of osteoclasts was found suggesting that enhanced bone resorption is responsible for the bone loss [34].

A characteristic feature of HCS is acroosteolysis of the distal phalanges of fingers and toes. This is associated with an inflammatory reaction, pain and swelling, and the process results in the loss of the distal phalanges and shortening of hands and feet. Biopsies of the lesions have revealed an inflammatory process, neovascularization and fibrosis, but the mechanism responsible for the lesions is not entirely clear. It might be related to effects on Notch2 on B-cell allocation, and subjects with HCS can display splenomegaly [35]. The mechanisms of the acroosteolysis are not considered to be the same as those responsible for the generalized

bone loss and fractures observed in HCS. Patients with HCS can present with cardiovascular defects, including patent ductus arteriosus, septal defects, and mitral and aortic valve abnormalities associated with valvular insufficiency or stenosis [36,37]. About 10% of subjects with HCS have polycystic kidneys, and serpentine fibula-polycystic kidney syndrome is most likely the same disorder as HCS [38–40].

3. Hajdu Cheney Syndrome – Genetic Aspects

Mutations in Notch receptors, their ligands, intracellular partners and factors that modify Notch signaling have been associated with genetic disorders, many of them affecting the skeleton. The mutations result either in a gain- or loss-of-Notch function and include genes encoding enzymes that modify the Notch receptor as well as its target genes (Table 2). Genes encoding glycosylating enzymes include *EOGT* (EGF domain specific O-linked N acetylglucosamine transferase) and *LFNG* (Lunatic fringe); *CHSYS1* encodes chondroitin sulfate synthase, a glucuronyltransferase that downregulates Notch signaling. *MESP* (mesoderm posterior bHLH transcription factor) and *HES7* are Notch target genes, and *HES7* regulates *LFNG* expression.

The inheritance of HCS is autosomal dominant although sporadic cases have been reported frequently. Exome-wide sequencing of families affected by HCS demonstrated that HCS is associated with mutations in exon 34 of *NOTCH2* [35,41–45]. The mutations are nonsense mutations or short deletions leading to the formation of a termination codon upstream of the PEST domain. Since the PEST domain is required for the ubiquitination and degradation of *NOTCH2*, the mutations lead to the translation of a truncated *NOTCH2* protein that is presumably stable; and as a consequence, a gain-of-*NOTCH2* function occurs. The truncated *NOTCH2* intracellular domain retains all sequences necessary to form a complex with *CSL* and *MAML*, and regulate transcription. Similar *NOTCH2* mutations in exon 34 leading to a truncated protein devoid of the PEST domain have been associated with B-cell lymphoma and were shown to have enhanced *NOTCH2* signaling [46,47]. The increased *NOTCH2* activity implies the existence of *NOTCH* ligand interactions under basal conditions with the subsequent activation of Notch signaling which is enhanced by the prolonged life of the mutated *NOTCH2* protein. The diagnosis of HCS can be established by documentation of mutations in exon 34 of *NOTCH2* that lead to the creation of a termination codon upstream of the PEST domain.

4. Hajdu Cheney Syndrome and Related Genetic Disorders

Lateral Meningocele (LMS) or Lehman Syndrome (OMIM 130720), is a rare genetic disorder with some phenotypic overlap with HCS [48]. LMS is characterized by craniofacial anomalies, hypotonia and meningocele with related neurological dysfunction [49] (Table 3). Skeletal manifestations are numerous, and they include craniofacial developmental defects, short stature, scoliosis and osteopenia [48,50]. Although LMS and HCS are distinct disorders, select phenotypic characteristics overlap [51]. Indeed, a case of LMS was reported initially as having HCS [48]. The subject, a young child, presented with severe scoliosis and dural ectasia surrounding the spinal cord as well as features of HCS [52]. Because of the spinal cord meningoceles, the diagnosis was questioned and the subject was subsequently

confirmed to have LMS [51,52]. Exome sequencing of subjects afflicted by LMS has demonstrated the presence of point mutations or short deletions in exon 33 of *NOTCH3*, upstream of the PEST domain. Like in HCS, the mutations lead to the translation of a truncated and stable protein, devoid of the PEST domain, and presumably a gain-of-*NOTCH3* function [51–53]. Interestingly, no genetic disorders associated with similar mutations in *NOTCH1* or *NOTCH4*, leading to the translation of a truncated protein devoid of the PEST domain and gain-of-function, have been reported. Somatic mutations in exon 34 of *NOTCH1* are associated with T-cell acute lymphoblastic leukemia [54].

5. Experimental Models of Hajdu Cheney Syndrome

To gain an understanding of the mechanisms responsible for the HCS phenotype, our laboratory created a mouse model reproducing the mutation found in a subject affected by the disease and presenting with bone loss and fractures. The subject harbored a 6949C>T mutation, (translation start site = 1) in exon 34 of *NOTCH2* upstream of the PEST domain. To create a mouse model of HCS, we introduced the human mutation into the corresponding nucleotide of the mouse genome, namely a 6955C>T mutation, by homologous recombination and verified by DNA sequencing in F1 pups [55]. The mutation created a STOP codon in exon 34 of *Notch2*, and the translation of a predicted truncated Notch2 Q2319X protein of 2318 amino acids. Homozygous *Notch2HCS* mutant mice died at birth, and heterozygous mice were smaller and had shorter limbs than controls. This is in accordance with the known inhibitory effects of Notch on chondrogenesis and is reminiscent of the short stature displayed by humans with HCS [56].

