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# A mutation in the heparin-binding site of noggin as a novel mechanism of proximal symphalangism and conductive hearing loss ☆

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ABSTRACT

The access of bone morphogenetic protein (BMP) to the BMP receptors on the cell surface is regulated by its antagonist noggin, which binds to heparan-sulfate proteoglycans on the cell surface. Noggin is encoded by *NOG* and mutations in the gene are associated with aberrant skeletal formation, such as in the autosomal dominant disorders proximal symphalangism (SYM1), multiple synostoses syndrome, Teunissen–Cremers syndrome, and tarsal–carpal coalition syndrome. *NOG* mutations affecting a specific function may produce a distinct phenotype. In this study, we investigated a Japanese pedigree with SYM1 and conductive hearing loss and found that it carried a novel heterozygous missense mutation of *NOG* (c.406C > T; p.R136C) affecting the heparin-binding site of noggin. As no mutations of the heparin-binding site of noggin have previously been reported, we investigated the crystal structure of wild-type noggin to investigate molecular mechanism of the p.R136C mutation. We found that the positively charged arginine at position 136 was predicted to be important for binding to the negatively charged heparan-sulfate proteoglycan (HSPG). An *in silico* docking analysis showed that one of the salt bridges between noggin and heparin disappeared following the replacement of the arginine with a non-charged cysteine. We propose that the decreased binding affinity of *NOG* with the p.R136C mutation to HSPG leads to an excess of BMP signaling and underlies the SYM1 and conductive hearing loss phenotype of carriers.



## 1. Introduction

Proximal symphalangism (SYM1, OMIM 185800) is a hereditary disease showing ankylosis of the proximal interphalangeal (PIP) joints and is frequently accompanied by conductive hearing loss; the disease results from mutations of *NOG* (OMIM 602991) [1] or *GDF5* (OMIM 601146) [2]. The noggin binds to bone morphogenetic protein (BMP) family, a subtype of the TGF-β superfamily, and antagonizes binding of these proteins to the BMP receptors (BMPRs) in a dose-dependent manner [3,4]; this interaction controls a cascade of developmental processes including morphogenesis and body patterning [4,5], middle ear formation [6,7], as well as chondrogenesis of the digits and interdigital apoptosis [6,8,9]. Another important feature of noggin for BMP-mediated morphogenesis is that the noggin–BMP complex associates with heparan-sulfate proteoglycan (HSPG), a major proteoglycan on the cell surface and in extracellular matrices [10], and is regulated by sulfatases to control the local activity of BMPs [11].

Mutations in *NOG* are associated with various autosomal dominant syndromes that are characterized by a spectrum of skeletal defects, conductive hearing loss, and synostoses, such as multiple synostoses syndrome (SYNS1, OMIM 186500) [1], Teunissen–Cremers syndrome (TCS, OMIM 184460) [12,13], tarsal–carpal

*Abbreviations:* ABR, auditory brainstem response; BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; CT, computed tomography; DIP, distal interphalangeal; DPOAE, distortion product otoacoutic emission; HSPG, heparan-sulfate proteoglycan; PIP, proximal interphalangeal; SYM1, proximal symphalangism; SYNS1, multiple synostoses syndrome; TCC, tarsal–carpal coalition syndrome; TCS, Teunissen–Cremers syndrome.

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coalition syndrome (TCC, OMIM 186570) [14,15], Fibrodysplasia ossificans progressive (FOP, OMIM 135100) [16], Brachydactyly type B (BDB, OMIM 113000) [17], and Stapes ankylosis with broad thumb and toes (OMIM 184460) [18]. According to a previous *in vitro* study, *NOG* with p.P35R mutation has a diminished affinity for BMP-7 due to reduced hydrophobic interaction [19]. Each noggin with mutation of p.G189C, p.W217G, or p.P223L is either poorly or not secreted in cultured mammalian cells [20], indicating a defect in protein folding and maturation.

Mutation-induced structural change of a protein and prediction of its pathogenicity can be explored through molecular modeling. This approach is effective especially when examining structure of molecules that are extremely difficult to be crystallized, for instance, proteins associated with structurally mobile sugar chain such as heparan-sulfate, whereas it can be investigated by docking simulations.

Here, we report a patient with a novel heterozygous missense mutation (c.406C > T) in *NOG*, which replaces the arginine residue at position 136 with a cysteine (p.R136C) in the heparin-binding site of noggin. A simulation of the binding mode of the mutated noggin to heparin successfully predicted its molecular dysfunction. We propose a novel molecular mechanism for the etiology of the SYM1 phenotype: *NOG* with the p.R136C mutation allows excess BMP signaling through a decreased binding affinity of the noggin–BMP complex to HSPG on cell surfaces and leads to aberrant bone formation in PIP joints and the middle ear.

## 2. Materials and methods

### 2.1. Ethics statements

All the procedures were approved by the Ethics Review Committee of National Mie Hospital and National Tokyo Medical Center, and were conducted only after written informed consent had been obtained from each individual or from the parents of the children.

