Otitis Media: The Case for Human Evolution in its Pathogenesis

Charles D. Bluestone

I am Charles Bluestone, I am a Professor at the University of Pittsburgh School of Medicine and have been invited to give you a story that I have been working on for many years that I hope will help you in your practice and to understand the pathogenesis, the causes of otitis media that is related to human evolution. So the title of my chapter is Otitis Media: The Case for Human Evolution in its Pathogenesis.

One of the most important articles that relates to this discussion today was published in December of 2010 in the Otolaryngology Head and Neck Surgery[1], by my colleague Dr. Swarts who is an anthropologist, and myself. Dr. Swarts has been important in my understanding of the evolutionary and anthropologic issue related to human evolution related to otitis media.

Otitis media (OM), the most common diagnosis for children by health professionals, is also frequently encountered in adults. Indeed, OM is a major health care problem for which new measures of prevention and treatment are needed. There is general agreement that the etiology and pathogenesis of middle ear (ME) disease is multifactorial, but the consequences of human evolutionary history for the presence and prevalence of OM have not been addressed. We have recently posited that OM is most likely a disease that occurs at such a high prevalence only in humans, in contrast to its infrequent expression in other wild species. We attribute low levels of OM in wild species to the associated hearing loss, which would have dramatic consequences for 2 either a predator or its prey.[1] Normal hearing is essential in the wild.

The ubiquity of OM in humans suggests to us that it is a "normal" feature of our life history. We hypothesize that design compromises resulting from two human adaptations resulted in ubiquitous ME disease: the interaction of bipedalism and our big brain, and the loss of facial prognathism. We also describe an animal model, the Cavalier King Charles Spaniel, which through artificial selection has been bred to have a short snout (reduced facial prognathism) and is reported to have a high prevalence of OM, potentially lending insight into the pathogenesis of ME disease in humans [1].

First of all is my hypothesis that humans are the only species in nature with the high incidence of otitis media. Other animals do get ear infections but it's primary in the ear canal, external otitis, but unusualy they get otitis media. Now why would that be? Well, first of all fish have no middle ear, so how are they going to get middle ear disease? But when we leave the water and got up into the land as amphibians we needed to transfer sound from the air medium to liquid which was in the inner ear, and you need a middle ear for that. Now a middle ear if it

gets fluid in it causes hearing loss, so therefore **hearing is essential in the wild**. Why is that? If you've ever been in East Africa or South Africa where there are animals that are like chimpanzees (*Pan troglodytes*), monkeys and so forth and they are also panthers and snakes. The panthers and snakes love protein and they love to eat monkeys and chimpanzees, so **if you had a hearing loss living in the wild life you would be eaten up by your predator**. We have no absolute proof that other animals have had no significant amount of ear disease with associated with hearing loss. But if otitis media was common in animals, hearing would be poor, it would be unfavorable for survival, and would be selected out, and that is just common sense.

So why do humans have this unique incidence of otitis media? What is different about us, humans, apes, gorillas and orangutan? **There are three consequences of human adaptation**. One adaptation is the **bipedalism** [2], one is the **speech** [3] and one is the **facial flattening** [2]. I will discuss all three.

Bipedalism and Big Brain

One distinguishing feature of hominids when compared with our predecessors, including our nonhuman primate ancestors, is habitual bipedalism (**Figure 1**). **Adaptations for bipedality** are evident in one of our ancient ancestors, *Ardipithecus ramidus*, who lived more than four million years ago.[4] There is no consensus on the evolutionary advantages of either walking and running on two legs or knuckle walking, as in the great apes, over quadrupedal locomotor patterns. However, among the hypothesized advantages of bipedality are improved thermoregulation due to decreased exposure of the body to the sun, the ability to carry (including food and infants), the ability to see over the savannah for food and predators, and freeing of the hands, allowing tooland weapon-making.