Microcomputed tomography (μ CT) of the distal femur revealed that *Notch2HCS* mutant mice of both sexes have a pronounced decrease in trabecular bone volume due to a reduction in the number of trabeculae, and to a lesser extent to a decrease in trabecular thickness. The decreased cancellous bone volume was associated with decreased connectivity. Cortical bone of *Notch2HCS* mutants was thin and porous, and total area as well as bone area were reduced. Cancellous bone histomorphometry of femurs from *Notch2HCS* mutant mice demonstrated decreased bone volume/tissue volume, an increase in osteoclast number and eroded surface, and no change in osteoblast number or bone formation rate. The results indicated that enhanced bone resorption without a coupled bone-forming response was responsible for the skeletal phenotype. Cortical bone histomorphometry confirmed an increase in endocortical osteoclast number and eroded surface in *Notch2HCS* mutant mice.

The expression of the Notch target genes *Hey1*, *Hey2* and *HeyL* and the activity of transiently transfected Notch reporter constructs were increased in cells from *Notch2HCS* mice confirming enhanced activation of Notch signaling. In accordance with the resorptive phenotype observed, there was an increase in the expression of *Tnfrsf11*, encoding for Rankl, in femurs, osteoblasts and osteocytes from *Notch2HCS* mutant mice, and no changes in *Tnfrsf11b*, encoding for the Rankl decoy receptor osteoprotegerin. The increase in *Tnfrsf11* was dependent on the activation of Notch signaling because it was prevented by the addition of the γ -secretase inhibitor LY450139, known to prevent the cleavage of Notch and release of the NICD [57]. The *Notch2HCS* mutation resulted in enhanced osteoclastogenesis *in*

vitro, and there was an increase in the number of osteoclasts in bone marrow cell cultures from *Notch2HCS* mutants compared to controls.

The phenotype of the *Notch2HCS* mutant mouse recapitulates aspects of HCS in humans. The osteopenic phenotype of the *Notch2HCS* mutant mouse can be explained by an increase in bone resorption and osteoclastogenesis [17,58]. The increased number of osteoclasts and bone resorption appeared to be secondary to enhanced Rankl expression in cells of the osteoblast lineage and possibly to direct effects of Notch2 on osteoclastogenesis. Notch2 enhances osteoclastogenesis directly by interacting with nuclear factor κ B on *Nfatc1* regulatory elements in cells of the osteoclast lineage [58]. Currently, it is not known whether the induction of Rankl by Notch2 is mediated by Notch canonical signaling and Notch2 NICD interactions with Rbpj κ and Maml. In contrast to the resorptive phenotype, neither bone histomorphometry nor *in vitro* studies demonstrated changes in osteoblast differentiation or in the bone-forming capacity of mice carrying the *Notch2HCS* mutation. Bone formation was decreased slightly and not increased as it would be expected in a state of high bone remodeling due to increased bone resorption. It is, therefore, possible that Notch2 inhibited bone formation and uncoupled bone resorption from bone formation.

To determine the individual contributions of osteoclasts and osteoblasts to the osteopenia of the *Notch2HCS* mouse model, we created a conditional by inversion model where Cre recombination generates a *Notch2* mutant allele lacking the PEST domain [59]. The model was designed to introduce a premature STOP codon in exon 34 of *Notch2* following Cre-mediated recombination. This would lead to the translation of a truncated Notch2 protein, mimicking the genetic defect associated with HCS. To activate the *Notch2* mutation in osteoclasts or osteoblasts, conditional mice were bred with mice expressing Cre under the control of the *Lyz2* (encoding for Lysozyme 2) or the *BGLAP* (encoding for osteocalcin) promoter, respectively. The induction of the Notch2 mutation in *Lyz2*-expressing cells had no skeletal consequences and did not affect the capacity of bone marrow macrophages to form osteoclasts *in vitro*, suggesting that the gain-of-function mutation does not have direct effects on cells of the myeloid lineage. In contrast, induction of the mutation in osteoblasts led to generalized osteopenia associated with enhanced cancellous bone resorption and increased *Tnfsf11* expression in osteoblasts from *Notch2* mutant mice. These experiments reveal that introduction of the HCS mutation in osteoblasts is responsible for the osteopenia due to increased Rankl expression and bone resorption. The results are congruent with the observation that the deletion of *Notch2* in *Lyz2*-expressing cells does not cause a skeletal phenotype [60]. Therefore, neither the activation nor the inactivation of *Notch2* in cells of the myeloid lineage *in vivo* has an effect on skeletal homeostasis, and the effect of Notch2 on bone resorption is secondary to its actions on cells of the osteoblast lineage through the increased expression of Rankl. However, the *in vivo* observations are not fully congruent with *in vitro* studies demonstrating that Notch2 enhances osteoclastogenesis directly [58]. This would suggest that the overall effect of Notch2 on osteoclastogenesis is complex and may be derived from its actions on cells of various lineages.