### 2.2. Subjects

Medical history and results of clinical examination (physical, audiological, and radiological) were obtained in four members of a Japanese SYM1 family, three affected (proband, old sister, and father) and one unaffected (mother). Audiological examinations included pure tone audiometry, distortion product otoacoustic emission (DPOAE) test, tympanometry, and stapedial reflex test. Radiological examinations included X-rays of hand and feet and computed tomography (CT) of temporal bones. Genetic analysis was also conducted in the four family members. The normal controls comprised 183 subjects who had healthy hands, feet, and normal hearing level according to pure tone audiometry. A comprehensive family history was obtained by interviewing parents of the proband.

### 2.3. Genetic analysis

Genomic DNA was extracted from blood samples using the Gentra Puregene Blood kit (QIAGEN, Hamburg, Germany), and primers specific for *NOG* (GenBank NG_011958.1) were designed. For PCR amplification of *NOG* exon, the primer sets 5′-tgtaaaacgacggccagtgTGCGGCCAACTTGTGTGCCTTTCT-3′ and 5′-caggaaacagctatgaccACAGCCACATCTGTAACTTCCTCC-3′, and 5′-tgtaaaacgacggccagtgGCCATGCCGAGCGAGATCAAAGG-3′ and 5′-caggaaacagctatgaccAGAGGGTGGTGGAACTGGTTGGAGGC-3 were used with the PC-818 Program Temp Control System (ASTEC, Shizuoka, Japan). Each primer is specific for a given genomic sequence (upper case) and was used in combination with either a universal forward M13 (lower case) or reverse M13pUC (lower case) primer. The following PCR program was used: 98 °C for 10 min; 37 cycles of 98 °C for 10 s, 62 °C for 10 s, and 72 °C for 1 min; and then 72 °C for 3 min. PrimeSTAR HS DNA polymerase (Takara Bio, Shiga, Japan) was used for the PCR. The amplicons were sequenced using the ABI 3730 DNA sequence analyzer with the ABI Prism Big Dye Terminator Cycle Sequencing kit (Applied Biosystems). Characterization of the sequences was undertaken using SeqScape software v2.6 (Applied Biosystems) and DNASIS Pro (Hitachisoft, Tokyo, Japan) with the *NOG* sequence (NG_011958.1, NCBI Build37.1) as the reference. Control DNA was obtained from 96 Japanese subjects with normal hearing. To study amino acid conservation among different species, noggin orthologs from the chimpanzee (XP_523802), mouse (NP_032737), rat (XP_343955), cow (XP_582573), chicken (NP_989454), African clawed frog (AAI69670), and zebrafish (NP_571058) were compared by ClustalW [21].

### 2.4. Docking study of noggin and heparin

The crystal structure of wild-type noggin (PDB: 1M4U) [19] was used as the input data for the receptor molecule in the docking study and as a template for modeling the structure of the p.R136C mutant. The p.R136C mutant and the missing residues in the PDB file of wild-type noggin were modeled using the Swiss-Model modeling server [22]. The pentasaccharide (fondaparinux) in the crystal structure of the antithrombin-S195A factor Xa-pentasaccharide complex (PDB: 2GD4) [23] is very similar to naturally-occurring heparin. Hence, this pentasaccharide structure was used here as a substitute for naturally-occurring heparin. Rigid body docking of heparin (ligand) to noggin (receptor protein) was performed using AutoDock4.2 [24]. The noggin–heparin complex conformation that scored highly in terms of van der Waals and electrostatic interactions was selected. Energy optimization was then performed for the conformation using Discovery Studio 3.1 (Accelrys Inc., San Diego, Ca). All structural figures were generated with Chimera [25]. Electric surface potentials were rendered with Pov-Ray [26].

## 3. Results

### 3.1. Clinical features of the family

The family included five individuals with SYM1 (Fig. 1A). The proband (IV:6) was an 10-year-old daughter of non-consanguineous Japanese parents. She had limited mobility of the second to fifth fingers and toes bilaterally at birth. Photographs and radiographs of her hands and feet indicating ankylosis of the PIP joints and the distal interphalangeal (DIP) joints are shown (Fig. 1B–F). She did not have hyperopia or strabismus, and her face was not dysmorphic. From age 8 to 10, bilateral, low-frequency, progressive hearing loss was identified (Fig. 1G and Supplementary Fig. S1A). DPOAE tests showed abnormal response in both ears (Supplementary Fig. S1B). The stapedial reflex was absent in both ears (Fig. 1H). CT of the temporal bones revealed no abnormalities (data not shown). These audiological and radiological findings suggested stapes ankylosis. Her father (III:5) and elder sister (IV:5) also had ankylosis of the PIP joints and conductive hearing loss (Supplementary Fig. S1C–G).

