The Theory of Maternal Administration of Meclizine:

An Achondroplasic Review and the Proposed Treatment of Foramen Magnum Stenosis within a Murine Model

Ava Katharine Perez Erickson

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_____
Kimberly Ann Parrott Mitchell, Ph.D.
Thesis Chair

_____
Kyle James Harris, Ph.D.
Committee Member

_____
Christopher Nelson, M.F.A.
Assistant Honors Director

_____
Date

Abstract

Arising from mostly *de novo* mutations, achondroplasia (ACH) is one of the most common, non-lethal forms of chondrodysplasia. The short stature indicative of ACH stems from a gain of function mutation within the complex *FGFR3* signaling pathway—mutations mitigating the too-rapid ossification of cartilage to bone. Meclizine, an FDA-approved drug long prescribed for motion sickness, halts such a conversion and allows the reconstitution of chondrodysplasia cell lines in attempts at following a normal growth pattern. Evinced by various cell line rescues as well as increased long bone growth, it can be hypothesized that maternally administered meclizine can rescue the ACH phenotype enough to attenuate the largest danger to ACH infants, foramen magnum stenosis (FMS).

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Achondroplasia Pathology and Subsequent Physiology

Characterized by an autosomal dominant pattern of inheritance and complete penetrance, achondroplasia (ACH) is the most common, non-lethal chondrodysplasia with an estimated incidence of 2.6:100,000. Curiously, higher rates of the disease are markedly noted in both Denmark and Latin America *(1)*. ACH has been recognized for thousands of years, as evinced through various artifacts of ancient cultures; evidence of which has been found amongst the populations of ancient Egypt, circa 2500 BC, and in the early state societies of Latin America as early as 100 BC *(2)*. First named formally in the nineteenth century, achondroplasia remains the most studied and best characterized instance of dwarfing skeletal dysplasias *(3)*. Although achondroplasia means “without cartilage formation”, the problem lies not in the formation of cartilage—which primarily makes up the skeleton during early development—but the expediated process of bone conversion, ossification, notably within the long bones of the body. Spontaneous instances of ACH make up the majority of the mutations within the diseased population—80% resulting from *de novo* mutations rather than inherited ones *(3)*. Regardless, no clinical findings favor *de novo* or inherited mutations; the severity of symptoms relies on the diseased state of the individual rather than the aforementioned mutation circumstances. Across the whole of the ACH population clinical findings include most notably: disproportionate short stature, frontal bossing, midfacial retrusion, brachydactyly, trident appearance of hands, lumbar hyperlordosis, genu

varum, and joint hypermobility *(1),* as well as an array of lethal and near-lethal secondary complications.

Ubiquitous in their role in development, secreted mammalian fibroblast growth factors (*FGF*) regulate the fundamental cellular processes that "include the positive and negative regulation of proliferation, survival, migration, differentiation, and metabolism" *(4)* in mammals. There are 22 recorded human fibroblast growth factors (FGF); the signaling components of such FGFs are comprised of 18 secreted proteins that interact with four *FGF* tyrosine kinase receptors, FGFRs *(4)*. Canonical FGFs are bound tightly to their cofactors, HS and HSPGs, and thus are limited in their ability to diffuse through the extracellular matrix, whereas endocrine *FGFs* require protein cofactors αKlotho, βKlotho, or KLPH *(4)* for proper receptor binding. *FGFs* are grouped according to biochemical function and sequence similarities; subfamilies *FGF1, FGF4, FGF8,* and *FGF9* produce several FGFR3 splice variants as a result of their splicing abilities and affinities. One of four fibroblast growth receptors in humans, the telocentric *FGFR3* gene is located on the *p* arm of chromosome 4. The FGFR3 protein itself is comprised of an extracellular domain with three immunoglobin-like regions, a transmembrane domain, and an intracellular tyrosine kinase, as seen simplified in Figure 1. Particularly prevalent on the surface of chondrocytes that give rise to cartilaginous bone *(5)*, most notably long bones, this receptor protein can be pictured as a cup that spans the surface of such cells leaving an empty end jutting out from the outer surface. Under non-mutated conditions, this cup remains empty and thus the FGFR3 protein is silent until various other FGFs—namely FGFs 2,9,18, and 23 *(6)*—can act as ligands to bind to FGFR3 and effectively fill the cup. Ligand-activated FGFR3 is dimerized through the formation of a ternary *FGF-FGFR-HS* complex, which allows the activation of

internal tyrosine kinase domains by phosphorylation of specific residues *(4)*, thus propagating an intracellular signal *(7)* throughout the cartilaginous bone cells.

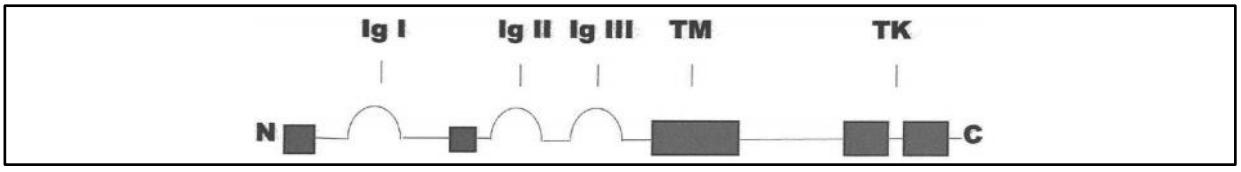


Figure 1. Simplified diagram of the FGFR3 protein, including three immunoglobulin-like domains (Ig), a transmembrane domain (TM), and the split tyrosine kinase (TK) region. Figure modified from reference 3.