6. Hajdu Cheney Syndrome, B-cells and the Spleen

Notch receptors play an important role in the fate and function of cells of the immune system. Notch1 is expressed preferentially by T-cells, and its inactivation prevents T-cell formation and causes ectopic B-cell development in the thymus, whereas its constitutive activation is associated with T-cell acute lymphoblastic leukemia [61]. Notch1 is an important modulator of T cell-mediated immune responses [62]. Notch2 is expressed preferentially in mature B-cells, and Notch2 signaling is indispensable for marginal zone (MZ) B-cell development in the spleen [63,64]. MZ B-cells reside in the spleen at the junction of the red pulp, constituted by red cells, and white pulp, constituted by follicular cells; MZ B-cells originate from circulating B-cells [65,66]. *Notch2* haploinsufficiency and the inactivation of *Notch2* in Mx- or CD19-expressing B-cells result in a marked reduction in MZ B-cells of the spleen [67–69]. The inactivation of *Rbpj κ* in CD19-expressing cells phenocopies the inactivation of *Notch2* indicating that canonical signaling and Notch2 NICD interactions with Rbpj κ are responsible for changes in MZ B cell allocation [64]. Moreover, the overexpression of the Notch2 NICD in CD19-expressing cells leads to the allocation of B-cells to the MZ of the spleen at the expense of follicular cells [27]. Individuals with *NOTCH2* loss-of-function mutations have decreased MZ B-cells, whereas subjects with gain-of-*NOTCH2* function mutations, such as those occurring in HCS, may present with splenomegaly [35,69]. Importantly, somatic mutations in the same region of exon 34 of *NOTCH2*, as those found in HCS, are associated with diffuse large B-cell lymphomas and lymphomas of the MZ of the spleen [46,47]. *Notch2HCS* mutant mice have altered B-cell allocation with an increase in MZ B-cells and a proportional reduction in follicular B-cells in the spleen. ADAM10 expression results in activation of Notch signaling and the commitment of T1 B transitional cells to MZ B-cells [70]. Accordingly, the MZ does not develop following the deletion of *Adam10* in CD19-expressing cells [71]. Treatment of *Notch2HCS* mutant and wild type mice with anti-Notch2 antibodies have confirmed the dependency of the MZ B-cell population on Notch2 signal activation [72]. The mechanisms responsible for the effect of Notch2 activation on B-cell allocation in the spleen have not been elucidated, but may involve canonical Notch signaling and the induction of the Notch target genes *Hes1* and *Hes5*, since they are expressed in the spleen and are Notch-dependent.

Although subjects with HCS have not been reported to have alterations in B-cell allocation, a recent report documents splenomegaly in an affected individual [35]. It is of interest that subjects with Alagille Syndrome associated with *JAG1* mutations do not have abnormalities in B-cell populations, whereas individuals with Alagille Syndrome associated with *NOTCH2* haploinsufficiency display a marked reduction in MZ B-cells arguing for a role of NOTCH2 in B-cell allocation also in humans [69]. However, it is important to note that humans have a less well-developed MZ of the spleen than rodents, and the organization of the marginal and follicular zone in the human spleen differs from that in the mouse [73]. Genome-wide association studies have identified *CSL* as a risk allele for rheumatoid arthritis, but it is not known whether subjects with HCS are at an increased risk of autoimmune disorders [74].

7. Controlling Notch Signal Activation

The approach to downregulate Notch signaling has been diverse, and includes the use of biochemical inhibitors of Notch activation, antibodies to Notch receptors or to Notch ligands, and the use of small permeable molecules that prevent the formation of an NICD/Rbpjk/Maml ternary complex [75]. γ -secretase inhibitors are frequently used to block the cleavage and prevent activation of the Notch receptor induced by presenilins [76]. The limitation of γ -secretase inhibitors is their lack of specificity since nearly 100 substrates of the γ -secretase complex are known to exist [77]. Thapsigargin is an inhibitor of the sarco/endoplasmic reticulum Ca^{2+} -ATPase that precludes the maturation and folding of the Notch receptor and its exit from the endoplasmic reticulum; and as a consequence, it has been used to prevent Notch activation [78,79]. Synthetic small cell permeable molecules that prevent the assembly of an active Notch transcriptional complex can be used for the inhibition of all Notch signaling, but their long-term efficacy is unknown [80]. A limitation of γ -secretase inhibitors, thapsigargin and small permeable molecules that prevent the formation of a Notch transcriptional complex is that they interfere with the indiscriminate activation of all four Notch receptors.

To target specific Notch receptors, antibodies to the NRR of Notch1, Notch2 and Notch3 have been developed [81,82]. The NRR consists of three LIN-12 Notch repeats (LNR) and a heterodimerization domain (HD), and contains the initial cleavage sites (S1 and S2) of Notch which are required for protein maturation and signal activation (Figure 1) [5,83]. The epitope for the anti-Notch NRR bridges the LNR and HD so that the antibody locks the receptor in its quiescent state preventing Notch activation [82,84]. The targeting of this region prevents the initial cleavage and activation of specific Notch isoforms, making the use of anti-Notch NRR antibodies ideal for the neutralization of individual Notch receptors. However, one needs to be cautious with the extrapolation of results from studies in preclinical models to their application to human disease since information on the inhibition of Notch signaling in humans is quite limited. Moreover, Notch neutralization is not without unwanted events, and it may cause vascular tumors and gastrointestinal toxicity [85,86].

Recently, anti-Notch2 NRR antibodies were tested for their effects on the skeletal phenotype of *Notch2HCS* mutant and control mice. Treatment of 1 month old mice with an anti-Notch2 NRR antibody for a 4 week period reversed the osteopenic phenotype of *Notch2HCS* mutant mice. Although encouraging, extrapolation of these results to the human condition is not possible and one should exert caution with their interpretation and applicability beyond the species studied.

8. Treatment of Hajdu Cheney Syndrome

The treatment and management of HCS is defined by the organs affected by the disease. Realistically this would entail the repair of cardiac defects and treatment of osteoporosis with the goal of preventing fractures. There are no controlled clinical trials on the management of the disease since the number of subjects affected is limited. There are no known ways to either prevent or treat the acroosteolysis, and no reports on the treatment of the growth failure. There are no trials on the treatment of the bone loss or fractures in HCS.

Only anecdotal cases treated with bisphosphonates, teriparatide or both exist although the benefit of these therapies is uncertain [87,88]. There should be caution with the use of teriparatide because experimental models of HCS reveal a resorptive phenotype, which could be augmented. Moreover, there is evidence that Notch signaling is enhanced in human osteosarcoma and the prolonged activation of Notch in osteoblasts results in osteosarcoma in experimental preclinical models [89,90]. Although there are no reports of osteosarcoma in HCS, it would seem prudent not to treat with teriparatide a population with a theoretical risk of osteosarcoma. Since *Notch2HCS* mutant mice exhibit increased osteoclastogenesis due to enhanced expression of RANKL by osteoblasts, a treatment to consider is denosumab, which by binding RANKL can reduce osteoclast formation. There is a case report demonstrating the beneficial effects of denosumab in HCS [35]. In this woman, there was an increase in femoral and lumbar spine bone mineral density, and fractures did not occur while the subject was receiving denosumab. It is of interest that the individual reported had increased serum levels of RANKL, confirming that increased osteoclastogenesis and bone resorption are mechanistically relevant to the skeletal manifestations of HCS.