### 3.2. Results of genetic analysis

The constellation of clinical features in this family was consistent with a diagnosis of SYM1, and prompted us to carry out a screen for mutations of *NOG*. Sequence analysis of the *NOG*

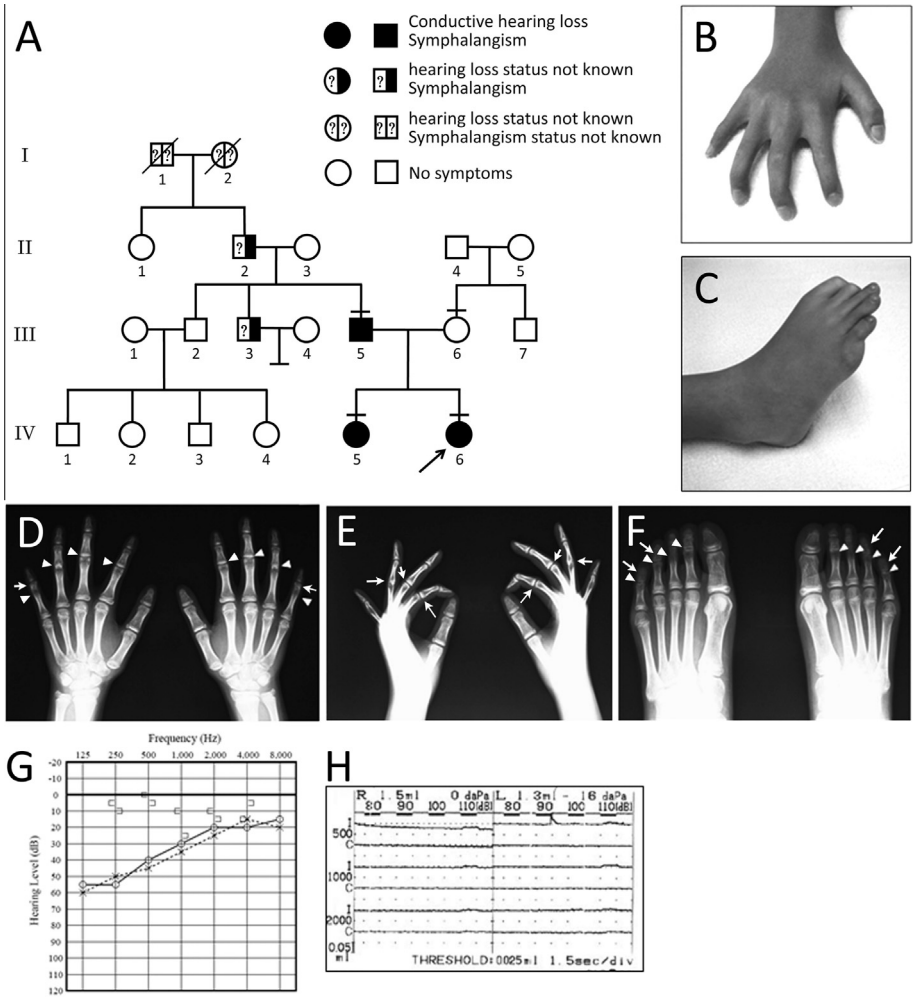


**Fig. 1.** Pedigree and clinical features of the proband. (A) The pedigree of the family. The family included five individuals with SYM1 (II:2, III:3, III:5, IV:5, and IV:6). The arrow indicates the proband. Horizontal bars above the symbols indicate the subjects who participated in the genetic analysis. (B) Limited mobility of the PIP joints and shortened fifth fingers in the hand. (C) Limited mobility of the PIP or the DIP joints in the foot. (D) Symmetrical fusions of the PIP joints (arrowheads) and hypoplastic middle phalanxes of fifth fingers (arrows) in both hands. (E) Limited mobility of the PIP joint of the fingers (arrows). (F) Symmetrical fusion of the PIP joints (arrowheads) and DIP joints (arrows) of both feet. (G) Pure tone audiometry at the age of 10. (H) Stapedial reflex.

protein-coding region in IV:6 revealed a heterozygous C to T transition at nucleotide 406 (c.406C > T), which led to replacement of arginine residue with cysteine at position 136 (p.R136C) in noggin (Fig. 2A). This mutation was also found in her father (III:5) and older sister (IV:5) (data not shown), who showed the similar clinical features, but not in her asymptomatic mother (III:6) (Fig. 2B). No other nucleotide variants within the *NOG* were identified. This mutation was not found on the 1000 Genome Browser [27], the Exome Variant Server [28], nor in the control group that consisted of 183 unrelated Japanese individuals with normal hearing as examined by pure-tone audiometry. Examination of noggin orthologs in other animal species showed that R136 was conserved as a positively charged arginine or lysine residue (Fig. 2C).

### 3.3. Location of p.R136C mutation in the noggin crystal structure

The R136 is located at putative heparin-binding site of noggin with reference to the crystal structure [19]. The putative heparin-binding site of noggin (K133–K144, which includes eight positively charged amino acids) spans the α-helix 4 and the adjacent loop (Fig. 2D) [19], and deletion of this region markedly reduces the binding of noggin to heparin [29]. The noggin–BMP7 complex is a tetramer consisting of two noggin and two BMP7 subunits [19] (Fig. 3A). The positively-charged side-chain of R136 appeared to protrude from the lateral surface region of the putative heparin-binding site of noggin (indicated in yellow and by arrows in Fig. 3B, yellow in Fig. 3C) and seemed to participate in determining the electric surface potential for association with the negatively-charged heparin.