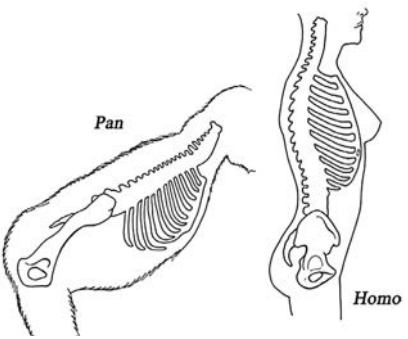

Figure 1. Locomotor posture from chimpanzee (*Pan troglodytes*) and humans (*Homo sapiens*) [5]

Now what about bipedalism related to this otitis media? Well, first of all when we stood upright a couple of million years ago, the issue was not a big problem in terms of being birthed, but when we developed a big brain, which makes us unique in the animal kingdom, we became born too soon. Now what does that mean? Well, if you look at chimpanzees who have almost the same genetic code as we do (98,4%). **Figure 1** shows the chimpanzee, and the **homo sapiens** (humans), and as we stand up: now we have bipedalism.

We are all familiar with the disadvantages that develop as a result of bipedality, including bad backs and joints of the lower limbs, both of which develop after reproductive age. But, an even more significant disadvantage arising from bipedality is the constriction of the pelvic outlet. The narrowing of the outlet is thought to arise as a consequence of the need for osseous support of the

abdominal contents and changes that increase biomechanical efficiency in locomotion. For early hominids, these anatomical changes did not impair the delivery of newborns because their brains and bodies were small relative to their mother's size. However, during the subsequent two million years, the hominid brain approximately doubled in size, such a large increase that the human newborn is born 12 months too early due to the constraints imposed by our big brain on passing through the relatively small pelvic outlet. This sequence of events is well known to anthropologists, as concluded by Martin,[5] who stated that, based on brain development, humans should have a 21-month gestation period; nine months in the uterus and 12 months outside the womb. **Figure 2** depicts the relative sizes of the female bony pelvic birth canal to the size of the brain in the chimpanzee (*Pan troglodytes*) in humans (*Homo sapiens*) and in Lucy or AL 288-1 (predecessor of *Homo sapiens*).[6] Delivery of human newborns is so tight that almost all pregnancies require birth attendants and a cesarean section. We are the only species that needs assistance during parturition.

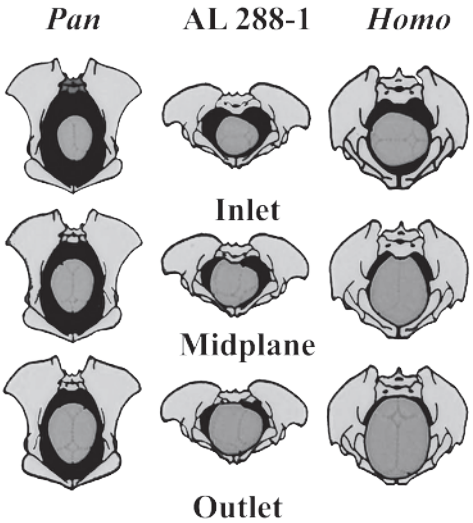

Figure 2. Different sizes of of the female bony pelvic birth canal and the size of the brain in humans (*Homo sapiens*), chimpanzee (*Pan troglodytes*) and in AL288-1 (Lucy), which was a predecessor of *homo-sapiens*. Tague and Lovejoy, 1986.[4A]

We previously described, in detail, the comparative anatomic and birthing differences between humans and our primate relatives, as well as the consequences of being "born too soon" for the ears, nose, and throat.[7] Among these consequences is that the eustachian tube (ET) is too short and floppy during the first year of life. This structural and functional immaturity, in the context of an immature immune system, helps to explain the high incidence of acute OM in the first year of life, especially now that child daycare attendance exposes these highly susceptible babies to respiratory pathogens. A recent report from Norway finds recurrent acute OM more prevalent during the first 18 months of life in premature infants when compared with normal-term babies.[8] This difference was attributed to gestational age differences, not weight at birth, thus, premature infants are "born *way* too soon." But, being born too soon does not explain why OM remains common throughout childhood and, in some individuals, into adulthood.

