



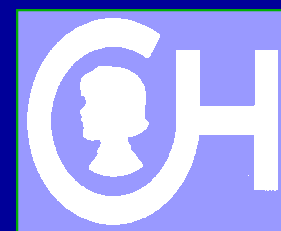
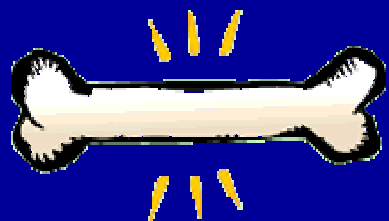
Bone Health and Alagille Syndrome

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Sam's Story

5 year old boy with Alagille Syndrome (AGS)
Sam had many characteristic features of AGS,
including:

- Liver disease
- Heart disease: Tetralogy of Fallot
- Kidney disease
- Growth failure
- Butterfly vertebrae in the spine
- Posterior embryotoxon in the eyes

Fracture History

Fracture history:

- Age 3 years: Stress fractures of right fibula and tibia. Buckle fracture right tibia
- Age 4 years: Fracture left tibia
Poor healing with 25% angulation
- Age 5 years: Recurrent fracture left tibia



Overview

- Functions of the skeleton
- Bone growth during childhood
- Requirements for healthy bones
- Measuring bone health in children
- Weak bones and fractures in AGS
- Possible reasons for weak bones in AGS
- Ongoing research studies
- Prevention strategies

Functions of the Skeleton



Specialized connective tissue with 3 important functions:

- 1) Structural support for the body
- 2) Protection for vital organs (brain, heart, lungs)
- 3) Reservoir for minerals, such as calcium and phosphorus

The Skeleton is a “Calcium Bank”

“If we don’t get a continuous daily intake of calcium, our bodies know to go to the piggy bank to get more. In our bodies that piggy bank is our bones and our teeth,”

Paul Burstein, M.D.



Calcium

Essential for many body functions, including

- Regulation of the heartbeat
- Conduction of nerve impulse
- Stimulation of hormones
- Clotting of the blood
- Building and maintaining healthy bones

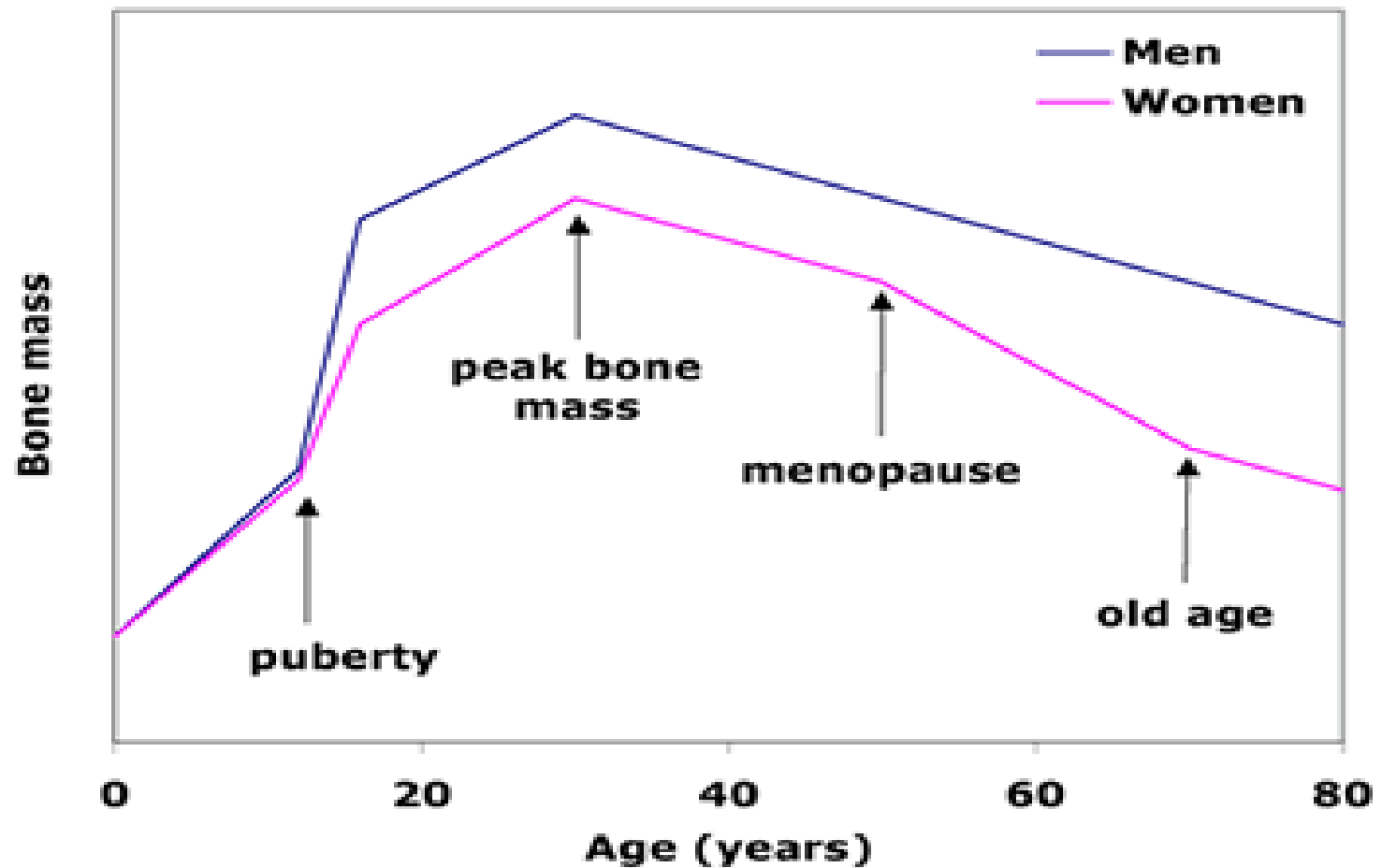
.....and 99% of the body's calcium is found in bone