### 3.4. Interaction between noggin and heparin

To examine the possibility that noggin with p.R136C mutation changes its affinity for association with HSPG, we carried out a docking simulation to identify the amino acid residues of noggin (used as the receptor protein) that are critical for the stable interaction with the pentasaccharide analogue of heparin. The putative heparin-binding site has previously been proposed (Fig. 2D, blue) [19,29]. In our model, the heparin analogue appeared to associate with the putative heparin-binding site of wild-type noggin (Fig. 3D). Several stable intermolecular salt bridges were present between K133, K134, R136 and K140 of wild-type noggin and $-NHSO_3^-$ or $-OSO_3^-$ groups of the heparin analogue (Fig. 3D, E). The mutation site R136 was predicted to interact with $-NHSO_3^-$ of sugar unit 1 of the heparin analogue. Q135 and S138 were predicted to be important for forming hydrogen bonds to heparin, instead of

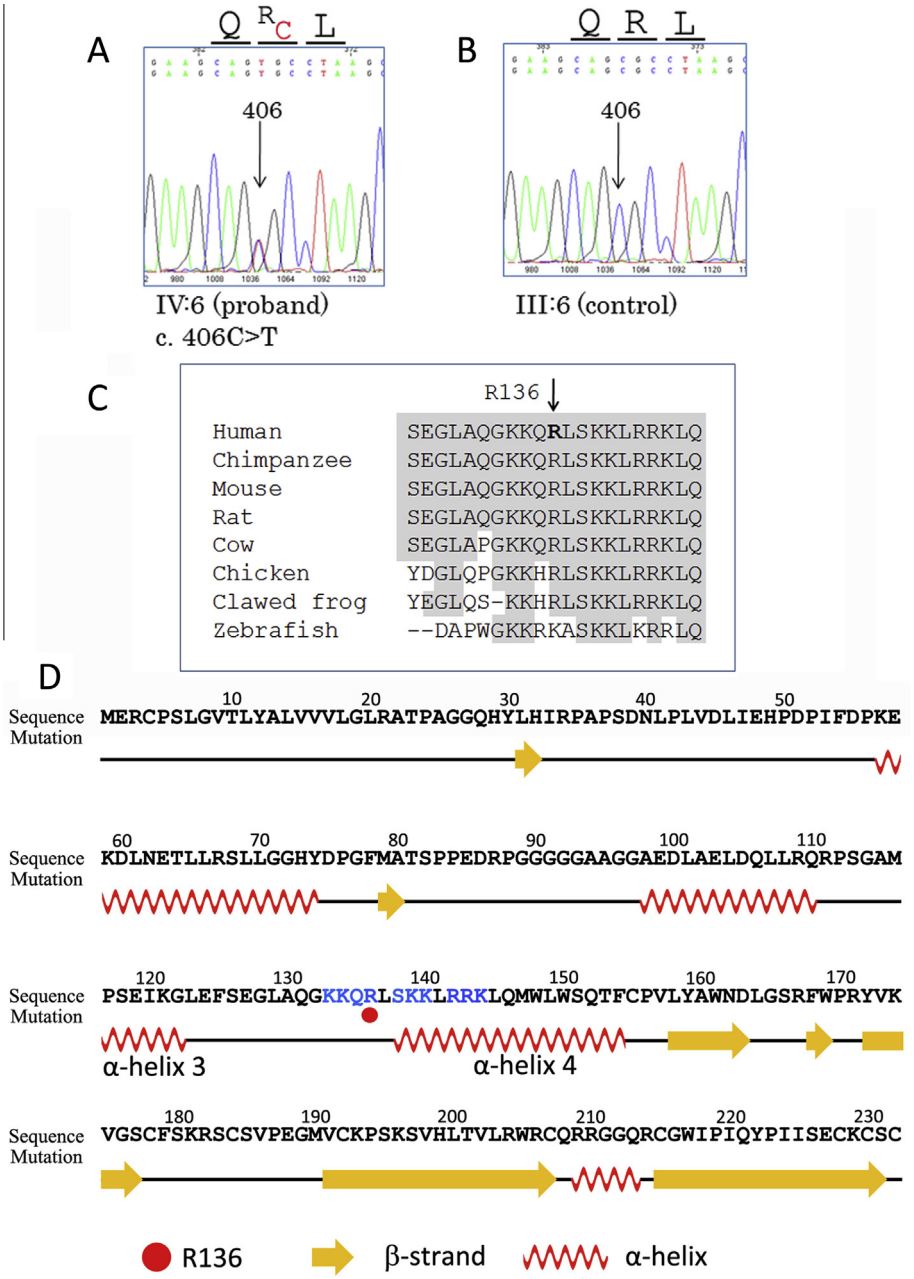


**Fig. 2.** Results of genetic analyses of the family members. (A, B) Partial chromatogram of DNA sequencing for *NOG* in the proband (IV:6) and an asymptomatic individual (III:6) as a control. The arrows indicate the nucleotide position 406. (C) Conservation of R136 in noggin in different species. The protein sequences of noggin orthologs at positions 126–146 are aligned. Amino acids identical to human noggin are highlighted in gray. The position of R136 (bold) is indicated by an arrow. (D) Secondary structure of noggin. R136 is indicated by the red circle. Putative heparin-binding residues are shown in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

forming salt bridges as found for the four amino acid residues described above. The binding form of the sugar units (numbers 3–5 in Fig. 3E) at the reducing end of the heparin analog and basic residues of the protein has been repeatedly shown in antithrombin [23] or basic fibroblast growth factor (PDB: 1BFC) [30].