9. Conclusions

In conclusion, HCS is a rare genetic disorder associated with gain-of-function mutations in exon 34 of *NOTCH2*. HCS influences the development of multiple organs and affected subjects present with fractures, acroosteolysis and classic craniofacial features. Neurological complications can be fatal. Preclinical murine models have offered an understanding of the pathogenesis of the syndrome. Treatment is defined by the organs affected.

Acknowledgments

Funding: This work was supported by grants from the National Institutes of Health [DK045227; AR063049 and AR068160]. The author thanks Mary Yurczak for secretarial assistance.

Abbreviations

ANK	Ankyrin
CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CSL, CBF1	Suppressor of Hairless Lag 1
CHYS1	chondroitin sulfate synthase
Dll	Delta-like
EGF	epidermal growth factor
EOGT	EGF domain specific O-linked N acetylglucosamine transferase
HCS	Hajdu Cheney Syndrome
Hes	hairy and enhancer of split
Hey	HES-related with YRPW

HD	heterodimerization domain
Jag	Jagged
LP	leader peptide
LFNG	Lunatic fringe
LMS	Lateral Meningocele Syndrome
LNR	LIN-12 Notch repeats
MZ	marginal zone
Maml	mastermind-like
MESP	mesoderm posterior bHLH transcription factor
μCT	microcomputed tomography
NRR	negative regulatory region
NICD	Notch intracellular domain
NLS	nuclear localization sequence
PEST	proline (P), glutamic acid (E), serine (S) and threonine (T)
RAM	Rbpjκ-association module
Rankl	receptor activator of nuclear factor kappa B ligand
Rbpjκ	recombination signal-binding protein for Ig of κ region
TMD	transmembrane domain

References

1. Lindsell CE, Boulter J, diSibio G, Gossler A, Weinmaster G. Expression patterns of Jagged, Delta1, Notch1, Notch2, and Notch3 genes identify ligand-receptor pairs that may function in neural development. *Mol Cell Neurosci.* 1996; 8:14–27. [PubMed: 8923452]
2. Cordle J, Redfieldz C, Stacey M, van der Merwe PA, Willis AC, Champion BR, et al. Localization of the delta-like-1-binding site in human Notch-1 and its modulation by calcium affinity. *J Biol Chem.* 2008; 283:11785–11793. [PubMed: 18296446]
3. Sanchez-Irizarry C, Carpenter AC, Weng AP, Pear WS, Aster JC, Blacklow SC. Notch subunit heterodimerization and prevention of ligand-independent proteolytic activation depend, respectively, on a novel domain and the LNR repeats. *Mol Cell Biol.* 2004; 24:9265–9273. [PubMed: 15485896]
4. Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell.* 2009; 137:216–233. [PubMed: 19379690]
5. Zanotti S, Canalis E. Notch and the Skeleton. *Mol Cell Biol.* 2010; 30:886–896. [PubMed: 19995916]
6. Kovall RA. More complicated than it looks: assembly of Notch pathway transcription complexes. *Oncogene.* 2008; 27:5099–5109. [PubMed: 18758478]
7. Kovall RA. Structures of CSL, Notch and Mastermind proteins: piecing together an active transcription complex. *Curr Opin Struct Biol.* 2007; 17:117–127. [PubMed: 17157496]

8. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. *Science* (New York, NY). 1999; 284:770–776.
9. Pursglove SE, Mackay JP. CSL: A Notch above the rest. *Int J Biochem Cell Biol*. 2005; 37:2472–2477. [PubMed: 16095948]
10. Ohtsuka T, Ishibashi M, Gradwohl G, Nakanishi S, Guillemot F, Kageyama R. Hes1 and Hes5 as notch effectors in mammalian neuronal differentiation. *EMBO J*. 1999; 18:2196–2207. [PubMed: 10205173]
11. Iso T, Sartorelli V, Poizat C, Iezzi S, Wu HY, Chung G, et al. HERP, a novel heterodimer partner of HES/E(spl) in Notch signaling. *Mol Cell Biol*. 2001; 21:6080–6089. [PubMed: 11486045]
12. Iso T, Kedes L, Hamamori Y. HES and HERP families: multiple effectors of the Notch signaling pathway. *J Cell Physiol*. 2003; 194:237–255. [PubMed: 12548545]
13. Kovall RA, Gebelein B, Sprinzak D, Kopan R. The Canonical Notch Signaling Pathway: Structural and Biochemical Insights into Shape, Sugar, and Force. *Developmental cell*. 2017; 41:228–241. [PubMed: 28486129]
14. Morel V, Schweisguth F. Repression by suppressor of hairless and activation by Notch are required to define a single row of single-minded expressing cells in the *Drosophila* embryo. *Genes Dev*. 2000; 14:377–388. [PubMed: 10673509]
15. Wu J, Bresnick EH. Bare rudiments of notch signaling: how receptor levels are regulated. *Trends Biochem Sci*. 2007; 32:477–485. [PubMed: 17920278]
16. Yuan Z, Friedmann DR, Vanderwielen BD, Collins KJ, Kovall RA. Characterization of CSL (CBF-1, Su(H), Lag-1) Mutants Reveals Differences in Signaling Mediated by Notch1 and Notch2. *J Biol Chem*. 2012; 287:34904–34916. [PubMed: 22915591]
17. Bai S, Kopan R, Zou W, Hilton MJ, Ong CT, Long F, et al. NOTCH1 regulates osteoclastogenesis directly in osteoclast precursors and indirectly via osteoblast lineage cells. *J Biol Chem*. 2008; 283:6509–6518. [PubMed: 18156632]
18. Uyttendaele H, Marazzi G, Wu G, Yan Q, Sassoon D, Kitajewski J. Notch4/int-3, a mammary proto-oncogene, is an endothelial cell-specific mammalian Notch gene. *Development*. 1996; 122:2251–2259. [PubMed: 8681805]
19. Zanotti S, Canalis E. Parathyroid hormone inhibits Notch signaling in osteoblasts and osteocytes. *Bone*. 2017; 103:159–167. [PubMed: 28676438]
20. Swiatek PJ, Lindsell CE, del Amo FF, Weinmaster G, Gridley T. Notch1 is essential for postimplantation development in mice. *Genes Dev*. 1994; 8:707–719. [PubMed: 7926761]
21. Weinmaster G, Roberts VJ, Lemke G. Notch2: a second mammalian Notch gene. *Development*. 1992; 116:931–941. [PubMed: 1295745]
22. McCright B, Gao X, Shen L, Lozier J, Lan Y, Maguire M, et al. Defects in development of the kidney, heart and eye vasculature in mice homozygous for a hypomorphic Notch2 mutation. *Development*. 2001; 128:491–502. [PubMed: 11171333]
23. Bellavia D, Checquolo S, Campese AF, Felli MP, Gulino A, Screpanti I. Notch3: from subtle structural differences to functional diversity. *Oncogene*. 2008; 27:5092–5098. [PubMed: 18758477]
24. Canalis E, Parker K, Feng JQ, Zanotti S. Osteoblast Lineage-specific Effects of Notch Activation in the Skeleton. *Endocrinology*. 2013; 154:623–634. [PubMed: 23275471]
25. Canalis E, Adams DJ, Boskey A, Parker K, Kranz L, Zanotti S. Notch Signaling in Osteocytes Differentially Regulates Cancellous and Cortical Bone Remodeling. *J Biol Chem*. 2013; 288:25614–25625. [PubMed: 23884415]
26. Fujimura S, Jiang Q, Kobayashi C, Nishinakamura R. Notch2 activation in the embryonic kidney depletes nephron progenitors. *J Am Soc Nephrol*. 2010; 21:803–810. [PubMed: 20299358]
27. Hampel F, Ehrenberg S, Hojer C, Draeseke A, Marschall-Schroter G, Kuhn R, et al. CD19-independent instruction of murine marginal zone B-cell development by constitutive Notch2 signaling. *Blood*. 2011; 118:6321–6331. [PubMed: 21795747]
28. Hajdu N, Kauntze R. Cranio-skeletal dysplasia. *Br J Radiol*. 1948; 21:42–48. [PubMed: 18918373]
29. Cheney WD. Acro-Osteolysis. *Am J Roentgenol Radium Ther Nucl Med*. 1965; 94:595–607.

30. Descartes M, Rojnueangnit K, Cole L, Sutton A, Morgan SL, Patry L, et al. Hajdu-Cheney syndrome: phenotypical progression with de-novo NOTCH2 mutation. *Clin Dysmorphol*. 2014; 23:88–94. [PubMed: 24608068]
31. Canalis E, Zanolini S. Hajdu-Cheney syndrome: a review. *Orphanet journal of rare diseases*. 2014; 9:200. [PubMed: 25491639]
32. Elias AN, Pinals RS, Anderson HC, Gould LV, Streeten DH. Hereditary osteodysplasia with acro-osteolysis. (The Hajdu-Cheney syndrome). *Am J Med*. 1978; 65:627–636. [PubMed: 707523]
33. Nunziata V, di GG, Ballanti P, Bonucci E. High turnover osteoporosis in acro-osteolysis (Hajdu-Cheney syndrome). *J Endocrinol Invest*. 1990; 13:251–255. [PubMed: 2365959]
34. Udell J, Schumacher HR Jr, Kaplan F, Fallon MD. Idiopathic familial acroosteolysis: histomorphometric study of bone and literature review of the Hajdu-Cheney syndrome. *Arthritis Rheum*. 1986; 29:1032–1038. [PubMed: 3527178]
35. Adami G, Rossini M, Gatti D, Orsolini G, Idolazzi L, Viapiana O, et al. Hajdu Cheney Syndrome; report of a novel NOTCH2 mutation and treatment with denosumab. *Bone*. 2016; 92:150–156. [PubMed: 27592446]
36. Kaler SG, Geggel RL, Sadeghi-Nejad A. Hajdu-Cheney syndrome associated with severe cardiac valvular and conduction disease. *Dysmorph Clin Genet*. 1990; 4:43–47.
37. Sargin G, Cildag S, Senturk T. Hajdu-Cheney syndrome with ventricular septal defect. *Kaohsiung J Med Sci*. 2013; 29:343–344. [PubMed: 23684141]
38. Gray MJ, Kim CA, Bertola DR, Arantes PR, Stewart H, Simpson MA, et al. Serpentine fibula polycystic kidney syndrome is part of the phenotypic spectrum of Hajdu-Cheney syndrome. *Eur J Hum Genet*. 2012; 20:122–124. [PubMed: 21712856]
39. Isidor B, Le MM, Exner GU, Pichon O, Thierry G, Guiochon-Mantel A, et al. Serpentine fibula-polycystic kidney syndrome caused by truncating mutations in NOTCH2. *Hum Mutat*. 2011; 32:1239–1242. [PubMed: 21793104]
40. Majewski F, Enders H, Ranke MB, Voit T. Serpentine fibula-polycystic kidney syndrome and Melnick-Needles syndrome are different disorders. *Eur J Pediatr*. 1993; 152:916–921. [PubMed: 8276023]
41. Isidor B, Lindenbaum P, Pichon O, Bezieau S, Dina C, Jacquemont S, et al. Truncating mutations in the last exon of NOTCH2 cause a rare skeletal disorder with osteoporosis. *Nat Genet*. 2011; 43:306–308. [PubMed: 21378989]
42. Majewski J, Schwartzentruber JA, Caqueret A, Patry L, Marcadier J, Fryns JP, et al. Mutations in NOTCH2 in families with Hajdu-Cheney syndrome. *Hum Mutat*. 2011; 32:1114–1117. [PubMed: 21681853]
43. Simpson MA, Irving MD, Asilmaz E, Gray MJ, Dafou D, Elmslie FV, et al. Mutations in NOTCH2 cause Hajdu-Cheney syndrome, a disorder of severe and progressive bone loss. *Nat Genet*. 2011; 43:303–305. [PubMed: 21378985]
44. Zhao W, Petit E, Gafni RI, Collins MT, Robey PG, Seton M, et al. Mutations in NOTCH2 in patients with Hajdu-Cheney syndrome. *Osteoporos Int*. 2013; 24:2275–2281. [PubMed: 23389697]
45. Stathopoulos IP, Trovas G, Lampropoulou-Adamidou K, Koromila T, Kollia P, Papaioannou NA, et al. Severe osteoporosis and mutation in NOTCH2 gene in a woman with Hajdu-Cheney syndrome. *Bone*. 2013; 52:366–371. [PubMed: 23117206]
46. Lee SY, Kumano K, Nakazaki K, Sanada M, Matsumoto A, Yamamoto G, et al. Gain-of-function mutations and copy number increases of Notch2 in diffuse large B-cell lymphoma. *Cancer Sci*. 2009; 100:920–926. [PubMed: 19445024]
47. Rossi D, Trifonov V, Fangazio M, Bruscaggini A, Rasi S, Spina V, et al. The coding genome of splenic marginal zone lymphoma: activation of NOTCH2 and other pathways regulating marginal zone development. *J Exp Med*. 2012; 209:1537–1551. [PubMed: 22891273]
48. Avela K, Valanne L, Helenius I, Makitie O. Hajdu-Cheney syndrome with severe dural ectasia. *Am J Med Genet A*. 2011; 155A:595–598. [PubMed: 21337686]
49. Lehman RA, Stears JC, Wesenberg RL, Nusbaum ED. Familial osteosclerosis with abnormalities of the nervous system and meninges. *J Pediatr*. 1977; 90:49–54. [PubMed: 830893]