Bone growth



- Throughout most of childhood, increases in bone mass are closely related to increases in height
- Bone mass increases most rapidly during adolescence, with 25% of peak bone mass accrued during the two years surrounding peak height velocity
- Peak bone mass is the heaviest bone mass that an individual achieves in his/her lifetime

Changes in bone mass with age



Source: <http://www.mrc-hnr.cam.ac.uk>

What do we need for a healthy
skeleton?

Calcium Requirements*

<u>Age Group</u>	<u>Adequate Intake Values (mg)</u>
Birth to 6 months	200
6-12 months	260
1-3 years	700
4-8 years	1,000
9-13 years	1,300
14-18 years	1,300

**Recommendations are meant for healthy individuals. People with AGS (especially those with severe fat malabsorption) may have higher calcium requirements.*

Dietary Sources of Calcium

- Dairy Products
 - Milk
 - Cheese
 - Yogurt
- Fortified foods
 - Certain brands of orange juice
 - Certain brands of waffles
- Other foods
 - Broccoli
 - Salmon
 - Sardines

Determining the amount of calcium from food labels

Labels assume that 100% of the DV equals 1,000 mg of calcium a day

Nutrition Facts			
Serving Size 1/2 cup (67g)			
Servings Per Container 16			
Amount Per Serving			
Calories	100	Calories from Fat	0
% Daily Value			
Total Fat	0g		0%
Saturated Fat	0g		0%
Cholesterol	0g		0%
Sodium	60mg		3%
Total Carbohydrate	22g		7%
Dietary Fiber	0g		
Sugars	15g		
Protein	3g		
Vitamin A	2%	* Vitamin C	0%
Calcium	45%	* Iron	0%

* Percent Daily Values are based on a 2,000 calorie diet.

Great calcium/bone websites

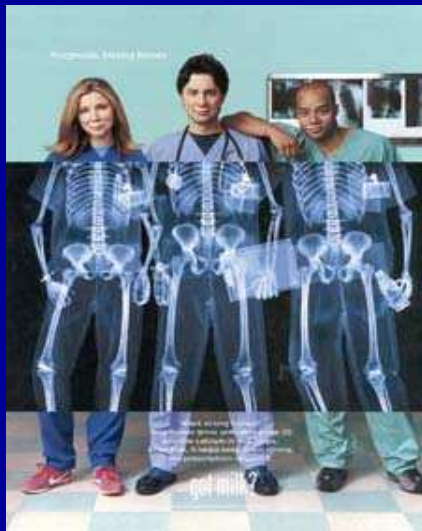
www.3aday.org



3-A-Day of Dairy is a campaign to remind families to get 3 daily servings of milk, cheese or yogurt for stronger bones and better bodies

www.cdc.gov/powerfulbones

Website targeted for girls to teach them about how to have healthy bones. It has games and recipes and is interactive.