### 3.5. Alteration of heparin-binding affinity of noggin by the p.R136C mutation

On the basis of the structural model simulating the docking of noggin to the heparin analogue, we can now suggest how the p.R136C mutation causes an adverse effect on noggin–heparin binding. The shortest distance between the side chain atoms of R136 (cyan residue) and the $-NHSO_3^-$ group of the heparin analogue was estimated to be 2.51 Å based on the wild type noggin–heparin complex model (Fig. 3D). In noggin with the p.R136C mutation (Fig. 3D), the shortest distance between the side chain atoms of C136 (orange residue) and the heparin analogue was estimated to have increased to 6.01 Å, a distance that is too great for salt bridge formation. Replaced cysteine did not form a strong electrostatic interaction (i.e. a salt bridge) with a heparin $-NHSO_3^-$ group. For these reasons, the p.R136C mutation of noggin is predicted to cause a marked decrease in the electrostatic interaction with heparin.

To investigate the mutation-associated change in the electrostatic environment of the heparin binding site, we compared the

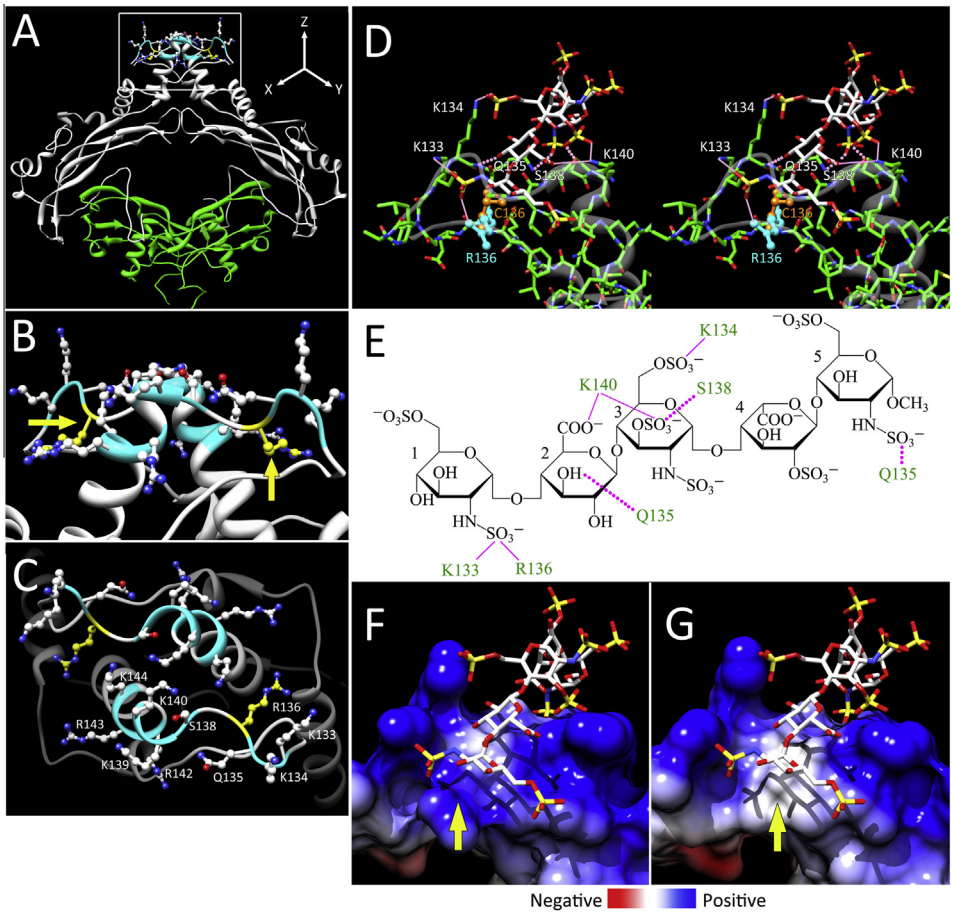


**Fig. 3.** Structure of the noggin–BMP-7 complex. (A) Ribbon diagram of the noggin (white) and BMP-7 (green) complex viewed with a twofold symmetry axis. Cyan colored residues on the main and side chains indicate putative heparin-binding residues and are surrounded by a white box. All atoms in the white box are displayed by ball and stick models (red, oxygen; blue, nitrogen; white, carbon). (B) Higher magnification image of the area shown in the white box in (A). Homomeric R136s are highlighted in yellow and indicated by arrows. (C) *Z*-Axis view of the area shown in the white box in (A). (D) A stereo view of the structural model of the noggin–heparin complex. The carbon atoms of noggin are indicated by the green sticks and the main chain of the heparin-binding site of noggin by the semi-transparent gray tube. The carbon atoms of R136 and C136 are shown in cyan and orange, respectively. Other critical residues are described in white letters. Salt bridges and hydrogen bonds are represented by pink solid lines and dashed lines, respectively. The carbon atoms of heparin are indicated in white. All the other atoms of heparin are displayed in different colors (red, oxygen; blue, nitrogen; yellow, sulfur). (E) Schematic diagram of molecular interactions of heparin (black) and amino acid residues of noggin (green). Salt bridges and hydrogen bonds are represented by pink solid lines and dashed lines, respectively. Sugar units are labeled 1–5 from the non-reducing end to the reducing end. (F, G) Electrostatic surface potential of wild type (F) and noggin with p.R136C mutation (G) at the heparin-binding site. The position of R136 and C136 is indicated by the yellow arrow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

electrostatic potential map between the wild-type and the p.R136C mutant. Wild-type noggin was estimated to have a positively charged electrostatic surface potential around the position of R136 (indicated by the arrow in Fig. 3F). In noggin with the p.R136C mutation, the electrostatic surface potential around the position of C136 (indicated by the arrow in Fig. 3G) was predicted to be neutral under physiological conditions. The elimination of the positively charged surface potential may also reduce the binding affinity of heparin to noggin molecules.

## 4. Discussion

Although mutations of noggin that cause defective binding to BMPs, and mutations that influence the folding stability of noggin have been reported [19], no mutations in the heparin-binding site have been found to date. This is the first study to demonstrate a change in the binding affinity of noggin to heparin that is associated with clinical features. As a result of our successful docking simulation of noggin to the heparin analogue and by estimating the change in the interaction following replacement of the arginine at position 136 with a cysteine residue, we propose that the positively charged R136 in the heparin-binding site is important for the retention of the noggin–BMP complex on the negatively charged HSPG on the plasma membrane.

Noggin-mediated inhibition of BMP signaling has been shown to be regulated by a two-step process [11]. First, noggin binds to BMPs and antagonizes their interaction with BMPRs [3,4]. The noggin–BMP complex binds to HSPG via the heparin binding site of noggin [29], and as a result, BMPs are localized on cell surface (Fig. 4A). Second, sulfatase releases the localized noggin–BMP complexes near the plasma membrane, resulting in an increased accessibility of BMPs to the BMPRs at the plasma membrane and an increased BMP signaling [11] (Fig. 4B). The local concentration of free BMPs that can bind to BMPRs is regulated in proportion to the local concentration of free noggin–BMP complexes that is controlled by sulfatase. *In vitro* studies showed that sulfatase selectively removes sulfate groups from the 6-*O* position of sugars within the highly sulfated domains of heparan-sulfate [31], which catalyzes the dissociation of noggin from cell surface [11]. It is unlikely that the p.R136C mutation had a significant effect on the binding affinity of noggin to BMPs, because position 136 is located