Speech

What other unique adaptation did *Homo sapiens* acquire during evolution? **Humans are the only species that developed speech**. So why is speech maybe related to otitis media? During our evolution, in a short 40,000 years, our larynx descended, elongating the supralaryngeal vocal tract into a two-tube configura-

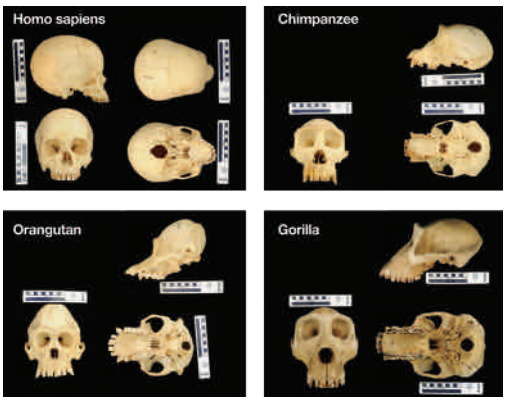

Figure 3. *Homo sapiens* (humans), chimpanzee, orangutan and gorilla maxilla and jaw. Size differences

tion that enhanced speech.[9] This adaptation narrowed the pharyngeal airway and shortened the palate, which probably aids in the production of vowels and consonants, but also has consequences for the palatal muscles.

Loss of Facial Prognathism (Facial Flattening)

The third relevant difference observed in modern humans, when compared with our hominid ancestors and extant nonhuman primates, is **facial flattening, or the loss of facial prognathism**.[10] This is evident in **Figure 3** a human-chimpanzee skull comparison that shows the reduction and repositioning of the maxillofacial complex in the human. Facial flattening, along with descent of the hyoid, contributed to shortening of the palate.[9] So why have we lost our facial prognathism?

Figure 4 shows a cartoon with the chimpanzee, the gorilla, and the human. We can see that the humans maxilla that is rather flattened as compared to the chimpanzee. Look at the chimp's huge prognathic jaw, that is the upper jaw, a huge difference between the chimpanzee's prognathic jaw and the humans face, which is flat. And the orangutan is even worse, with prolonged jaw. So take a look at the gorilla, look at the size of that jaw compared humans. The gorilla has a sort of a big head, gets through the pelvic outlet easily when he is born, but in humans it is even trouble getting out and many children have to have Cesarian section to get out.

Figure 4. Chipanzee, gorilla and human

Face differences between chimpanzees and humans

If you look at **Figure 5** comparing chimpanzee with human, the red being the palate and the bluish green is the epiglottis, and the chimpanzee is on the left and the human is on the right you see that the human who is on the right, has a gap between the epiglottis. The green areas and the soft palate (in red), compared to the chimpanzee and the human baby before 3 months of age, there's a so-called lockup between the epiglottis and the palate. And that allows the animals and babies up until 3 months to be able to such, breath, swallow without choking. But after 3 months of age our larynx drops as on the right side as you can see the human. The larynx drops to a level, almost to the level of the hyoid and therefore we are probably the only species that has choking and aspiration because of that issue [9].

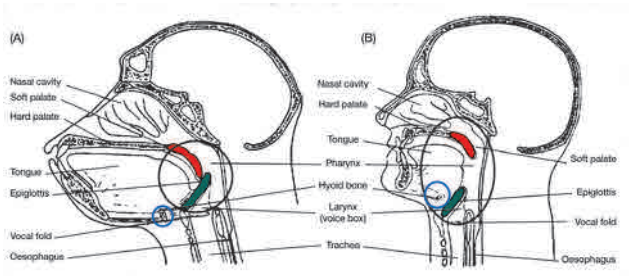

Figure 5. Chimpanzee (left) and human (right)

So how else are we different our ancestors? What other problems do we have that we have adapted to? Well **we have lost our prognathism**, or **we have gotten facial flattening**. How flat our face is compared to the chimpamzee? We have lost our facial flattening from our ancestors who are the hominids that preceded *Homo-erectus* and *Homo-sapiens*, and also the great apes. So why is that a problem?