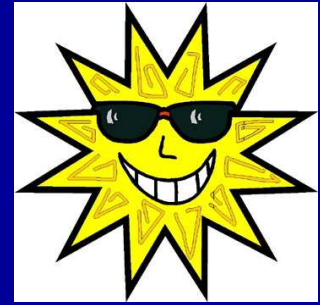


<http://www.whymilk.com>

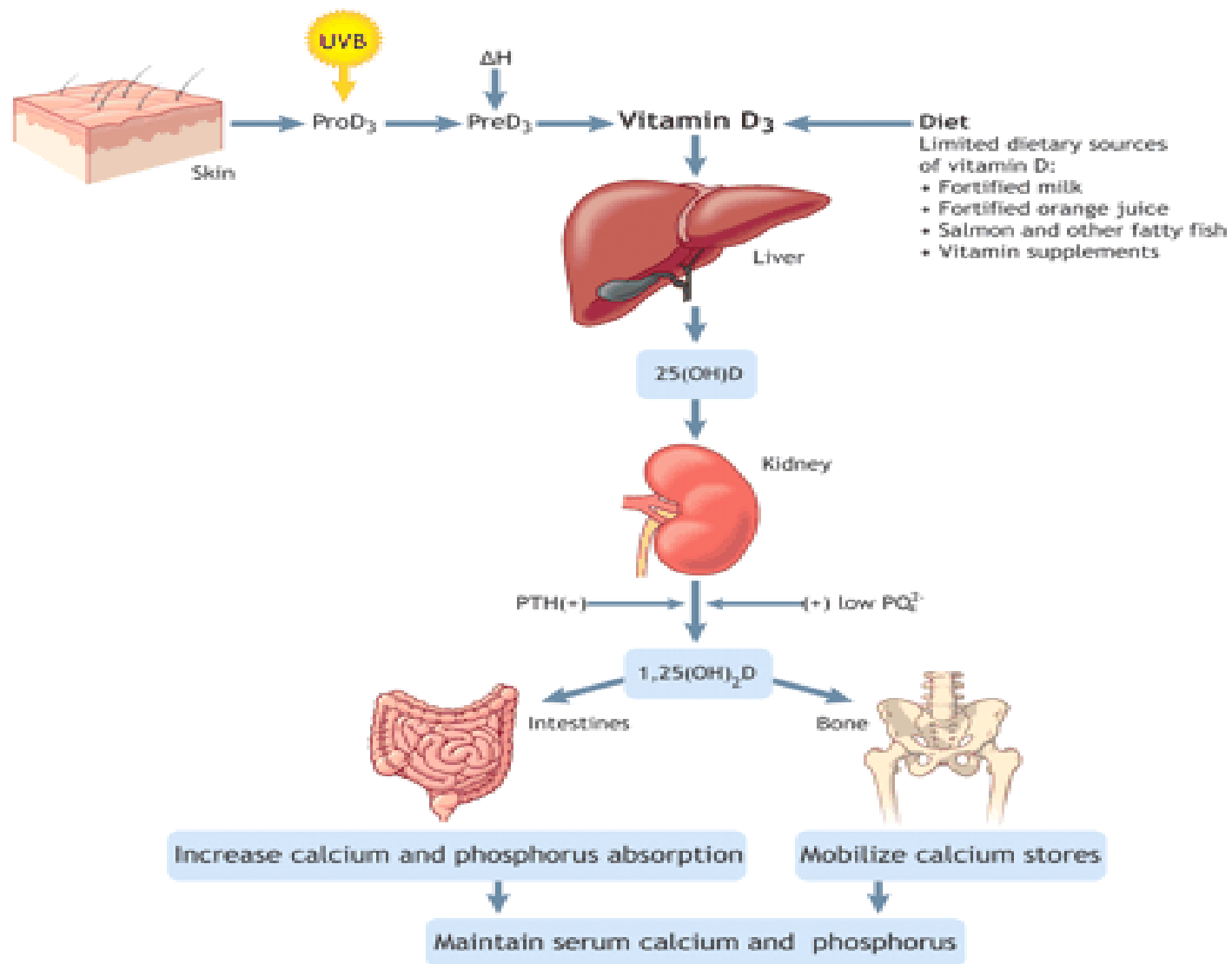
Has celebrities endorsing milk. Also has information about health benefits of milk, recipes and quizzes.