50. Gripp KW, Scott CI Jr, Hughes HE, Wallerstein R, Nicholson L, States L, et al. Lateral meningocele syndrome: three new patients and review of the literature. *Am J Med Genet.* 1997; 70:229–239. [PubMed: 9188658]
51. Gripp KW. Lateral meningocele syndrome and Hajdu-Cheney syndrome: different disorders with overlapping phenotypes. *Am J Med Genet A.* 2011; 155A:1773–1774. author reply 1775. [PubMed: 21671395]
52. Gripp KW, Robbins KM, Sobreira NL, Witmer PD, Bird LM, Avela K, et al. Truncating mutations in the last exon of NOTCH3 cause lateral meningocele syndrome. *Am J Med Genet A.* 2015; 167A:271–281. [PubMed: 25394726]
53. Ejaz R, Qin W, Huang L, Blaser S, Tetreault M, Hartley T, et al. Lateral meningocele (Lehman) syndrome: A child with a novel NOTCH3 mutation. *Am J Med Genet A.* 2016; 170A:1070–1075. [PubMed: 26754023]
54. Pear WS, Aster JC. T cell acute lymphoblastic leukemia/lymphoma: a human cancer commonly associated with aberrant NOTCH1 signaling. *Curr Opin Hematol.* 2004; 11:426–433. [PubMed: 15548998]
55. Canalis E, Schilling L, Yee SP, Lee SK, Zanotti S. Hajdu Cheney Mouse Mutants Exhibit Osteopenia, Increased Osteoclastogenesis and Bone Resorption. *J Biol Chem.* 2016; 291:1538–1551. [PubMed: 26627824]
56. Zanotti S, Canalis E. Notch suppresses nuclear factor of activated T cells (Nfat) transactivation and Nfatc1 expression in chondrocytes. *Endocrinology.* 2013; 154:762–772. [PubMed: 23264614]
57. Olsauskas-Kuprys R, Zlobin A, Osipo C. Gamma secretase inhibitors of Notch signaling. *Onco Targets Ther.* 2013; 6:943–955. [PubMed: 23901284]
58. Fukushima H, Nakao A, Okamoto F, Shin M, Kajiya H, Sakano S, et al. The association of Notch2 and NF-kappaB accelerates RANKL-induced osteoclastogenesis. *Mol Cell Biol.* 2008; 28:6402–6412. [PubMed: 18710934]
59. Zanotti S, Yu J, Sanjay A, Schilling L, Schoenherr C, Economides AN, et al. Sustained Notch2 signaling in osteoblasts, but not in osteoclasts, is linked to osteopenia in a mouse model of Hajdu-Cheney syndrome. *J Biol Chem.* 2017; 292:12232–12244. [PubMed: 28592489]
60. Yorgan T, Vollersen N, Riedel C, Jeschke A, Peters S, Busse B, et al. Osteoblast-specific Notch2 inactivation causes increased trabecular bone mass at specific sites of the appendicular skeleton. *Bone.* 2016; 87:136–146. [PubMed: 27102824]
61. Radtke F, Wilson A, Stark G, Bauer M, van Meerwijk J, MacDonald HR, et al. Deficient T cell fate specification in mice with an induced inactivation of Notch1. *Immunity.* 1999; 10:547–558. [PubMed: 10367900]
62. Radtke F, Fasnacht N, MacDonald HR. Notch signaling in the immune system. *Immunity.* 2010; 32:14–27. [PubMed: 20152168]
63. Witt CM, Hurez V, Swindle CS, Hamada Y, Klug CA. Activated Notch2 potentiates CD8 lineage maturation and promotes the selective development of B1 B cells. *Mol Cell Biol.* 2003; 23:8637–8650. [PubMed: 14612407]
64. Tanigaki K, Han H, Yamamoto N, Tashiro K, Ikegawa M, Kuroda K, et al. Notch-RBP-J signaling is involved in cell fate determination of marginal zone B cells. *Nat Immunol.* 2002; 3:443–450. [PubMed: 11967543]
65. Pillai S, Cariappa A. The follicular versus marginal zone B lymphocyte cell fate decision. *Nat Rev Immunol.* 2009; 9:767–777. [PubMed: 19855403]
66. Pillai S, Cariappa A, Moran ST. Marginal zone B cells. *Annu Rev Immunol.* 2005; 23:161–196. [PubMed: 15771569]
67. Saito T, Chiba S, Ichikawa M, Kunisato A, Asai T, Shimizu K, et al. Notch2 is preferentially expressed in mature B cells and indispensable for marginal zone B lineage development. *Immunity.* 2003; 18:675–685. [PubMed: 12753744]
68. Witt CM, Won WJ, Hurez V, Klug CA. Notch2 haploinsufficiency results in diminished B1 B cells and a severe reduction in marginal zone B cells. *J Immunol.* 2003; 171:2783–2788. [PubMed: 12960298]