So **why did we lose our prognathic jaw**? **Cooking of food** may be another possible explanation for our facial flattening. **Our diet changed**. What is so important about **cooking food**? Well, it is been said that gorilla's spend almost 6 hours a day trying to chew food to get enough protein to support their need for calories, As recently described by Wrangham,[11] the earliest evidence of cooking by hominids dates back almost two million years to *Homo erectus*. He hypothesizes that cooking provided the necessary caloric density to meet the energy requirements of our rapidly growing brain. An ancillary effect of this change in dietary processing was an alteration in the size and shape of our teeth, a shorter maxilla and mandible, and relatively small oral cavity in comparison to other hominids. Although speculative, this is an attractive hypothesis to explain both the increase in human neonatal body and brain size and the loss of facial prognathism.[11]

I went to **Galapagos** and wanted to see what **Darwin** did about finches and how it relates to humans[12]. Well, Darwin noted that there are 13 specific species of finches that are different from each other **Figure 6**. And it's related to the islands that they are on, and that eating is related to their niche, their eating niche on that island. Some eat seeds of – ground seeds, some eat from the cactus and the finch beak can be made similar, homologous to the maxilla and mandible. So the point is that **the face changed in these animals and what was it caused by a difference in the kind of eating**.

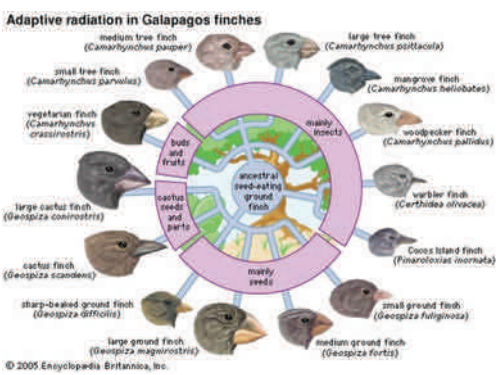

Figure 6. 13 specific species of finches with changed beaks

Darwin also described in his famous book, The Origin of Species, the **Galapagos marine iguana** that is a unique species because the iguana in Latin and South America is a land animal, but the iguana in the Galapagos has learned, adapted to eating algae off the lava rocks under the

Figure 7. Galapagos iguana

water and by doing that it got a short blunt nose. So there is an adaptation for scraping algae or food from the lava rocks under the ocean (**Figure 7**). So **you can change the shape of your face and your eating habits, change the shape of your teeth and your jaw**[12].

Canine Model of Otitis Media and Its Implications for Human Disease

Among veterinarians, chronic ME effusion (termed *primary secretory otitis media)* is a well-known disease in the Cavalier King Charles Spaniel.[13] It has been reported to be present in up to 40 percent of these animals. The effusion is mucoid and fills the entire ME. Diagnosis is made by operating microscopic examination, computed tomography scanning, or magnetic resonance imaging (MRI), and has been confirmed at the time of myringotomy. Myringotomy and tympanostomy tube placement has been recommended for treatment.[14] This breed has been artificially selected to have a shortened front-toback diameter of the skull, a shape termed *brachycephaly*, which arises due to premature fusion of the coronal sutures. The term *neotenous* (retention of juvenile characteristics into adulthood) is also appropriate for these breeds.[15] The Cavalier snores habitually like other brachycephalic dogs, including the English Bulldog, a breed that has been reported to be the only animal known to develop obstructive sleep apnea. Obstructive sleep apnea only occurs in humans in nature. There is one animal, the English Bulldog is the only one to get obstructive sleep apnea

The snoring is undoubtedly secondary to its constricted pharynx, a consequence of the shortening of the snout. As reported by Davidson *et al*,[16] we are also prone to obstructive sleep apnea due to our reduced pharyngeal airway. **Figure 8** compares the head shape of a Cavalier King Charles Spaniel, with its extremely short face, to that of a Golden Retriever, which has a classic prognathic snout.