Vitamin D



- Enhances the intestinal absorption of calcium and phosphorus
- Promotes the release of calcium from the bone into the bloodstream
- Found in very few foods naturally
- Humans can make vitamin D from exposure to sunlight



Vitamin D Requirements*

<u>Age</u>	<u>Requirement</u>
Birth – 1 year	400 IU
1 - 18 years	600 IU
18-69 years	600 IU
70 + years	800 IU

**Recommendations are meant for healthy individuals. People with AGS (especially those with severe fat malabsorption) typically have higher vitamin D requirements.*

Food Sources of Vitamin D

<u>Food</u>	<u>International Units</u>
Cod Liver Oil, 1 Tbs.	1,360 IU
Salmon, cooked 3 1/2 oz	360 IU
Mackerel, cooked, 3 1/2 oz	345 IU
Sardines, canned in oil, 3 1/2 oz	270 IU
Vitamin D fortified Orange Juice 8 oz.	200 IU
Milk, vitamin D fortified, 1 c	98 IU
Margarine, fortified, 1 Tbs.	60 IU
Cereal grain bars, fortified	50 IU
Egg, 1 whole (vitamin D is in the yolk)	25 IU

Other Important Vitamins and Minerals

Vitamin/Mineral

Common Food Sources

Vitamin K

leafy greens, broccoli

Magnesium

legumes, whole grains,
nuts, fish, meat

Zinc

meats, whole grains, some
seafood, dried beans, and
nuts

Vitamin C

citrus fruits, tomatoes

Physical Activity

- Exercise helps build bone, and weight-bearing exercise is particularly helpful.
- Weight-bearing exercise includes any activity in which your feet and legs carry your own weight.
- Examples of weight bearing exercise are:
 - Walking
 - Running or Jogging
 - Jumping rope
 - Dancing
 - Climbing stairs
 - Aerobics
 - Hiking

How can we measure bone health in children?



Biochemical tests to assess bone health

- Serum calcium (Ca^{++})
- Serum Vitamin D levels (25-OH)
- Serum PT/INR and PIVKA
- Serum magnesium
- Serum zinc
- Parathyroid hormone (PTH)
 - Hormone secreted by the parathyroid gland which is the most important regulator of body calcium and phosphorus

Assessing bone mineral density (BMD)

- An x-ray is not a sensitive test for diagnosing low bone density.
- There are several different machines that measure bone density.
- DEXA (dual energy x-ray absorptiometry) is the most common method.



DEXA



WHO Definition of osteoporosis in adults

TABLE I World Health Organization Diagnostic Criteria for Osteoporosis

Group	Diagnostic Criteria
Normal	Bone mineral density within 1 standard deviation of the mean of a young adult reference population
Osteopenia (low bone mass)	Bone mineral density between 1.0 and 2.5 standard deviations below the mean of a young adult reference population
Osteoporosis	Bone mineral density <2.5 standard deviations below the mean of a young adult reference population
Severe osteoporosis	Osteoporosis with one or more fragility fractures

NO DEFINITION OF OSTEOPENIA OR OSTEOPORIS EXISTS IN CHILDREN!!!

Qualities of a Good Pediatric DEXA Scan

- Performed in a center where DEXA is done on children
- Separate measurements are made for the spine and whole body
- Results are reported as a “z-score” and not a “t-score”
- Results are adjusted for height and weight

What is known about bone health and fractures in children with Alagille Syndrome?



