FGF components and FGF signaling are upregulated in enhanced BMP signaling mutants.

Because upregulated FGF signaling results in craniosynostosis (15,16,32-34), as exemplified by Apert syndrome (14,17,35), we speculated that enhanced BMP signaling through BMPR1A might positively regulate FGF signaling or components of its pathway leading to premature suture fusion. We first analyzed the expression of FGF pathway components in the skull by immunohistochemistry at embryonic day (E) 17.5. In the AF sutures of mutant embryos, levels of FGF2, FGFR1 and FGFR2 were significantly upregulated (Fig. 2A). Consistent with the upregulation of FGF ligand and receptors in mutant embryos, phosphorylated ERK1/2 (*p*-ERK1/2), a known effector of FGF signaling, was dramatically increased in mutant skull bones and metopic sutures (Fig. 2B). Using preosteoblasts established from CNC-derived skull bones (nasal and frontal), we observed that higher levels of *p*-ERK1/2 were induced with BMP2 stimulation in mutant preosteoblasts compared with control preosteoblasts (Fig. 2C, D). Taken together, these results suggest that enhanced BMP signaling through BMPR1A in CNC cells might upregulate or synergize with FGF signaling to promote craniosynostosis, which we examined further as below.

Augmentation of FGF signaling is not the direct cause to develop skull malformation in enhanced BMP signaling mutants.

To help confirm that the *ca-Bmpr1a* transgene exerted its effects on both skull and suture abnormalities via excess BMP signaling, we tested the impact of decreased wild-type *Bmpr1a* gene dosage by superimposing *Bmpr1a* heterozygosity (*Bmpr1a*$^{+/-}$) (36) on the *ca-Bmpr1a:P0-Cre* double transgenic background. Notably, *ca-Bmpr1a:P0-Cre* carrying one copy of endogenous *Bmpr1a* (*ca-Bmpr1a:P0-Cre:Bmpr1a*$^{+/-}$, rescued; R, n=7) showed normal head morphology that was comparable with controls (control; CT, n=7), whereas *ca-Bmpr1a:P0-Cre* littermates with two

copies of endogenous *Bmpr1a* retained the craniosynostosis phenotype (mutant; MT, n=7) (Fig. 3A). We confirmed the patency of AF sutures in rescued mice by skeletal staining and histological analysis (Fig. 3A). We also noted that skull bone thickness in rescued mice was comparable to that of control mice (Fig. 3A). We further examined the expression level of *Bmpr1a* among control, *ca-Bmpr1a:P0-Cre* and *ca-Bmpr1a:P0-Cre:Bmpr1a*$^{+/-}$ preosteoblast cells by quantitative real time RT-PCR (qRT-PCR). *Bmpr1a* expression was reduced by more than half in *ca-Bmpr1a:P0-Cre:Bmpr1a*$^{+/-}$ cells as compared with both control and *ca-Bmpr1a:P0-Cre* preosteoblast cells (Fig. 3B). Expression levels of the transgene were very low when assessed using transgene-specific primers by qRT-PCR (data not shown), explaining the observation that levels of *Bmpr1a* expression in the mutants (when measuring transcripts both from the endogenous locus and the transgene) were comparable with those in controls. These results demonstrate that the increase in BMP signaling mediated by *ca-Bmpr1a* transgene is relatively modest in that the loss of one wild-type allele may compensate, and consequently that precise regulation of BMP signaling is critical for maintaining suture patency and normal skull morphogenesis.

Since FGF signaling was also upregulated in mutant skulls (Fig. 2), we speculated that increased BMP signaling in CNC cells might enhance FGF signaling to cause premature suture fusion in *ca-Bmpr1a:P0-Cre* mice. To compare the regulation of FGF signaling molecules in normal and mutant skulls, we examined their levels of expression in nasal-frontal bones by qRT-PCR. On one hand, the expression of *Fgf2*, *Fgfr1*, *Fgfr2,* components for the FGF signaling, and *Sprouty1/2/3/4*, downstream targets for the FGF signaling, in mutant (MT) mice were enhanced compared with control (CT) mice (Fig. 3C), suggesting enhanced FGF signaling accompanies enhanced BMP signaling in this model. However, the expression of these genes was comparable between mutant (MT) and rescued (R) mice (Fig. 3C). Furthermore, we found comparable levels of *p*-ERK1/2 in mutant (MT) and rescued (R) preosteoblasts, which still showed a higher degree of

ERK1/2 activation as compared with control (CT) preosteoblasts (Fig. 3D). These results suggest that the rescue of the craniosynostosis phenotype in compound inducible-transgenic and haploinsufficient mice was not due to the normalization of FGF-ERK1/2 signaling.