Figure 8. Photographs of the head of a Cavalier King Charles Spaniel (left) and a Golden Retriever (right) showing the Cavalier with an extremely short snout compared to the Golden Retriever. (With permission from Lynette Cole, DVM.)

The Cavalier King Charles Spaniel is an animal model of chronic OM with effusion. It has been "artificially selected" (Charles Darwin's term) for its

short snout and globular head, but an unintended consequence of breeding for this characteristic is the propensity for chronic OM with effusion. In a recently reported study using MRI, veterinarians from England found that not only did the Cavalier have OM (54%), but another brachycephalic breed, the Boxer, also had ME disease (32%), which was not present in Cocker Spaniels, a mesaticephalic breed. The investigators suggested that the reduced nasopharyngeal space in the Cavalier and Boxers, when compared with the Cocker Spaniel, predisposed them to OM.[17] It might be that one or both of the paratubal muscles is dysfunctional due to the abnormal palatal anatomy in these breeds and is the cause of their OM. The underlying pathogenesis of the Cavalier's ME disease is currently under investigation in our laboratory. Analogously, one could speculate that with the loss of their prognathic face, humans became susceptible to OM, an unintended consequence of "natural selection" for another adaptation (again, Darwin's term), as described above.

Risk factors for otitis media

What are the problems with otitis media related to the first year of life? During the first year of life we have an **immature Eustachian tube** (ET), which **is too short** that structure and function that is **too floppy**, the cartilage support of the ET is very floppy. We have shown that in temporal bone specimens and in function tests. Also, not related to the ET, but the **immune system is very immature** in the first year of life, as you know, and doesn't really get more mature until about 1 year of age, better maturity by 6, and fully matured by 10 years of age. So **prematurity is also a risk factor** for otitis media. So if you are born with 9 months, that's one risk factor, but if you are born at 7 or 8 months, that's even worse, which I call born way too soon because it's before 9 months even.

Also **the impact of nurture [11]**, our predecessors who were *homo-sapiens* in the cave days, mothers would **breast feed**, we never heard of such things as milk, bottle – **bottle feeding**, and we know that **breast feeding is good for prevention of ear disease and bottle feeding is with cow's milk**. And the **environment**, they didn't have day care centers, and they rarely **smoked early on in our span of life** for *Homo sapiens*. We have **increased exposure to viruses and bacteria in day care** and smoking causes probably – problems with cilia in the nose and ET. So That is why the ear disease is most common when it presents itself in the first year of life, so those are three good reasons why (**day care, poor breast feed and smoking**). Also the **use of pacifiers** are related to sucking on the pacifier. When your nose is not obstructed is not a problem, but sucking on a pacifier when your nose is blocked, you get a lot of negative pressure in the back of your throat and that can shut down the ET and when it finally opens up it sucks "junk" or secretion from the back of your nose into your ear.

Change in palate morphology related to Eustachian tube function

Our facial flattening changes palatal anatomy, including the muscles of the palate involved in Eustachian tube function (ETF), muscle tensor veli palatini (mTVP), and muscle levator veli palatini (mLVP). They became less effective physiologically than in the nonhuman primate (*Macaca mullata*).[2]

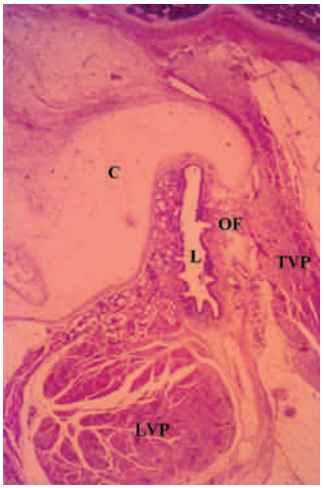

Figure 9. Cross-section through the mid-cartilaginous portion of a left human eustachian tube. Note the robust rounded belly of the levator veli palatini muscle abutting the inferior portion of the tubal lumen, and the rather thin slip of the attachment of the tensor veli palatini muscle to the lateral lamina of the tube. *C,* tubal cartilage; *L,* tubal lumen; *LVP,* levator veli palatini muscle; *OF,* Ostmann's fat pad; *TVP,* tensor veli palatini muscle. (Courtesy: I. Sando, MD.)