Evidence of Bone Fractures in Children with Alagille Syndrome

- Anecdotal
- Case studies: One study demonstrates severe post-fracture bone deformities in a young girl with AGS (deHalleux et al., 1998)
- Study of 10-year outcomes in AGS revealed that 6 of 8 patients undergoing liver transplantation experienced fractures (Hoffenberg et al., 1995)

Studies Demonstrating Bone Fragility in Alagille Syndrome

- X-ray evidence of osteopenia (Rosenfield et al., 1980)
- DEXA evidence of deficits in bone area and bone mineral content relative to size (Olsen et al, 2005)

CHOP Bone Study*

- 31 children with AGS (4.1 - 13.7 years) and 80 healthy children (4.1 - 12.5 years)
- Growth measurements
- DEXA measurements
- Coefficient of Fat Malabsorption

* Olsen IE, Ittenbach RF, Rovner AJ, Leonard MB, Mulberg AE, Stallings VA, Piccoli DA, Zemel BS. Deficits in size-adjusted bone mass in children with Alagille syndrome. *J Pediatr Gastroenterol Nutr.* 2005 Jan;40(1):76-82.

CHOP Bone Study

- AGS and healthy children were similar in age and pubertal status
- Children with AGS were small for their age and had decreased whole body bone area and bone mineral content for their age even after corrected for height
- There was an association between poor bone mineralization and fat malabsorption

CHOP Bone Study

	Controls	AGS	P-value*
Whole body scans†‡			
BA-for-HT Z-score	0.0 ± 1.0¶ (-1.7, 1.9)	-1.0 ± 1.4 (-4.4, 3.5)	<0.001
BMC-for-HT Z-score	0.0 ± 1.0 (-2.5, 1.7)	-0.9 ± 1.5 (-3.4, 2.0)	<0.001
Spine scans§			
BA-for-HT Z-score	0.0 ± 1.0 (-2.8, 2.4)	-0.4 ± 1.6 (-3.6, 2.8)	0.146
BMC-for-HT Z-score	0.0 ± 1.0 (-2.7, 2.5)	1.2 ± 3.8 (-12.0, 7.2)	0.202

*P-values by Student's *t*-test or Wilcoxon Rank-Sum test, as appropriate.

†Controls, n = 76; AGS, n = 30.

‡Whole body measures exclude the skull.

¶Mean ± SD (range).

§Controls, n = 80; AGS, n = 31.

AGS, alagille syndrome; BA, bone area; BMC, bone mineral content; Ht, height.

Fractures



Fracture Survey

Surveys were mailed to patient families in the CHOP research and Alagille Alliance databases

406 surveys sent

Survey title: “Alagille Syndrome Growth Study”

Survey included a check box (“yes” vs. “no”) to determine the patient’s history of fractures

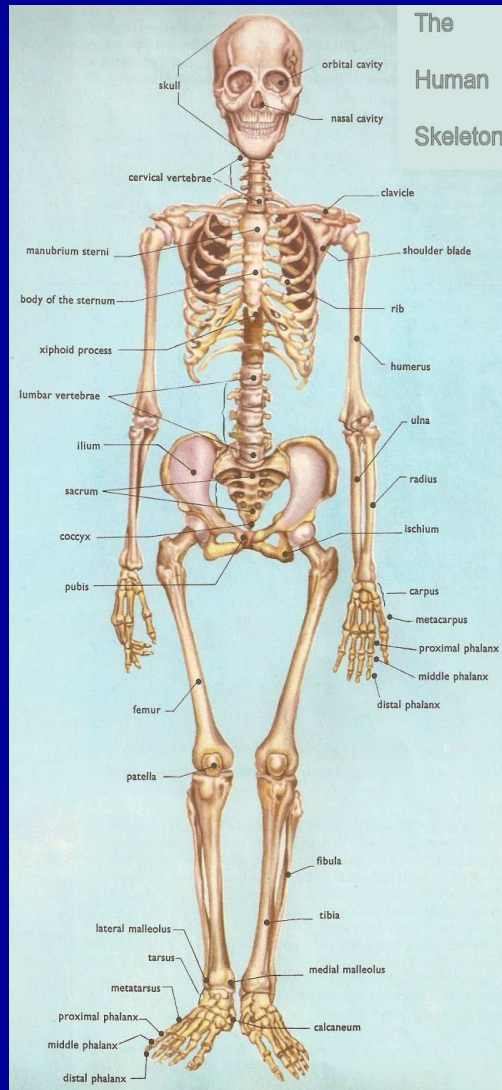
Surveys were reviewed to identify those patients with a positive history of fracture