Downstream of active FGFRs, the intracellular signaling cascade is also tightly regulated by specialized adaptor proteins such as FGFR substrate 2α and regulators of *RAS-MAPK* and *PI3K-AKT* pathways such as SPRY proteins; due in part to *FGFR3's* affinity to bind, *FGFR3* becomes coupled with other intracellular signaling pathways as seen in Figure 2. Other such intracellular signaling pathways include the RAS-MAPK, PI3K-AKT, PLCγ, and STAT pathways. Several isoforms of FGFR3 proteins are produced from the activation of the *FGFR3* gene and such isoforms are found in various tissues throughout the body; however, most of the isoforms are found in the cells that form bones.

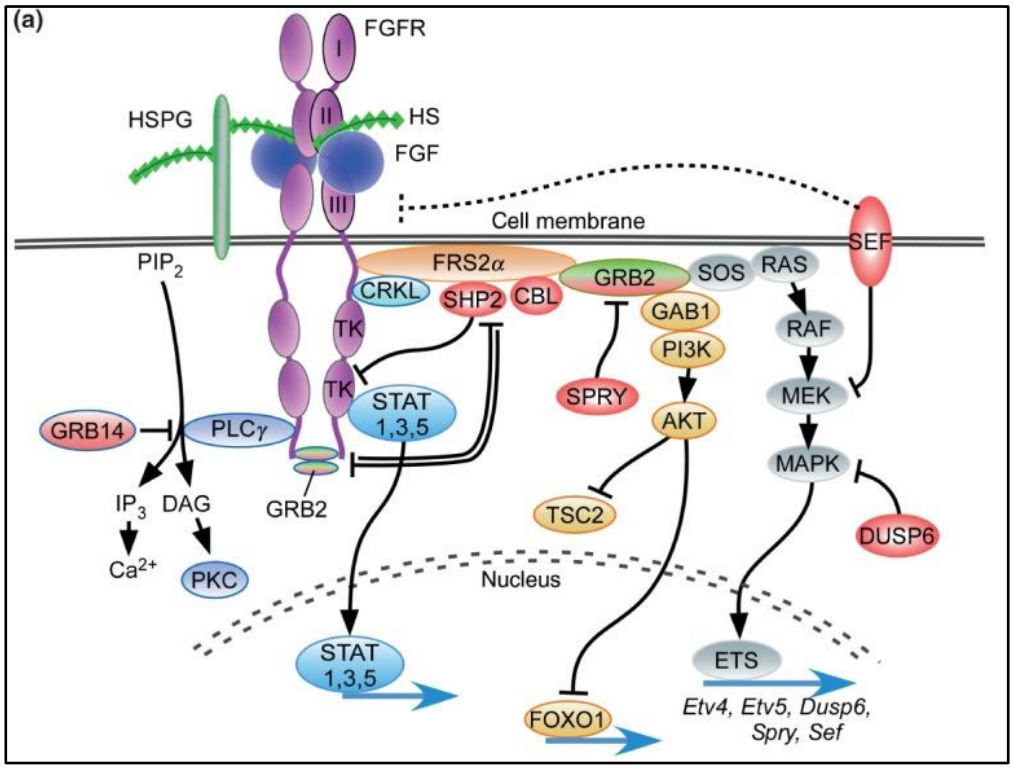


Figure 2. A dimerized *FGFR* signal transduction pathway is coupled with other, complementary signaling pathways to relay a negative signal to halt chondrocyte bone growth throughout the cell. Other intracellular signaling pathways with which the *FGFR3* pathway binds are RAS-MAPK, PI3K-AKT, PLCγ, and STAT pathways. Figure modified from reference 4.

The overall signal propagated within the growth plate of the cartilaginous bone is negative and results in *FGFR3* being a negative regulator of chondrocyte bone growth as its activation results in the shortening of the proliferative phase of growth and the propagation of terminal differentiation *(8)*. The *FGFR3* cup filled with ligands of various FGFs results in a halting signal to be propagated throughout relevant bone cells.