When we compared the ETF of monkeys and humans, monkey dilatory (opening) tubal function was consistently superior.[18] Relatively speaking, humans have poor tubal function. This becomes evident during activities that impose nonphysiologic stresses on the ET, such as flying in airplanes and scuba diving, during which equalizing ME negative pressure becomes problematic. By contrast, even the application of sudden large negative ME pressures in the monkey, were easily equilibrated with a single swallow.

The physiologically inferior function of the human ET compared with that of the monkey is most likely due to differences in the anatomy of the paratubal muscles. **Figure 9** is a photograph of a cross-section of the ET showing a relatively slim mTVP attached to the lateral lamina of the tubal cartilage and the relatively large belly of the mLVP, a rounded mass that abuts the inferior portion of the tubal lumen. Even though the rhesus monkey's genome is similar to ours, and although we have employed them in our laboratory as a model for humans in studies involving the physiology and pathophysiology of the ET and the pathogenesis of OM, the paratubal muscles of the rhesus are *not* identical to humans. Comparison of the anatomy of these two muscles between the monkey and the human reveals two major differences: 1) in the monkey, the mTVP has more bulk and attaches to the entire length of the cartilaginous ET, whereas in the human this muscle is not as robust and attaches to only the mid-portion of the ET; and 2) the belly of the mLVP is not as prominent and does not impinge on the inferior portion of the tubal lumen in the monkey in contrast to the human condition (**Figure 10**).[19] The humans have a shorter insertion in the tensor, so therefore the tensor is anatomically different than the monkey.

Doyle and Rood [19] dissected human and monkey specimens and **Figure 10** shows human on the left (**Figure 10A**) you see the marking for mLVP on the human is huge and the mTVP is a slight slip. If you look at the Rhesus Monkey on your right side (**Figure 10B**) you will see that the mTVP is huge and the mLVP is small. So these are differences.

Figure 10A, Human **10B**, Rhesus monkey

Eustachian Tube Muscles Related to Pathogenesis of Human Middle-Ear Disease

As opposed to the extraordinarily high incidence of OM in humans, particularly in childhood, we have almost never observed spontaneous ME disease in the outbred animals (e.g., ferrets and monkeys) we have had in our laboratory over more than 30 years. Is this remarkable difference in the rate of OM related to the comparatively poor ET function a consequence of differences in paratubal muscular anatomy in *Homo sapiens* as compared with the monkey? Experiments with the monkey in our laboratory undergirded our understanding of the pathogenesis of ME disease in the human.[20] We have successfully created OM in this animal model by inactivating the mTVP by either severing the tendon at the hamulus of the pterygoid bone or by injecting botulinum toxin into its belly. Since the mTVP is the only muscle that opens (dilates) the tubal lumen during swallowing, ME effusion develops when it is rendered nonfunctional. We concluded that a healthy mTVP is important in the prevention of OM[20]. Hypothetically, since the monkey's mTVP is larger and has a longer attachment to the tube, producing excellent tubal function, impairment of ETF due to inflammation should not result in OM. Humans, on the other hand, with comparatively poor tubal function, are susceptible to inflammatory conditions that degrade ETF, resulting in OM. In other words, the insertion of the mTVP in the ET cartilage in the monkey is much longer than the insertion in humans. The humans have a shorter insertion in the tensor, so therefore the tensor is anatomically different than the monkey.