Patients were contacted by telephone and available medical records were reviewed

Preliminary Results

- 42 Surveys Returned (10%)
- Demographic characteristics
 - Gender : 18 female, 24 male
 - Age: 1 to 35 years (mean 10 years)
- 12 patients reported bone fractures (28.5%)
 - Gender: 6 female, 6 male
 - Age at fracture: 9 mos to 21 years (mean 5 years)
 - Total number of fractures: 27
 - Number of fractures per patient: 1 to 7 (mean 2)
 - Mechanism of fracture: 74% were atraumatic

Anatomic Distribution of Fractures



- 70% of fractures in the lower extremities

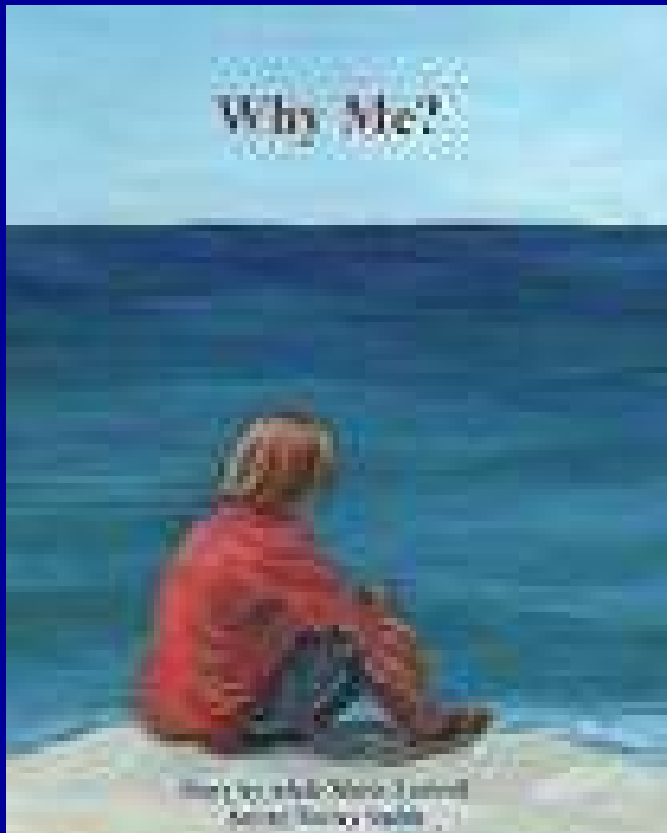
8 femur (30%)

11 tibia or fibula (40%)

- Only 6 fractures (22%) occurred in the upper extremities, where healthy children are most likely to fracture

The fracture survey suggests that children with Alagille syndrome may....

- Break bones without preceding trauma
- Experience fractures at a younger age than healthy children
- Experience more fractures in the leg bones than healthy children



Why do children
with Alagille
syndrome have weak
bones?

Nutritional Deficiencies: The Culprits

- Poor intake
- Intestinal Malabsorption (due to liver and sometimes pancreas disease) can cause deficiencies in:
 - Calcium (fat binds calcium to make “soap”)
 - Fat soluble vitamins, including vitamins D and K
- Kidney disease
- Certain medications
 - Steroids
 - Immunosuppressants
 - Cholestyramine

Other Possible Mechanisms Underlying Weak Bones in Alagille Syndrome

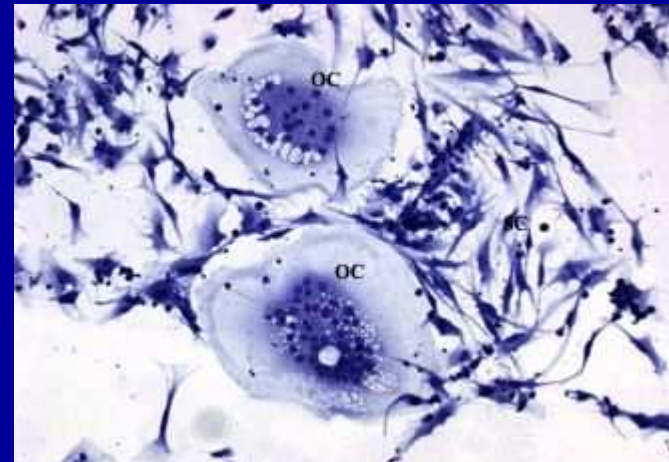
(1) Bilirubin or Bile Salt Toxicity

(2) Genetic Factors: Do mutations in *Jag1* affect the developing bone?