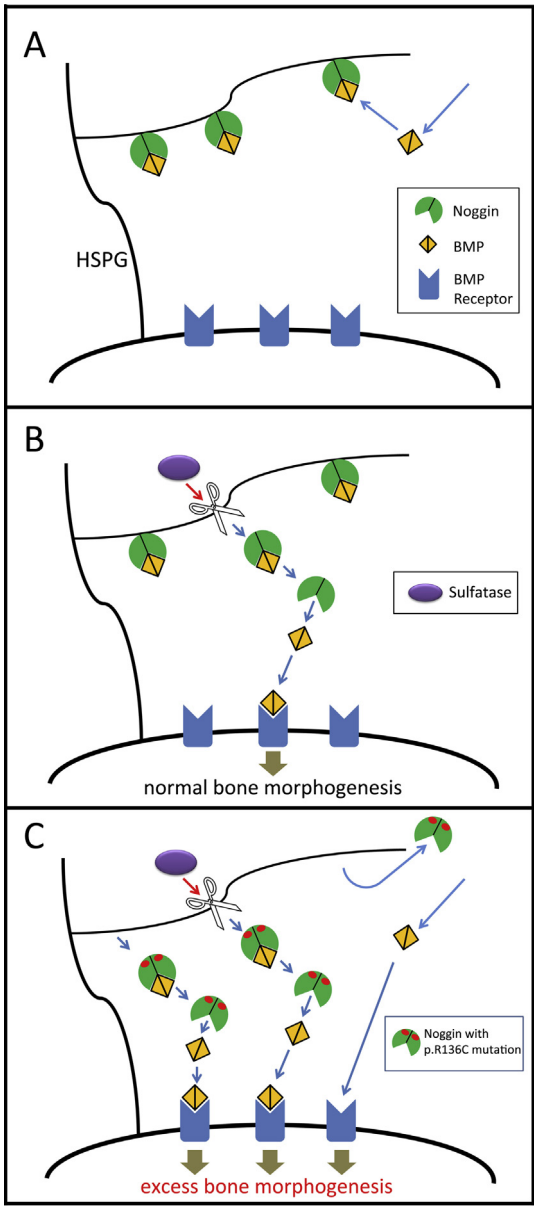


**Fig. 4.** Model for pathology of SYM1 caused by noggin with p.R136C mutation. (A) Wild type noggin–BMP complex is tethered to HSPG. Accessibility of BMPs to BMPRs is antagonized by the binding of noggin to BMP. (B) Accessibility of BMPs to BMPRs is also controlled by sulfatase that releases the noggin–BMP complex from HSPG. BMPs released from noggin–BMP complex bind to BMPRs. (C) Flow pattern on the right: noggin with p.R136C mutation has decreased binding affinity to HPSG; the reduction in noggin mediated antagonism for BMPs allows excess BMPs to bind to BMPRs. Flow pattern on the middle and left: dissociation of noggin–BMP complex from HSPG by sulfatase is accelerated by the reduced binding affinity of noggin with p.R136C mutation to HPSG, and results in increased local concentration of free BMPs with access to BMPRs.

in a region separate from the BMP-binding site [19]. In addition, deletion of the heparin-binding site (K133–K144) of noggin allows normal BMP binding affinity but causes reduced binding affinity to heparin and to the cell surface [29]. Thus, noggin with a p.R136C mutation is very likely to retain the same BMP binding affinity as wild-type noggin. This conclusion leaves unanswered the questions of how and why the p.R136C mutation affects bone morphogenesis?

On the basis of our docking simulation model, we hypothesize that the number of mutant noggin–BMP complexes that are tethered to HSPG on the cell surface are decreased because of the low binding affinity of the mutant noggin to HSPG. As a result, some free BMPs, untrapped by noggin, would gain access to BMPRs, even in the absence of sulfatase activity (Fig. 4C, flow pattern on the right, compare Fig. 4A). In addition, when sulfatase is active, free BMPs would be increased in proportion to the excess numbers of free noggin–BMP complexes because of the accelerated dissociation of the noggin–BMP complexes from HSPG. This too would result in excessive access to BMPRs (Fig. 4C, flow pattern on the left, compare to Fig. 4B). An increased amount of free BMPs could bind to BMPRs and activate excess BMP signals to cause the joint malformations that underlie SYM1. Noggin with a p.R136C mutation, however, is associated with a limited range of developmental defects such as ankylosis of PIP/DIP joints and stapes, but not other skeletal deformities that are found in TCC, TCS, or SYNS1. This limited effect might be explained by the level of dysfunction of the noggin mutant or the contribution of HSPG during development of a specific joint.