There exist two common pathological variants *(9)* by which true achondroplasia manifests itself, both of which result in a mutation at the 1138th nucleotide position *(10)* of the *FGFR3* gene. The most common form of the mutation, with 98% *(10)* of all ACH individuals possessing this specific error, substitutes the amino acid guanine with that of an adenine. This

particular nucleotide location corresponds to the 380th amino acid of the FGFR3 protein, and due to the missense mutation, the amino acid at the particular position changes from glycine to arginine in the transmembrane domain, resulting in protein malfunction. The other possible variant identified in approximately 1% *(10)* of affected individuals is a substitution of guanine by a cytosine, resulting in the same glycine to arginine protein substitution. Unexpectedly, virtually all mutations within the *FGFR3* gene arise in the same nucleotide pair and result in the same amino acid substitution despite the fact that various point mutations often give way to an array of protein functionalities and identities *(11)*. Classified as a gain of function mutation, "the effect of the ACH mutation on *FGFR3* signaling demonstrates increased ligand-independent activation" *(12)* in areas of the cell with low ligand concentrations; areas of high ligand concentration remain unaffected by the ACH mutation. The transmembrane domain (TM) protein substitution causes drastic protein malfunction due to the critical role TM domains have in stabilizing FGFR dimers. Without tightly regulated ligand control, *FGFR3* signaling increases activation and dimerization *(13,14)* as FGFR3's two kinase domains are brought in close proximity to be cross-phosphorylated and activated without the use of the aforementioned FGF ligands. In terms of the previously mentioned cup analogy, *FGFR3* is activated regardless of whether the cup is filled or not. Independent from regulating ligases, a 2.5-fold increase *(12)* in dimerization and *FGFR3* activation leads to the autophosphorylation of selected tyrosine residues in the cytoplasmic domain of the receptor *(15)*, propagating the negative, slow down signal at an abnormally high rate. With the repressing functionality of *FGFR3* increased, chondrocyte proliferation is reduced, and growth of cartilage and long bones is significantly slowed *(16)*.

Abnormal Anatomy: Viable Infancy

Grouped among the RAMP disorders—recurrent, autosomal dominant, male biased, paternal age effect disorders *(3)*—the propensity for the mutated *FGFR3* gene to be positively selected for in spermatogonial cells increases with advancing paternal age *(17)*. However, whether ACH mutations are caused by paternal age-influenced mutations or allelic inheritance from an affected parent, non-ligand dimerization and activation of the *FGFR3* gene still occurs. Increased signals from the *FGFR3* pathway erroneously repress the chondrocyte proliferation and, consequently, bone growth during critical growth stages for humans, notably fetal and early child development, as well as in adolescent growth spurts. Repression is not total; however, the general growth of all long bones of the body is slowed and modified in comparison to the general development of average sized children *(18)*. It can be noted that the sitting height, and therefore torso, of ACH individuals is near normal *(19).* Such near normal measurements again indicate that mutated *FGFR3* genes primarily affect the osteocytes within the long bones of the body, rather than all osteocytes. If all bodily osteocytes were affected in a way that was still conducive to life, sitting height would be dwarfed along with the rest of the skeleton. It is the small stature, the direct result of the mutated *FGFR3* gene, that has "substantial consequences for the affected individual" *(3)*. Psychological sequelae as a result of drastic stature differences and ensuing physical limitations are prevalent in sufferers, diverging into an array of mental illnesses despite relatively normal cognitive functions *(20)*. Gross motor delays are substantial in ACH children as a direct result of the disproportionate growth stimulated by the mutated *FGFR3* gene. Secondary characteristics such as obesity, due in part to varying degrees of immobility and disproportioned features, become more prevalent with age and although not directly associated with the gene

mutation itself, “are far more prevalent in the ACH population at large” *(21)*. As ACH individuals age, their short stature—the primary result of the ACH mutation—can converge with various medical conditions and other physical limitations that may result in a need for assisted living and ultimately shorter life spans. Although length at birth may be normal, slow growth is both evident and inevitable shortly thereafter. The average height of ACH adult males ranges from 120 cm to 145 cm and 115 cm to 137 cm for adult females. Such measurements are -6 to -7 S.D. below the mean for average individuals.

ACH is most commonly diagnosed in early infancy— although prenatal recognition has become more frequent and gained increasing accuracy. Small stature, although the most distinguishing feature in affected adults, is not always a constant marker in ACH infants whose length could still be considered normal via the standard height chart range for newborns. Rhizomelic shortening is uniformly present, with varying severity, along with abundant skin folds of the upper arms and thighs. Macrocephaly both at birth and throughout life is coupled with frontal and parietal bossing of the cranium. Coupled with cranial bossing, midfacial retrusion results in the flattening of the entire midface and nasal bridge, which results in the anteversion of the nose. ACH infants often present with a smaller than average chest with overly compliant ribs, which results in paradoxical movement during respiration. Although not present at birth, both thoracolumbar kyphosis and lumbar hyperlordosis, or “swayback”, develop during infancy and when walking begins. Bowing of the mesial segments of the legs, although not congenital, most often arises in early childhood progressing until growth is completed. Virtually all joints within ACH individuals are able to be hyperextended except that of the elbow, which becomes progressively stiffer with age. A combination or select few characteristics can be

displayed in infants, characteristics that vary in each individual. The combination of overall joint hypermobility, with the exception of the elbow with hypotonia creates an unusual floppiness in dwarfed infants. Although such features strongly indicate ACH, diagnostic confirmation requires radiographic assessment. Such an assessment, if ACH is indeed present, will yield a physical description of "short, robust long bones; squared off iliac wings; flat, horizontal acetabula; narrowing of the sacrosciatic notch, proximal femoral radiolucency, narrowing of the interpediculate distance of the caudal spine; short proximal and middle phalanges" *(3)*.