Ongoing Research Studies

Cell Culture Studies: Exploring the Effects of Bile Acids on Developing Bone Cells



Hot off the press!

ARTICLES

nature
medicine

Notch signaling maintains bone marrow mesenchymal progenitors by suppressing osteoblast differentiation

Matthew J Hilton^{1,5}, Xiaolin Tu^{1,5}, Ximei Wu¹, Shuting Bai², Haibo Zhao², Tatsuya Kobayashi³, Henry M Kronenberg³, Steven L Teitelbaum²

Postnatal bone marrow houses mesenchymal progenitor potential, but they are difficult to maintain and expand, controlling their fate decisions. To investigate the potential to genetically remove components of the Notch signaling in the limb skeletogenic mesenchyme, markers for mesenchymal progenitors were undetectable in the bone marrow of severe osteopenia as they aged. Moreover, Notch signaling, which diminished Runx2 transcriptional activity via p19^{INK4}, normally acts to maintain a pool of these mesenchymal progenitors that may be expanded *in vitro* by enhanced by transiently suppressing this pathway.

Notch signaling mediates communication between neighboring cells and controls cell fate decisions during embryogenesis¹ and in postnatal life. In the canonical Notch pathway, the single-pass transmembrane surface receptors (Notch 1–4 in mammals) undergo two proteolytic cleavages upon binding of its ligands (Jagged-2 and Delta-like-1, Delta-like-3 and Delta-like-4 in mammals) presented on a neighboring cell surface². As a result, the intracellular domain (NICD) is released from the plasma membrane and translocates to the nucleus, where it interacts with a transcription factor of the CSL family (RBP-J κ or CBF-1 in mammals).

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nature
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Dimorphic effects of Notch signaling in bone homeostasis

Feyza Engin¹, Zhenqiang Yao^{2,6}, Tao Yang^{1,6}, Guang Zhou¹, Terry Bertin¹, Ming Ming Jiang^{1,3}, Yuqing Chen^{1,3}, Lisa Wang⁴, Hui Zheng¹, Richard E Sutton⁵, Brendan F Boyce² & Brendan Lee^{1,3}

Notch signaling is a key mechanism in the control of embryogenesis. However, its *in vivo* function during mesenchymal cell differentiation, and, specifically, in bone homeostasis, remains largely unknown. Here, we show that osteoblast-specific gain of Notch function causes severe osteosclerosis owing to increased proliferation of immature osteoblasts. Under these pathological conditions, Notch stimulates early osteoblastic proliferation by upregulating the genes encoding cyclin D, cyclin E and Sox7 (osterix). The intracellular domain of Notch1 also regulates terminal osteoblastic differentiation by directly binding Runx2 and repressing its transactivation function. In contrast, loss of all Notch signaling in osteoblasts, generated by deletion of the genes encoding presenilin-1 and presenilin-2 in bone, is associated with late-onset, age-related osteoporosis, which in turn results from increased osteoblast-dependent osteoclastic activity due to decreased osteoprotegerin mRNA expression in these cells. Together, these findings highlight the potential dimorphic effects of Notch signaling in bone homeostasis and may provide direction for novel therapeutic applications.

Conditional Knockout Mouse Studies: Exploring the Effects of *Jag1* mutation in the Developing Bone



Prevention: What Can We Do Now to
Protect Bone Health and Minimize
Fracture Risk in Your Child?

Preventive Strategies

- Be aware of signs of possible fracture
 - Limping
 - Complaining of pain
 - Refusing to walk or use a limb
- Talk to your doctor about a DEXA Scan (age 4-5 years)
- Talk to your doctor about measuring vitamin and mineral levels (generally every 3 months)
 - calcium
 - vitamin D (D25-OH)
 - INR/PT
 - consider PIVKA, zinc, magnesium in certain cases
- Talk to your doctor about looking for kidney disease

Preventive Strategies

- Encourage safe weight-bearing exercise to build strong bones
- Incorporate sources of calcium, vitamin D, vitamin K, vitamin C, zinc, and magnesium into the diet
- Supplement vitamins as needed
 - Separate vitamin (D, E, and K) are preferred to ADEK's
 - Talk to your doctor to help you get insurance coverage
- Seek care of an experienced orthopaedic doctor if your child has a fracture



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