In conclusion, we identified a novel *NOG* mutation in the heparin binding site of noggin in a family with SYM1 and conductive hearing loss. Computational analyses predicted that the alteration of the binding interaction of the mutant noggin and HSPG might have caused excess BMP signaling that ultimately resulted in ankylosis of PIP/DIP joints and stapes.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.04.015.

## References

[1] Y. Gong, D. Krakow, J. Marcelino, D. Wilkin, D. Chitayat, R. Babul-Hirji, L. Hudgins, C.W. Cremers, F.P. Cremers, H.G. Brunner, K. Reinker, D.L. Rimoin, D.H. Cohn, F.R. Goodman, W. Reardon, M. Patton, C.A. Francomano, M.L. Warman, Heterozygous mutations in the gene encoding noggin affect human joint morphogenesis, Nat. Genet. 21 (1999) 302–304.
[2] P. Seemann, A. Brehm, J. Konig, C. Reissner, S. Stricker, P. Kuss, J. Haupt, S. Renninger, J. Nickel, W. Sebald, J.C. Groppe, F. Ploger, J. Pohl, M. Schmidt-von Kegler, M. Walther, I. Gassner, C. Rusu, A.R. Janecke, K. Dathe, S. Mundlos, Mutations in GDF5 reveal a key residue mediating BMP inhibition by NOGGIN, PLoS Genet. 5 (2009) e1000747.
[3] W.C. Smith, R.M. Harland, Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in Xenopus embryos, Cell 70 (1992) 829–840.
[4] L.B. Zimmerman, J.M. De Jesus-Escobar, R.M. Harland, The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4, Cell 86 (1996) 599–606.
[5] B.L. Hogan, Bone morphogenetic proteins: multifunctional regulators of vertebrate development, Genes Dev. 10 (1996) 1580–1594.
[6] F. Declau, J. Van den Ende, E. Baten, P. Mattelaer, Stapes ankylosis in a family with a novel NOG mutation: otologic features of the facioaudiosymphalangism syndrome, Otol. Neurotol. 26 (2005) 934–940.
[7] C.H. Hwang, D.K. Wu, Noggin heterozygous mice: an animal model for congenital conductive hearing loss in humans, Hum. Mol. Genet. 17 (2008) 844–853.
[8] L.J. Brunet, J.A. McMahon, A.P. McMahon, R.M. Harland, Noggin, cartilage morphogenesis, and joint formation in the mammalian skeleton, Science 280 (1998) 1455–1457.
[9] H.H. Weekamp, H. Kremer, L.H. Hoefsloot, A.M. Kuijpers-Jagtman, J.R. Cruysberg, C.W. Cremers, Teunissen–Cremers syndrome: a clinical, surgical, and genetic report, Otol. Neurotol. 26 (2005) 38–51.
[10] J.R. Bishop, M. Schuksz, J.D. Esko, Heparan sulphate proteoglycans fine-tune mammalian physiology, Nature 446 (2007) 1030–1037.
[11] B.L. Viviano, S. Paine-Saunders, N. Gasiunas, J. Gallagher, S. Saunders, Domain-specific modification of heparan sulfate by Qsulf1 modulates the binding of the bone morphogenetic protein antagonist Noggin, J. Biol. Chem. 279 (2004) 5604–5611.