Aside from the primary symptoms caused by the gene mutation, further complications include obesity, hydrocephalus, obstructive sleep apnea—which in itself can cause serious developmental consequences if left untreated—middle ear infections and spinal stenosis. When combined, mutational abnormalities and secondary complications yield an average ACH life expectancy of ten years less than that of the non-affected populace. Energy expenditure and caloric needs are less in those with ACH, resulting in a greater propensity for obesity in affected individuals; efforts for any weight loss, therefore, need to be more rigorous and strictly upheld than that of average-sized adults in order to avoid obesity and the implications which accompany it—namely cardiac disease. It is imperative that those with achondroplasia consume 2/3 of the caloric intake of a normal sized individual. This is in part due to the direct relationship between weight and cardiovascular disease—obesity being closely coupled with increased risk of heart disease. Heart disease is the leading cause of ACH deaths past infancy, with over a mortality rate greater than twofold that of the general population *(26)*. Cognitive function is normal in most ACH cases; however, it has been noted that motor delays are quite common. Along with the motor delays, unusual patterns of motor development are often observed. Marked rhizomelic

shortening of the arms and legs, limited elbow extension, generalized joint hypermobility, macrocephaly, and hypotonia underlie these differences. Together, such bioanatomic differences make typical preorthograde movement strategies senseless for a baby with achondroplasia—senselessness that results in delayed developmental milestones such as crawling. Many ACH children instead choose to "snowplow," a movement provided by the feet and the forehead or to "reverse snowplowing," by employing the back of the head and heels *(3)*. Such unique movements allow ACH infants to somewhat compensate for their developmental disadvantages. However, the gross motor delays are still substantial. The median age of independent walking in those with ACH is 18 to 19 months old compared to 9 to 12 months in a normally developing child. Fine motor differences have a biophysical base within the brachydactyly and trident configuration of the fingers along with small joint hypermobility. Due to brachydactyly and hypermobility of the wrist and fingers, a persistent two or four-finger grasp is found within ACH individuals. As a result, there is an inability to exert sufficient force on small objects like that of a pencil and a general fatigue during fine motor tasks.

Abnormal Anatomy: Inviable Infancy

Primary and secondary characteristics of ACH obviously cannot develop if the infant does not survive past birth. With at least a sixfold increase in infant mortality, the ACH populace faces a much higher risk for inviable pregnancies and sudden infant death than that of the general population (*27)*. Such a statistic is thought to be the result of premature synchondroses narrowing and closing— resulting in foramen magnum stenosis (FMS). In normal development, cartilaginous joints of the skull form when neighboring centers of ossification within a continuous mass of hyaline cartilage enlarge and encroach upon each other. This intervening

cartilage functions as a necessary proliferative growth spacer during bone development and is typically replaced by the developing bone as individuals age. In the skull, the synchondroses form between the occipital, temporal, sphenoid, and ethmoid bones of the developing chondrocranium and provide early support for the developing brain *(32)*. In an ACH infant, however, the heightened bone-to-cartilage conversion rate—which stunts the growth of individuals who make it past their birth—causes such cartilaginous spaces to disappear too quickly. Along with waning support for the brain, as a result of soft cartilage being rapidly replaced by hard bone, the foramen magnum also narrows dramatically *(33)*. The resulting FMS pinches the vertebral arteries, medulla and meninges, dural veins, and anterior and posterior spinal arteries that pass through the foramen, and the life of the affected neonate is quite literally pinched off *(33)*.

Rudimentary Treatments

Short stature as a result of stunted long bone growth has been treated with growth hormone (GH) therapy *(22),* and initial acceleration of growth is shown; however, the effect lessens over time and results in, at best, 3 cm of additional adult height. Limb lengthening procedures, the most effective treatment option *(23),* may increase the height of individuals up to 30-35 cm; however, complications are both frequent and potentially serious. Such procedures are performed on ACH individuals as early as 6 to 8 years of age when chance of recovery is higher and the child is still growing—albeit at an impaired rate. In addition to GH therapy, selective inhibitors have been proposed to counter the effects of "overactive *FGFR3* on endochondral bone formation" *(24)* by inhibiting FGFR3 tyrosine kinase or blocking one of the pathways that are coupled with the *FGFR3* pathway. Additionally, the use of blocking antibodies, which

interfere with the FGF-FGFR3 complex, provides another ACH treatment option. Recommendations for management of the various manifestations of ACH are individualistic in their approach to treat secondary characteristics like stature-induced obesity *(25)*. Preventative measures can be implemented during early childhood to avoid complications, such as obesity, by measuring caloric treatment and general awareness of BMI standards as generated for ACH individuals.