Enhanced Smad-dependent BMP signaling pathway is responsible for the etiology of craniosynostosis.

Upregulated FGF/ERK signaling did not appear to account for skull development abnormalities observed in *ca-Bmpr1a:P0-Cre* mice (Fig. 3C, D). We investigated other potential explanations of how enhanced Smad-dependent or -independent BMP signaling might induce premature suture fusion in mutants via downstream intracellular signaling. We assessed activation of other mitogen-activated protein kinase (MAPK) pathways including p38, which are thought to be effectors of Smad-independent signaling by BMP and TGF-β ligands (37). Consistent with findings of enhanced Smad activation in skull-derived preosteoblasts (Fig. S2B), mutant calvarium exhibited higher levels of phosphorylated SMAD1/5/8 (*p*-SMAD1/5/8) compared with controls (Fig. 4A). On the other hand, levels of phosphorylated p38 (*p*-p38) remained undetectable in both control and mutant skulls (Fig. 4A). In addition, BMP2 stimulation did not activate p38 or JNK beyond basal levels in skull-derived preosteoblasts (Fig. 4B). Moreover, levels of phosphorylated TGF-β-activated kinase 1 (TAK1), the MAPKKK functioning upstream of p38 and JNK (38), was comparable between control, mutant, and rescued preosteoblast cells (Fig. S9). The lack of modulation of TAK1 or specific MAPK pathways in mutant mouse tissues suggested that canonical Smad-dependent BMP signaling may be primarily responsible for the craniosynostosis phenotype. To investigate further the relationship between enhanced Smad-dependent BMP signaling and skull defects in *ca-Bmpr1a:P0-Cre*, we examined the kinetics of *p*-SMAD1/5/8 signaling in control, mutant and rescued preosteoblasts. As seen in the mutant calvarium showing

enhanced SMAD1/5/8 phosphorylation (Fig. 4A), mutant preosteoblasts (MT) displayed the higher basal levels of *p*-SMAD1/5/8 (at 0 min) and greater levels of *p*-SMAD1/5/8 in response to BMP2, which were restored in rescued presosteoblasts (R) to levels comparable to wild-type preosteoblasts (CT) (Fig. 4C, D). These findings strongly suggest that enhanced Smad-dependent BMP signaling is the principle etiology of craniosynostosis in *ca-Bmpr1a:P0-Cre* mice.

Treatment of selective chemical inhibitor of BMP type I receptor kinases partially rescues craniosynostosis phenotype in vivo.

To assess further the role of enhanced Smad-dependent BMP signaling in the skull malformation phenotype, we used a selective chemical inhibitor for BMP type I receptor kinases, LDN-193189 (22,39) as a means for normalizing Smad-dependent signaling. Since LDN-193189 and other chemical kinase inhibitors are known to possess various off-target effects (40), we validated the selectivity of LDN-193189 for Smad-dependent BMP signaling in our tissues. We measured levels of *p*-SMAD1/5/8 with varying concentrations of LDN-193189 using wild-type preosteoblasts from neural crest-derived bones in skull. Similar to what was previously reported (22), in skull-derived preosteoblasts low concentrations of LDN-193189 (~50 nM) were sufficient to inhibit BMP mediated phosphorylation of SMAD1/5/8 without affecting Smad-independent signaling pathways (Fig. 5A, left panel). We further examined the potential impact of LDN-193189 upon levels of *p*-TAK1, *p*-ERK1/2 and *p*-p38 induced by FGF stimulation (Fig. 5A, right panel), confirming the activity of LDN-193189 as a selective inhibitor of Smad-mediated BMP signaling in CNC-derived preosteoblasts. We next explored the efficacy of LDN-193189 in the pre-natal prophylaxis of craniosynostosis in *ca-Bmpr1a:P0-Cre*. LDN-193189 was administered (2.5 mg/kg per day i.p.) to pregnant *ca-Bmpr1a* females after timed mating with *P0-Cre* males starting at E14.5 (Fig. 5B). After birth, lactating dams continued to receive LDN-193189 through P15, and neonates were