69. Descatoire M, Weller S, Irtan S, Sarnacki S, Feuillard J, Storck S, et al. Identification of a human splenic marginal zone B cell precursor with NOTCH2-dependent differentiation properties. *J Exp Med*. 2014; 211:987–1000. [PubMed: 24733829]
70. Hammad H, Vanderkerken M, Pouliot P, Deswarte K, Toussaint W, Vergote K, et al. Transitional B cells commit to marginal zone B cell fate by Taok3-mediated surface expression of ADAM10. *Nat Immunol*. 2017; 18:313–320. [PubMed: 28068307]
71. Gibb DR, El Shikh M, Kang DJ, Rowe WJ, El Sayed R, Cichy J, et al. ADAM10 is essential for Notch2-dependent marginal zone B cell development and CD23 cleavage in vivo. *J Exp Med*. 2010; 207:623–635. [PubMed: 20156974]
72. Simonetti G, Carette A, Silva K, Wang H, De Silva NS, Heise N, et al. IRF4 controls the positioning of mature B cells in the lymphoid microenvironments by regulating NOTCH2 expression and activity. *J Exp Med*. 2013; 210:2887–2902. [PubMed: 24323359]
73. Zouali M, Richard Y. Marginal zone B-cells, a gatekeeper of innate immunity. *Front Immunol*. 2011; 2:63. [PubMed: 22566852]
74. Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP, et al. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. *Nat Genet*. 2010; 42:508–514. [PubMed: 20453842]
75. Ryeom SW. The cautionary tale of side effects of chronic Notch1 inhibition. *J Clin Invest*. 2011; 121:508–509. [PubMed: 21266769]
76. De Strooper B, Annaert W, Cupers P, Saftig P, Craessaerts K, Mumm JS, et al. A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature*. 1999; 398:518–522. [PubMed: 10206645]
77. Duggan SP, McCarthy JV. Beyond gamma-secretase activity: The multifunctional nature of presenilins in cell signalling pathways. *Cell Signal*. 2016; 28:1–11.
78. Ilagan MX, Kopan R. Selective blockade of transport via SERCA inhibition: the answer for oncogenic forms of Notch? *Cancer Cell*. 2013; 23:267–269. [PubMed: 23518343]
79. Largaespada D, Ratner N. Interweaving the strands: beta-catenin, an HIV co-receptor, and Schwann cell tumors. *Cancer Cell*. 2013; 23:269–271. [PubMed: 23518344]
80. Moellering RE, Cornejo M, Davis TN, Del BC, Aster JC, Blacklow SC, et al. Direct inhibition of the NOTCH transcription factor complex. *Nature*. 2009; 462:182–188. [PubMed: 19907488]
81. Li K, Li Y, Wu W, Gordon WR, Chang DW, Lu M, et al. Modulation of Notch signaling by antibodies specific for the extracellular negative regulatory region of NOTCH3. *J Biol Chem*. 2008; 283:8046–8054. [PubMed: 18182388]
82. Wu Y, Cain-Hom C, Choy L, Hagenbeek TJ, de Leon GP, Chen Y, et al. Therapeutic antibody targeting of individual Notch receptors. *Nature*. 2010; 464:1052–1057. [PubMed: 20393564]
83. Zanotti S, Canalis E. Notch Signaling and the Skeleton. *Endocr Rev*. 2016; 37:223–253. [PubMed: 27074349]
84. Aste-Amezaga M, Zhang N, Lineberger JE, Arnold BA, Toner TJ, Gu M, et al. Characterization of Notch1 antibodies that inhibit signaling of both normal and mutated Notch1 receptors. *PLoS One*. 2010; 5:e9094. [PubMed: 20161710]
85. Ridgway J, Zhang G, Wu Y, Stawicki S, Liang WC, Chanthery Y, et al. Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. *Nature*. 2006; 444:1083–1087. [PubMed: 17183323]
86. Yan M, Callahan CA, Beyer JC, Allamneni KP, Zhang G, Ridgway JB, et al. Chronic DLL4 blockade induces vascular neoplasms. *Nature*. 2010; 463:E6–7. [PubMed: 20147986]
87. Galli-Tsinopoulou A, Kyrgios I, Giza S, Giannopoulou EM, Maggana I, Laliotis N. Two-year cyclic infusion of pamidronate improves bone mass density and eliminates risk of fractures in a girl with osteoporosis due to Hajdu-Cheney syndrome. *Minerva Endocrinol*. 2012; 37:283–289. [PubMed: 22766895]
88. McKiernan FE. Integrated anti-remodeling and anabolic therapy for the osteoporosis of Hajdu-Cheney syndrome: 2-year follow-up. *Osteoporos Int*. 2008; 19:379–380. [PubMed: 17874031]
89. Engin F, Bertin T, Ma O, Jiang MM, Wang L, Sutton RE, et al. Notch signaling contributes to the pathogenesis of human osteosarcomas. *Hum Mol Genet*. 2009; 18:1464–1470. [PubMed: 19228774]