Treatments that target the signaling pathways that produce the ACH phenotype are oftentimes the most effective and progressive treatment for ACH. This is due in part by attacking the mutation at its source rather than simply treating the symptoms that subsequently arise as ACH individuals age. Two such treatments include: the CNP/NPR-*B* system and soluble FGFR3s. Although the *de novo* missense mutations of ACH cannot be predicted, it is the complicated nature of the FGFR3 signaling pathway, and its coupled pathways, that can be targeted in order to remediate overactive signaling. Blocking FGFR3 signaling pathways would theoretically adjust or attenuate skeletal dysplasia phenotypes according to Guo, et al. *(28).* However, due to redundant biochemical signals between downstream FGFR family signaling pathways, modulation of the activated FGFR3 pathway requires strategic interventions rather than brute force shutdown. When intracellular phosphorylation of the tyrosine kinase domains occurs during the dimerization and activation of FGFR3, cytoplasmic substrates including FRS2α, Grb2, and SOS are recruited to further activate the predominant downstream MAPK signaling pathway and delay endochondral ossification. The CNP/NPR-B system exhibits antagonistic properties that inhibit MAPK signaling *(28).* The system itself includes C-type natriuretic peptide (CNP), membrane receptor natriuretic peptide receptor-B (NPR-B), and

relevant signal transduction proteins. This pathway positively regulates endochondral ossification, contributing to longitudinal bone growth *(28).* Clinical reports and preclinical trials show that overexpressing CNP increases longitudinal endochondral bone elongation. When CNP physically binds to the NPR-B receptor, it induces increased production of cyclic GMP, which markedly inhibits the MAPK signaling cascade. Since overactive CNP harbors antagonism against the MAPK signaling pathway, it has been shown to rescue defective ACH phenotypes in mice by inducing endochondral bone growth, naso-anal elongation, and increasing weight and thickness of the growth plate; it also has fewer side-effects on blood pressure and the metabolic system than traditional growth hormones do *(28).* The soluble form of human FGFR3, sFGFR3, is a recombinant protein that mimics FGFR3 and competitively binds to the endogenous FGF ligands. SFGFR3 lacks the transmembrane domain and is secreted from cells unable to activate the signaling cascade *(27).* sFGFR3 binds to FGF ligands and decreases the levels of tyrosine kinase phosphorylation—indicative of intracellular *FGFR3* signaling transduction. *In vivo,* sFGFR3 permeates the cartilaginous matrix to reinstate the growth plate by boosting hypertrophic chondrocytes. Postnatally treated mice exhibited restored long bone lengths, increased survival rates and decreased spinal and skull related complications—with few adverse effects such as visceral toxicity. Additionally, sFGFR3- treated mice were fertile and showed rescued phenotypes in their offspring. Previously treated females were able to produce and bear normal sized litters due to a rescued pelvic size *(28)*. If this translates into humans, this would be a significant advantage, as during delivery achondroplasia patients must undergo C-section due to small pelvis size. The decoy approach, with further development, shows promise as a potential treatment for achondroplasia not only restoring stature but also preventing most of the

complications due to the characteristic features of achondroplasia. However, it is of note that despite promises of increased longevity such treatments are still within a very rudimentary phase, as they are unable to significantly alter the ACH phenotype in a manner that would cleanse the afflicted individual of the genetic differences that set them apart from the general populace.

ACH is typically considered a benign disorder *(1)*. Because of this classification, the medical complications that arise from the resulting phenotype are often neglected. ACH, despite being a disease recognized for thousands of years across many different cultures, lacks treatment with any well-established protocols necessary for the surveillance of affected people; most of the existing protocols are made based on the care provider's previous experience or simply the needs of the individual patient. There remains an absence of concrete data on the incidence of sudden unexplained death in infants and ways to detect and deter narrowing of the foramen magnum, which as mentioned previously, contributes to a high mortality rate in the ACH population as a whole.

A Meclizine Hypothesis

ACH manifests itself as a disease of notably disproportionate short stature; chief amidst the other serious complications associated with ACH and accompanying such stature issues is foramen magnum stenosis (FMS) (*31*). Premature closing of the synchondroses, more specifically the spheno-occipital and anterior interoccipital, due to rapid ossification is to blame for narrow foramen magnum measurements recorded in ACH neonates, allowing for the fatal pinching of the spinal cord along with various vital vessels. Meclizine dihydrochloride, or simply meclizine, a drug long prescribed for motion sickness prevents bone loss by inhibiting

osteoclastogenic activity within the FGFR3 signaling pathway responsible for ACH. Drug repositioning strategy allows drugs currently being used to treat specific diseases to be repurposed and used to treat another instead. Such dual purpose allows drugs to be readily cleared for various clinical trials as their optimal doses and adverse effects have already been identified and investigated in prior trials in order to ensure the safety of original trial members (*31*). In addition to its effectiveness as an anti-emetic drug due to its properties as an antihistamine, meclizine suppresses osteoclastogenic activity in three separate chondrocytic cell lines, embryonic bone cultures, and FGFR2 mediated phosphorylation of ERK as evinced by the Department of Orthopaedic Surgery, Nagoya University (*29*). As delineated previously, the FGFR3 pathway that ultimately dictates an ACH phenotype is incredibly complex, as multiple gene pathways and second messenger cascades converge together. Meclizine affects multiple, early portions of this complex pathway, allowing mutated signals to be influenced and curbed to a greater degree. Supplying expectant mothers—either ACH individuals themselves or those who are suspected of carrying an ACH infant—with gestational treatments offers alleviation from both primary symptoms and major complications, like FMS, before significant postnatal developmental milestones begin (*29*). However, there currently exists very little information on optimal meclizine dosage. High levels of any drug, meclizine included, have the potential for causing adverse effects not only in the affected neonate, but in the mother as well (*31*). The determination of an optimal dose of meclizine administered pre-birth via the mother could lessen the occurrence of FMS and eliminate the fear of sudden infant death; additionally, meclizine usage slows the ossification process within the long bones, perhaps producing the same effect human growth hormone treatment has on increasing overall height.