euthanized at P16. Importantly, the cranial morphology of *ca-Bmpr1a:P0-Cre* treated in utero and perinatally with LDN-193189 was normalized (Fig. 5C). The length of nasal bones (n=5/6) and the shape of foramina in frontal bones (n=3/6) were normalized to that of control mice. Interestingly, prenatal LDN-193189 treatment did not visibly affect normal skull development in control pups (n=6/6) (Fig. 5C). Bone volume and trabecular number were decreased and trabecular spaces were wider in nasal and frontal bones of *ca-Bmpr1a:P0-Cre*, but were also normalized after LDN-193189 treatment (Fig. 5D). These results demonstrate that prenatal and perinatal treatment with a selective chemical inhibitor of BMP type I receptor kinases can partially rescue craniosynostosis in vivo, further supporting the importance of precisely regulated Smad-signaling in this phenotype.

Discussion

Our genetic study demonstrates that tightly controlled levels of Smad-dependent BMP signaling through BMPR1A in cranial neural crest (CNC) cells, not in committed osteoblasts, are critical for regulating suture patency and normal skull morphogenesis, whereas excessive signaling leads to craniosynostosis. Underneath molecular mechanisms of our findings are summarized in Fig. 6. In wild-type, BMPR1A exerts both Smad-dependent and -independent signaling upon BMP ligand binding. In the mutant mice (*ca-Bmpr1a:P0-Cre*), the basal levels of Smad-dependent signaling are upregulated and further increased upon BMP ligand binding. Increases of *p*-ERK1/2 are also observed, not due to the increase of Smad-independent signaling mediated by TAK1, but due to the increase of FGF signaling (Fig. S9). In the rescued mice (*ca-Bmpr1a:P0-Cre:Bmpr1a*$^{+/-}$), removal of one copy of endogenous *Bmpr1a* normalizes levels of Smad-dependent signaling leading to the phenotypic rescue of suture patency and skull morphology. Enhanced levels of FGF-ERK signaling are still observed in the rescued mice. There are two outstanding questions why Smad-signaling enhanced by the constitutively activated BMPR1A is normalized by removal of

one copy of the endogenous gene and why FGF signaling is still augmented in the rescued samples (see below for potential explanations). Although additional studies are necessary to answer these questions, our current results clearly demonstrate that a modest increase of BMP signaling via Smad-dependent signaling in CNC cells can be a cause of a premature suture fusion in our mouse model.

There is overwhelming evidence both in humans and mice that enhanced FGF signaling contributes to several types of craniosynostosis (1,2,41,42). Our mutant mice also exhibit upregulated FGF signaling; however, our model appears to be unique in that the enhanced levels of FGF signaling observed do not appear to be sufficient for the phenotype. Rather, the independent contribution of Smad-dependent BMP signaling in our model suggests an important and novel signaling mechanism for the etiology of craniosynostosis. Because associated gene mutations are identified in only 20-30% of human craniosynostosis (1,2) and each case shows diverse phenotypes such as positions of premature fusions and thickness of calvaria (26,35), some of the human cases may be developed by mutations in other signaling cascade than FGF signaling. Our findings suggest one of the possibilities that some of the human craniosynostosis are caused by misregulation of BMP signaling.

Since enhanced production of FGF ligands and receptors, and consequent activation of ERK1/2 are the causes of Apert syndrome (15-17,34), it is surprising that our compound transgenic/haploinsufficient mice exhibited upregulated FGF signaling in the face of essentially complete phenotypic rescue (Fig. 3B, C). One explanation may be that enhanced FGF signaling in *ca-Bmpr1a:P0-Cre* mice is not sufficient to induce morphological malformations, i.e., FGF signaling in *ca-Bmpr1a:P0-Cre* does not attain levels needed to develop craniosynostosis, as seen in $Fgfr2^{+/S252W}$ mice, for example (17). However, it is possible that the enhanced FGF signaling activity found in *ca-Bmpr1a:P0-Cre* mice may enhance pathogenesis of skull deformity, but only