[12] B. Teunissen, W.R. Cremers, An autosomal dominant inherited syndrome with congenital stapes ankylosis, Laryngoscope 100 (1990) 380–384.
[13] R. Merino, Y. Ganan, D. Macias, A.N. Economides, K.T. Sampath, J.M. Hurle, Morphogenesis of digits in the avian limb is controlled by FGFs, TGFbetas, and noggin through BMP signaling, Dev. Biol. 200 (1998) 35–45.
[14] H.N. Gregersen, G.B. Petersen, Congenital malformation of the feet with low body height. A new syndrome, caused by an autosomal dominant gene, Clin. Genet. 12 (1977) 255–262.
[15] J.P. Drawbert, D.B. Stevens, R.G. Cadle, B.D. Hall, Tarsal and carpal coalition and symphalangism of the Fuhrmann type. Report of a family, J. Bone Joint Surg. Am. 67 (1985) 884–889.
[16] M.Q. Xu, G. Feldman, M. Le Merrer, Y.Y. Shugart, D.L. Glaser, J.A. Urtizberea, M. Fardeau, J.M. Connor, J. Triffitt, R. Smith, E.M. Shore, F.S. Kaplan, Linkage exclusion and mutational analysis of the noggin gene in patients with fibrodysplasia ossificans progressiva (FOP), Clin. Genet. 58 (2000) 291–298.
[17] K.W. Kjaer, M. Tiner, S. Cingoz, V. Karatosun, N. Tommerup, S. Mundlos, I. Gunal, A novel subtype of distal symphalangism affecting only the 4th finger, Am. J. Med. Genet. A 149A (2009) 1571–1573.
[18] D.J. Brown, T.B. Kim, E.M. Petty, C.A. Downs, D.M. Martin, P.J. Strouse, S.E. Moroi, J.M. Milunsky, M.M. Lesperance, Autosomal dominant stapes ankylosis with broad thumbs and toes, hyperopia, and skeletal anomalies is caused by heterozygous nonsense and frameshift mutations in NOG, the gene encoding noggin, Am. J. Hum. Genet. 71 (2002) 618–624.
[19] J. Groppe, J. Greenwald, E. Wiater, J. Rodriguez-Leon, A.N. Economides, W. Kwiatkowski, M. Affolter, W.W. Vale, J.C. Belmonte, S. Choe, Structural basis of BMP signalling inhibition by the cystine knot protein Noggin, Nature 420 (2002) 636–642.
[20] J. Marcelino, C.M. Sciortino, M.F. Romero, L.M. Ulatowski, R.T. Ballock, A.N. Economides, P.M. Eimon, R.M. Harland, M.L. Warman, Human disease-causing NOG missense mutations: effects on noggin secretion, dimer formation, and bone morphogenetic protein binding, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 11353–11358.
[21] <http://www.genome.jp/tools/clustalw/> (accessed May 2013).
[22] <http://swissmodel.expasy.org/> (accessed February 2013).
[23] D.J. Johnson, W. Li, T.E. Adams, J.A. Huntington, Antithrombin-S195A factor Xa-heparin structure reveals the allosteric mechanism of antithrombin activation, EMBO J. 25 (2006) 2029–2037.
[24] <http://autodock.scripps.edu/> (accessed May 2013).
[25] <http://plato.cgl.ucsf.edu/chimera/> (accessed July 2013).
[26] <http://www.povray.org/> (accessed July 2013).
[27] <http://browser.1000genomes.org/index.html> (accessed May 2013).
[28] <http://evs.gs.washington.edu/EVS/> (accessed May 2013).
[29] S. Paine-Saunders, B.L. Viviano, A.N. Economides, S. Saunders, Heparan sulfate proteoglycans retain Noggin at the cell surface: a potential mechanism for shaping bone morphogenetic protein gradients, J. Biol. Chem. 277 (2002) 2089–2096.
[30] S. Faham, R.E. Hileman, J.R. Fromm, R.J. Linhardt, D.C. Rees, Heparin structure and interactions with basic fibroblast growth factor, Science 271 (1996) 1116–1120.
[31] M. Morimoto-Tomita, K. Uchimura, Z. Werb, S. Hemmerich, S.D. Rosen, Cloning and characterization of two extracellular heparin-degrading endosulfatases in mice and humans, J. Biol. Chem. 277 (2002) 49175–49185.