Drug Repositioning Data: Meclozine Facilitates Proliferation and Differentiation of Chondrocytes by Attenuating Abnormally Activated FGFR3 Signaling in Achondroplasia

As observed and proven by Matsushita and colleagues, meclizine suppresses the abnormal proliferation of three chondrocytic cell lines: rat chondrosarcoma chondritic cells (RCS), human chondrosarcoma cells (HCS-2/8), and ATDC5 chondrocytes for the select purpose of studying chondrocytes *in vitro*. RCS naturally express high levels of FGFR3 to produce cartilage-like sulfated proteoglycans, and by injecting the cells with FGF2, the cells can be induced into the erred FGFR3 pathways exhibited in skeletal dysplasia. Such a pathway induces the proliferation of metalloproteinases and decreases proteoglycans, both of which exacerbate the cartilage-to-bone conversation rate. Observed via an MTS assay, it was found that meclizine caused a 1.4-fold increase in the proliferation of proteoglycans despite the errant pathway within the untreated RCS cells where almost all proteoglycans were completely lost within 72 hours of FGF2 injection (*31*). As seen in Figure 3, the presence of round proteoglycans can be seen in RCS cells untouched by FGF2 injections and within the cells rescued by meclizine despite the aforementioned injections. The absence of round proteoglycans can also be noted in cells following FGF2 injections and left untreated by meclizine.

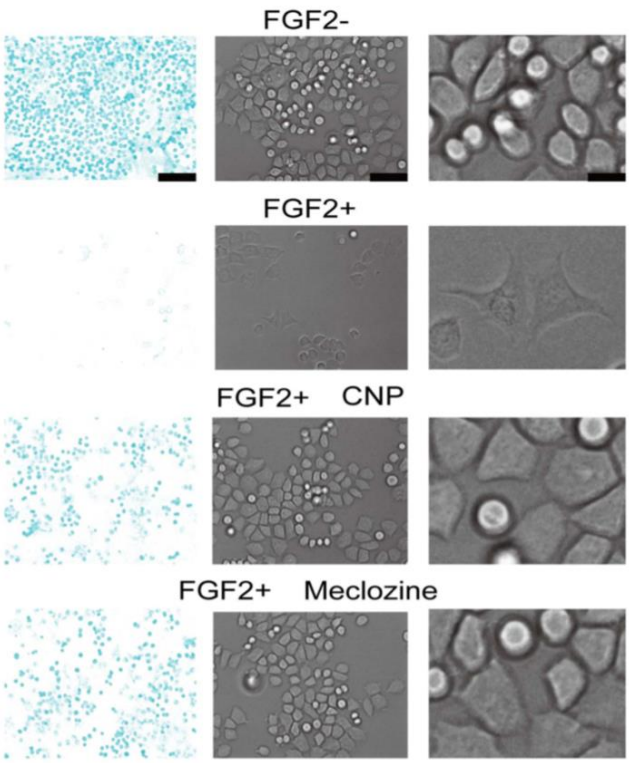
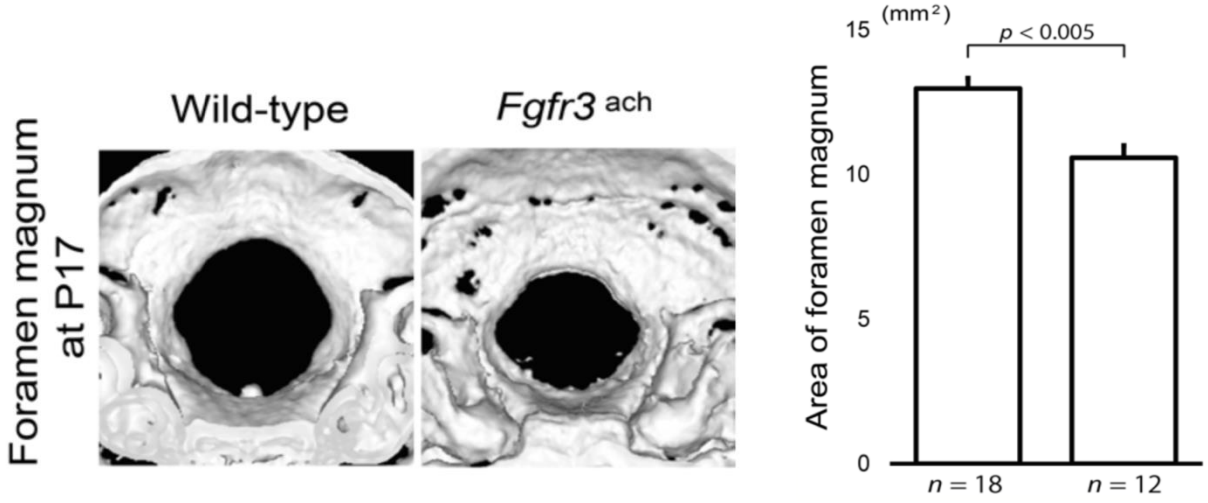