in the context of sufficient BMP signaling. While the mechanism of how enhanced BMP signaling upregulates the expression of FGF ligand, receptors and downstream components in mutant skulls is unclear, it has been reported that FGF receptor genes have Sp1 and Ap1 binding sequences in their promoters (43,44). Deletion of the Ap1 promoter sequence from a TGF-β and BMP-inducible gene disrupts BMP and TGF-β responsiveness (45), suggesting BMP signaling might regulate FGF via the Sp1/Ap1 promoter sequences. Thus, one could postulate that enhanced Smad-dependent BMP signaling may induce Sp1/Ap1 family transcriptional regulators that will bind to promoters of FGF receptors to enhance their expression in *ca-Bmpr1a:P0-Cre* mice. Subsequently, phospho-ERK1/2 may be evoked through the augmentation of FGF signaling in *ca-Bmpr1a:P0-Cre* mice. Further studies investigating the overlay of genetic or pharmacologic suppression of FGF signaling in our model would help to resolve this possibility.

The simplistic notion that excessive FGF-mediated signaling and cell growth drives the pathophysiology of craniosynostosis is complicated by the fact that craniosynostoses with enhanced FGF signaling are not always marked by enhanced proliferation and differentiation. Specifically, the FGFR2 C342Y mutation (Crouzon type craniosynostosis) inhibits preosteoblast differentiation and increases apoptosis in vitro (21). Moreover, Deng et al. found that the FGFR2 S252W mutation results in Apert type craniosynostosis in mice but did not observe changes in cell differentiation and proliferation, concluding that dysregulated apoptosis is involved in the pathogenesis of Apert type skull defects (26), consistent with our observations (Fig. S5). These observations and the present study support the concept that clinical phenotypes of craniosynostosis are quite divergent and cannot be accounted for by a single mechanism.

Craniosynostosis in *ca-Bmpr1a:P0-Cre* could be rescued by the loss of a single endogenous *Bmpr1a* allele (Fig. 3) suggests that a relatively small increment in levels of Smad-signaling results in the mutant phenotype, despite the use of an overexpressed and constitutively-

active *Bmpr1a* transgene. Expression levels of *ca-Bmpr1a* transgene were quite low when assessed by qRT-PCR (data not shown), which may lead to the very modest increase of signaling associated with transgene. The completeness of rescue by heterozygosity, and the mildly enhanced ligand-independent and ligand-dependent BMP signaling observed in the tissues of these mice, and with other constitutively-active BMP type I receptor transgenes (22,46) suggest that the levels of BMP signaling attained by these strategies are only mildly supraphysiologic. The results from our pharmacological rescue also support this notion because the dose of LDN-193198 that did not influence skull morphology in the controls was capable to rescue the craniosynostotic phenotypes in the mutants (Fig. 5). Substantially larger increases in BMP signaling, especially during embryogenesis, lead to devastating outcomes (18,47). In contrast, constitutively-activating GS domain mutations of the BMP type I receptor *ACVR1* found in fibrodysplasia ossificans progressiva (48) permit survival to term with only mild morphological abnormalities, likely as a result of mildly increased activity of mutant protein expressed under endogenous promoter control (49). We interpret these data to suggest that moderate increases BMP signaling, for example caused by mutations in enhancer regions, could lead to craniosynostosis in the appropriate context, a possibility that has yet to be investigated in human studies. One interesting issue that remains to be addressed is whether the phenotypic rescue by heterozygosity of *Bmpr1a* is due to cell autonomous or non-cell autonomous effects. Using *Wnt1-Cre*, it was reported that nearly all cells in nasal and frontal bones are derived from CNC cells during skull development (2,11,13). Similarly, as shown in Fig.S3, we confirmed that *P0-Cre*, which we used in our study, could efficiently and specifically target to the CNC-derived cells in skull. We expect that in *ca-Bmpr1a:P0-Cre:Bmpr1a*$^{+/-}$ mice, virtually all cells in nasal and frontal bones in addition to the metopic suture possess a constitutively active *Bmpr1a* transgene along with loss of one allele of

Bmpr1a. Thus, we are speculating that the skull abnormalities were rescued in a cell autonomous manner.