90. Tao J, Jiang MM, Jiang L, Salvo JS, Zeng HC, Dawson B, et al. Notch activation as a driver of osteogenic sarcoma. *Cancer Cell*. 2014; 26:390–401. [PubMed: 25203324]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1. Domains of the four Notch receptors

The upper panel shows the domain and motif organization of a generic human/murine Notch receptor before cleavage at the S1 site by furin-like convertases in the Golgi compartment. The extracellular domain contains a leader peptide (LP) and multiple epidermal growth factor (EGF)-like tandem repeats followed by Lin12-Notch repeats (LNR) and the heterodimerization domain (HD). The transmembrane domain (TMD) is located between the extracellular and intracellular domains. The Notch intracellular domain (NICD) contains an Rbpj κ -association module (RAM), a nuclear localization sequence (NLS), ankyrin (ANK) repeats and tandem NLS, which are followed by a proline (P)-, glutamic acid (E)-, serine (S)- and threonine (T)-rich (PEST) domain. The lower panel shows the domains and motifs of heterodimeric individual receptors, the negative regulatory region (NRR) is formed by the LNR and HD following cleavage at the S1 site. Notch1 and Notch2 have 36 EGF-like repeats; in green are those required for binding of Notch1 and Notch2 to cognate Delta/Serrate/Lag2 ligands. Notch1 and Notch2 have a similar NICD, and Notch3 has 34 EGF-like repeats and a shorter NICD than Notch1 and Notch2. Notch4 has 29 EGF-like repeats and an NICD that is shorter than that of other receptors and lacks the tandem NLS located between the ANK repeats and the PEST domain. Reproduced with permission from Zanotti and Canalis, *Endocrine Reviews* 37:223, 2016.

Figure 2. Canonical Notch Signaling

Under resting conditions, Notch receptors are intact and Rbpj κ binds histone deacetylases (HDAC) and co-repressors. Following Delta-like or Jagged interactions with the extracellular domain of Notch, the Notch intracellular domain (NICD) is released and translocates to the nucleus to form a complex with mastermind-like (Maml) and Rbpj κ . As a consequence, co-repressors and HDAC is displaced, CBP/p300 is recruited and histone acetylation ensues leading to the induction of *Hes* and *Hey*.

Table 1

Clinical manifestations of Hajdu Cheney Syndrome

<ul style="list-style-type: none">• Craniofacial Developmental Abnormalities<ul style="list-style-type: none">Platybasia, open sutures, wormian bonesCharacteristic and evolving facial featuresMaxillofacial and dental abnormalities
<ul style="list-style-type: none">• Skeletal Features<ul style="list-style-type: none">Osteoporosis with fracturesScoliosis, kyphosisOsteolysis of distal phalangesShort statureJoint hypermobility
<ul style="list-style-type: none">• Renal<ul style="list-style-type: none">Polycystic kidneys
<ul style="list-style-type: none">• Cardiac<ul style="list-style-type: none">Septal and valvular defects
<ul style="list-style-type: none">• Spleen<ul style="list-style-type: none">Splenomegaly

Table 2

Genetic disorders associated with mutations in the major components of the Notch signaling pathway.

Gene	Clinical Disorder	Presumed Change in Function
<i>Notch Receptors</i>		
<i>NOTCH1</i>	Adams Oliver Syndrome	Loss
<i>NOTCH2</i>	Alagille Syndrome	Loss
	Hajdu Cheney Syndrome	Gain
<i>NOTCH3</i>	CADASIL *	Uncertain
	Lateral Meningocele Syndrome	Gain
<i>NOTCH4</i>	Not reported	
<i>Notch Ligands</i>		
<i>JAG1</i>	Alagille Syndrome	Loss
<i>JAG2</i>	Not reported	
<i>DLL1</i>	Not reported	
<i>DLL3</i>	Spondylocostal Dysostosis	Loss
<i>DLL4</i>	Adams Oliver Syndrome	Loss
<i>Intracellular Signal</i>		
<i>CSL (Rbpjk)</i>	Adams Oliver Syndrome	Loss
<i>Modifiers of Notch Signaling and Notch Target Genes</i>		
<i>EOGT</i>	Adams Oliver Syndrome	Loss
<i>LFNG</i>	Spondylocostal Dysostosis	Loss
<i>CHSY1</i>	Brachydactyly	Gain
<i>MESP</i>	Spondylocostal Dysostosis	Loss
<i>HES7</i>	Spondylocostal Dysostosis	Loss

* CADASIL – Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy

Table 3

Clinical manifestations of Lateral Meningocele Syndrome.

<ul style="list-style-type: none"> • Craniofacial Features <ul style="list-style-type: none"> Hypertelorism High arched eyebrows Ptosis Low set eyes Cleft and high arched narrow palate * Micrognathia * Thick calvariae * Dense base of skull * Wormian bones *
<ul style="list-style-type: none"> • Skeletal Features <ul style="list-style-type: none"> Short stature * Scoliosis * Pectus excavatum Osteopenia *
<ul style="list-style-type: none"> • Other Features <ul style="list-style-type: none"> Developmental and intellectual delay Decreased muscle mass Hypotonia Lateral meningocele Syringomyelia Cardiac valve abnormalities *

* Clinical features also present in HCS.