Figure 3. RCS cells were treated for 72 hours with 5 ng/mL FGF2 in order to suppress the expression of the sulfated proteoglycan matrix as seen by a lack of round cellular masses when left untreated with either meclizine (20 μM) or a CNP (0.2 μM) control. Figure modified from reference 31.

As experimental design moved from a high level of induced wild-type FGFR3 from the RCS cell line to that of human chondrosarcoma cells, HCS-2/8 allowed for the examination of human, transduced FGFR3 mutants under meclizine treatments. MTS assays prior to meclizine showed that the FGFR3 mutant chondrosarcoma cells—mutated via a lentivirus carrying 3 active mutants of FGFR3: K650E, K650M, and G380R—demonstrated a severely arrested growth state (*31*). However, following 20 μM meclizine treatments, the stunted growth was partially rescued without any apparent cellular toxicity issues—issues that occurred with 50 μM meclizine injections and RCS cells. Using the same lentivirus, mutated ATDC5 cells were subsequently treated with meclizine to the same effect as RCS cells, exhibiting an increase in proteoglycans.

While the research described above demonstrates the great effect meclizine has on proteoglycan presence— a presence that assists in dictating the formation of cartilage—there still exists a wide gap in the application of such knowledge going from simple cell lines to expectant mothers and their ACH infants. Shifting from chondrocytic cell lines to pregnant murine models fed a meclizine-supplemented diet, in theory, should produce the same rescued proteoglycan production just on a grander scale evinced through lesser degrees of FMS narrowing and less bony bridges. Bony bridges themselves are bone structures that grow abnormally at the synchondroses in ACH; their development is evident in 4.5-day-old mutant mice (*29*). Bony bridge growth prematurely closes the synchondroses and further narrows the foramen magnum (FM) as seen in Figure 4. The extreme narrowing present in FMS is evident in 17-day-old mutant mice (*29*).



a. *b.*

Figure 4. A) The area of the foramen magnum (FM) is naturally narrowed in achondroplasia mice in comparison to their wildtype siblings at post-natal day 17. B) Of statistical significance, FM measurements for mutated mice are (10.58 +/- 0.42 mm^2) compared to wildtype measurements of (12.97 +/- 0.36 mm^2). Figure modified from reference 29.

As seen in Figure 5, bony bridges can be rated: 0, non-bridge; 1, minimal bridge; 2, incomplete bridge; 3, complete bridge. The higher the bony bridge score based on the 3 possible bridges present at the synchondroses within the cranial base, the more premature the closings become which, as mentioned previously, greatly increases the instances for FMS (*29*)

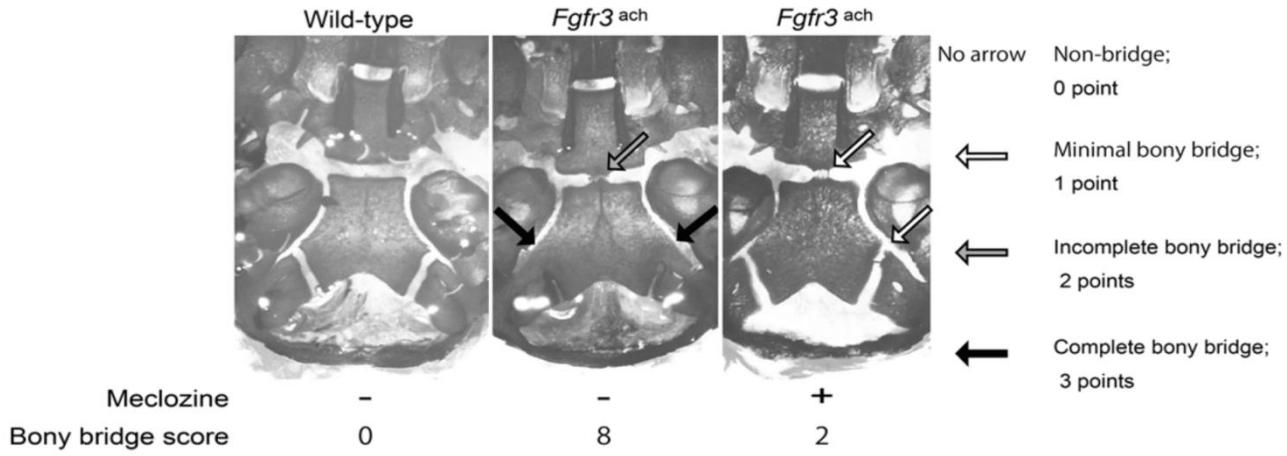


Figure 5. The cranial base of both wild-type and transgenic postnatal day 4.5 mice. Bony bridges can be seen rated 0 to 3 in both untreated wild-type and transgenic mice and treated transgenic mice. No abnormal bony bridges are observed within wild-type mice, while a high bony bridge score and premature synchondrosis closure is observable in untreated mutated mice. A significantly lower bony bridge score is observed in meclizine treated mice. Figure modified from reference 29.