Both Smad-dependent and -independent BMP signaling pathways orchestrate cell fate, proliferation and differentiation in many tissues. Previous studies show that BMP signaling is critical for skull development (5,6,10,50-52). However, molecular mechanisms by which Smad-dependent and/or -independent BMP signaling regulate cranial development remain unknown. Regarding Smad-independent BMP signaling pathway, X-chromosome-linked inhibitor of apoptosis protein (XIAP) bridges BMPR1A and TAK1 and induces the activation of MAPK including p38 (53,54). One might hypothesize that augmentation of BMP signaling via ca-BMPR1A modulates TAK1 activity, which subsequently activates MAPK signaling pathway to cause craniosynostosis. However, we did not observe significant activation of TAK1 or MAPK p38 in mutant preosteoblasts (Fig. S9). The absence of modulation of TAK1 strongly argues against a role of MAPKs including p38 and JNK in our model; however, we cannot fully exclude the participation of other Smad-independent pathways contributing to the *Bmpr1a:P0-Cre* phenotype.

It has been known that FGFR tyrosine kinase inhibitors including PD173074 and PLX052 significantly prevent the premature suture fusion in organ culture level (16,55), and ERK/MAPK specific blocker U0126 successfully inhibits craniosynostosis in $Fgfr2^{+/S252W}$ mice (17). However, there are no known therapeutic interventions for other mechanisms of craniosynostosis. Since craniosynostoses have very diverse phenotypes (and likely mechanisms), successful strategies would identify chemopreventive reagents that target the specific etiology of each type of craniosynostosis. Our study employing a selective chemical inhibitor of BMP type I receptor kinases suggests a potential therapeutic strategy for prophylaxis of craniosynostosis caused by enhanced BMP signaling. Although it has not yet been reported whether mutation(s) of *BMPRIA*

cause craniosynostosis in human, our results suggest that treatment of LDN-193189 might be useful in craniosynostosis that is either caused by or facilitated by gain-of-function in BMP signaling. Identification of mutations or biomarkers signifying enhanced BMP signaling in CNC-derived tissues corresponding with syndromic or non-syndromic craniosynostosis would permit novel strategies for early identification and intervention for this challenging and incompletely characterized set of conditions.

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Figure legends

Fig. 1. Enhanced BMP signaling through constitutively-active form of *Bmpr1a* causes craniosynostosis.

(A-D) *Ca-Bmpr1a:P0-Cre* (MT) displayed short broad snouts and hypertelorism at P17. (E, F) Skeletal staining at P17 revealed premature fusion in AF suture. AF suture was fused in *ca-Bmpr1a:P0-Cre* (F; white arrows), while AF suture in controls was still patent (E; white arrows). Along with premature fusion, foramina were developed in mutant's frontal bones (black dots). (G, H) H&E staining was performed on histological specimen at P8. AF suture in mutants displayed premature fusion (H) although AF suture in control showed patency (G; red arrows). The thickness of mutant skull was thinner than control (black lines). Abbreviations: AF, anterior frontal suture; F, frontal bone; N, nasal bone; PF, posterior frontal suture.

Fig. 2. Enhancement of BMP signaling results in upregulation of FGF ligand, receptors and phospho-ERK1/2.

(A) Immunohistochemistry was performed by using FGF2, FGFR1 and FGFR2 antibodies (green) at E17.5. Levels of FGF2, FGFR1 and FGFR2 were upregulated in anterior frontal suture and osteoblasts in *ca-Bmpr1a:P0-Cre* (MT). (B) The levels of phospho-ERK1/2 (P-ERK1/2) in *ca-Bmpr1a:P0-Cre* at E17.5 were highly activated in comparison to controls (CT). Osteogenic front was marked by white asterisk. (C) Kinetics of ERK1/2 pathway activation following BMP2 stimulation. Preosteoblasts were stimulated with recombinant BMP2 (100 ng/ml) for the time indicated (min). Levels of P-ERK1/2 along with levels of total ERK1/2 proteins were measured by western blotting. Peak and sustained levels of P-ERK1/2 in *ca-Bmpr1a:P0-Cre* preosteoblasts were much higher than in control preosteoblasts. (D) Results in C for P-ERK1/2 quantified by densitometry.

Fig. 3. Heterozygous mutation of *Bmpr1a* rescues the phenotype of craniosynostosis.