Hypothesis

It can be hypothesized that a maternal meclizine dosage of 0.4 g/ kg of standard feed for 240 hours, roughly 50% of the *Mus musculus* gestation period, is an optimal dosage to increase foramen magnum sizes in ACH-affected mice by limiting complete bony bridges and premature synchondrosis closure.

Experimental Design: From the groundwork of 'Maternal administration of meclizine for the treatment of foramen magnum stenosis in transgenic mice with achondroplasia'

A second study performed by Matsushita and colleagues further contemplated the effects of meclizine as a means to avoid FMS (*29*). A meclizine supplemented diet of 0.4 g/kg was administered maternal ad libitum for 72 hours, 15% of *Mus musculus* gestation period; the FM of both 17.5 embryonic day (ED) mice and 6.5 postnatal day (PD) was observed and marked according to their bony bridge scores, but no data of statistical value was obtained (*29*). While both placental (ED 17.5) and breast milk (PD 4.5) meclizine transportability or dosage evidence was confirmed by significant levels of the drug in the homogenized embryos and infants, proceedings with the aforementioned hypothesis are necessary in order to continue the research delineated by Nagoya University—as the discovery of optimal meclizine dosage was left unresolved (*29)*. When optimal dosage is considered, both patient health and medicinal effectiveness are to be taken into account. It can be noted that 0.4 g of meclizine with 1 kg of food is slightly below the mean drug peak concentration of 68.42 ng/ml after a single dose of 25 mg meclizine tablet in humans. Relative safety is assumed with dosages below the mean drug peak despite consistent ingestion for 50% of the *Mus musculus* gestational period (*29*). Meclizine levels that are over the current mean peak drug concentration could indicate a decline in drug effectiveness in comparison to maternal and pup health.

Discussion: Hypotheticals

As noted by previous studies, the premature closure of synchondroses at the cranial base began to develop in 4.5-day old mutant mice and deadly FMS was evident in 17-day old mutant mice (*29)*. The maternal administration of an FGFR3 pathway inhibiting drug, such as meclizine,

is indispensable to prevent premature closure of occipital synchondroses and subsequently, FMS. The limited effects of prenatal treatment on the suppression of the FGFR3 pathway—evinced through the 72-hour maternal meclizine supplemented diet—may be due to relatively low placental transmission of the drug. Such diffusion from mother to child is an indispensable component in the ability to deter FMS from dangerously narrowing; the physical properties of the placental membrane need to align with the pharmacological properties of certain drugs in order to determine the degree by which the drugs may diffuse. FMS poses its largest threat to infants, rather than any other ACH individuals; this leaves only one way to treat such an issue—prenatally. Bony bridge development and FMS cannot be predicted via prenatal scans; however, meclizine should be nonetheless employed as a preventative measure to begin some form of ACH treatment prior to birth or any postnatal developmental milestones. Despite a lack of concrete data surrounding FMS and meclizine, it was noted in a previous study conducted by Matsushita and colleagues that longitudinal bone length increased in the progeny of ad libitum-fed mothers despite this being the goal of meclizine employment *(31)*. From ED 14.5 to PD day 4.5, there was increased longitudinal bone growth—ulna, femur, tibia—by 1.6 to 4.3%. However, maternal administration showed less effect on longitudinal bone growth than oral administration on growing pups from PD 21 to PD 42 *(29)*. In theory, a higher dose of meclizine during the prenatal period would be necessary for treatment of FMS, although serious adverse effects would be of concern to the clinical feasibility of this drug. Rather than severely increasing dosage—despite already being an over-the-counter drug long approved for consumers—placental diffusion compatibility might be observed and used to tweak the pharmacological properties of meclizine in a manner to better facilitate diffusion.

Conclusion

Achondroplasia, despite being recognized for thousands of years, possesses no rational therapy *(30)*—nor, in fact, does any form of skeletal dysplasia possess such treatment. However, meclizine has the potential to present itself as an indisputable victor in its conquest of FMS, beginning with its preventative conquest still *in utero*—an *in vivo* race against quickly narrowed foramina. With the prevention of premature synchondrosis closure and fewer, complete bony bridges by the determination of an optimal meclizine dosage, the devastating effects of FMS can be alleviated antepartum. Departing from the concerns of FMS, the quality of life expected for ACH individuals, namely during infancy, greatly improves. Despite the mounting physical limitations prompted by an inevitable short stature, achondroplasia and viable life are still compatible when infancies are no longer plagued with the sudden and very real threat of death. Meclizine, as possibly one of the most attractive and potential therapeutic agents available (*30)*, must continually be studied in order to provide respite to such a long-recognized disease.

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