(A) Heterozygous null mice for *Bmpr1a* also expressing a *P0-Cre* transgene were crossed with *ca-Bmpr1a*-transgenic mice. Heterozygosity of *Bmpr1a* rescued morphological abnormalities of craniofacial region developed in *ca-Bmpr1a:P0-Cre*. Skeletal staining was performed for skulls. Skull bone shapes were similar between both control (CT) and *ca-Bmpr1a:P0-Cre* carrying heterozygous null *Bmpr1a* (R). Patency of anterior frontal suture was confirmed by histological analysis. Red arrows indicated anterior frontal suture in control (CT) and *ca-Bmpr1a:P0-Cre* carrying heterozygous *Bmpr1a* (R). Note that bone thickness and foramen in frontal bone were also recovered in rescued skull (R) (black lines). (B) Expression of *Bmpr1a* was measured by quantitative real time RT-PCR (qRT-PCR) in preosteoblast cells from CNC-derived skull tissues. Open columns (control; CT), black columns (*ca-Bmpr1a:P0-Cre*; MT) and gray columns (*ca-Bmpr1a:P0-Cre* carrying heterozygous null *Bmpr1a*; R) were shown respectively. Data presented were means ± s.e.m. by three different preosteoblast cells from skull and three independent experiments. *$p<0.05$. (C) Expression of FGF ligand (*Fgf2*), receptors (*Fgfr1, Fgfr2*) and downstream targets (*Sprouty1, 2, 3, 4*) were measured by qRT-PCR in nasal and frontal bones at P4. Open columns (control; CT), black columns (*ca-Bmpr1a:P0-Cre*; MT) and gray columns (*ca-Bmpr1a:P0-Cre* carrying heterozygous null *Bmpr1a*; R) were shown respectively. Data presented were means ± s.e.m. by three different skulls and three independent experiments. *$p<0.05$. (D) Preosteoblasts from the skull were stimulated with recombinant BMP2 (100 ng/ml) and levels of phospho-ERK1/2 (P-ERK1/2) along with levels of total ERK1/2 proteins were measured by western blotting. The levels of P-ERK1/2 were still comparable between mutants (*ca-Bmpr1a:P0-Cre*) and rescued (*ca-Bmpr1a:P0-Cre* carrying heterozygous null *Bmpr1a*).

Fig. 4. Enhanced Smad-dependent BMP signaling is responsible for developing craniosynostosis.

(A) Immunohistochemistry using phospho-Smad1/5/8 (P-SMAD1/5/8) and phospho-p38 (P-p38) antibodies (green) were performed for calvarial sections at E17.5. Samples were counterstained with DAPI (blue). Osteogenic front is marked by white asterisk. (B) Kinetics of MAPK pathways activation following BMP2 stimulation in preosteoblasts of control (CT) and mutant (*ca-Bmpr1a:P0-Cre*; MT). Preosteoblasts were stimulated with recombinant BMP2 (100 ng/ml) for the time indicated (min). Levels of phospho-P38 (p-P38) and phospho-JNK (p-JNK) along with levels of total MAPK proteins were measured by western blotting. (C) Phospho-Smad1/5/8 (P-SMAD1/5/8) levels were examined in preosteoblasts of control (CT), mutant (*ca-Bmpr1a:P0-Cre*; MT) and rescued (*ca-Bmpr1a:P0-Cre* carrying heterozygous null *Bmpr1a*; R). Preosteoblasts were stimulated by recombinant BMP2 (100ng/ml) for indicated time (min) then P-SMAD1/5/8 levels were examined by western blotting. GAPDH was used as a loading control (upper panel). (D) Results for P-SMAD1/5/8 from *C* were quantified by densitometry (lower panel).

Fig. 5. BMP type I receptor-specific chemical inhibitor LDN-193189 partially recovers the craniosynostosis phenotype in vivo.

(A) Wild-type preosteoblasts from skull were pretreated with LDN-193189 (0.005μM-20μM) for 30min. Subsequently preosteoblasts were stimulated by either BMP2 or FGF1 recombinant (100ng/ml) for 10min. Levels of phospho-Smad1/5/8 (P-SMAD1/5/8), phospho-ERK1/2 (P-ERK1/2), phospho-p38 (P-p38), phospho-TAK1 (P-TAK1) were examined by western blotting. GAPDH was used as a loading control. (B) Schematic representation of the dosing and harvesting schedule of LDN-193189 in vivo. (C) Lateral and top of view of face in control (CT) and *ca-Bmpr1a:P0-Cre* (MT) treated with LDN-193189. Note that short broad snouts and hypertelorism in *ca-Bmpr1a:P0-Cre* were partially recovered, which was comparable as control mice. (D) Bone