Thus, the relatively inefficient human mTVP is a viable candidate for the pathogenesis of ME disease in some individuals, but what role might the mLVP play in OM? We have reported that older children and adults with chronic OM with effusion had ET dysfunction characterized by *constriction* of the ET, as opposed to dilation, during swallowing on the forced response test.[21] This observation in the context of electrical stimulation of the monkey paratubal muscles[22] suggests that the constriction is most likely due to contraction of the mLVP, which collapses the tubal lumen during attempts to dilate the ET. This hypothesis is currently under investigation in our laboratory.

The possible role that mLVP plays in tubal constriction is illuminated further by our studies of children with cleft palate. The infant with an unrepaired cleft palate is an in vivo model of chronic ME disease, which has been shown to be a functional, as opposed to an anatomic, obstruction of the ET.[23] Constriction of the tube has been identified in these babies.[24] Following surgical clefting of the monkey palate, OM with effusion developed.[25] ET function tests revealed constriction of the tubal lumen that we now attribute to a dysfunction of the mLVP. Hypothetically, then, in an effort to prevent OM in these babies, surgical repair of the palate should focus on the mLVP as well as correcting their velopharyngeal insufficiency and speech defect

Buchman *et al*[26] reported that some adult volunteers who had **nasal challenge with virus developed ME disease**. **Why viral infection affected some subjects and not others may be explained by the results of a later study in which adult volunteers who had signs of ET dysfunction prior to a viral challenge were**

the ones who developed more severe dysfunction and ME under-pressures. Subjects with good tubal function before the challenge did not develop OM.[16]

Summary and conclusions

Human evolution has a role in otitis media. It is hereditable, we know that after Cassebrant study in children who were twins, triplets and who were homozygous and heterozygous. She reported that there is heritability of otitis media and that is probably high in females and still high but a little lower in males. Being otitis media prone it is also related to certain racial groups like the Aborigine of Australia, the Southwestern American Indian, Native Americans, the Navajos and the Apache. They have ear disease almost 100% by the time they are 1 year of age, as are Inuits, that are the Eskimos of North America, and Alaska especially and Canada. There are also other racial groups that are more prone to ear disease and that may be related to craniofacial anatomy and eustachian palatal muscles.

So **otitis media is a human condition related to the consequences of evolution that is adaptations of bipedalism and the big brain, speech and loss of prognathism which is probably related to cooking, genetic issues, immunologic and environmental factors**. So we've come a long way from being on all fours and our knuckles and then has led us to be bipedal with a big brain and we've got otitis media and some other diseases and disorders with our bipedalism. Sometimes I have back pain that is related to me standing up on two feet, because on all four I probably wouldn't have a bad back.

We hypothesize that **OM is primarily a human condition** and that **design compromises during our evolution predispose us to developing it**. We have presented two human evolutionary adaptations that could produce alterations of the structure and function of the ET and contribute to the unusual prevalence of this disease: being born too soon due to the development of a large fetal brain in the context of bipedalism, and the loss of facial prognathism due to speech or cooking, when compared with our ancestors. To reduce the incidence of OM, we should pinpoint possible anatomic differences (such as the paratubal muscles) in humans as compared to other species (e.g., the monkey) that do not have ME disease, and consider them as a target for correction.

We have proposed that the consequences of evolution have a role in the pathogenesis of otitis media (OM) in humans. But the phenomenal incidence of OM today cannot be explained solely by design compromises or the negative consequences of adaptation.[27] **Other factors, such as heredity and immune deficiency**, as well as those derived from our existence in novel environments (those we are not adapted to) such as a **decrease in breastfeeding, smoking in the household, use of pacifiers and, very importantly, child daycare attendance are well known to increase the risk of middle ear (ME) disease**.

References

1. Bluestone CD, Douglas Swarts JD. Human evolutionary history: Consequences for the pathogenesis of otitis media. Otolaryngology Head and Neck Surgery, December 2010; vol. 143, 6: pp. 739-744.
2. Bluestone CD. Impact of evolution on the eustachian tube. Laryngoscope 2008;118:522–7.
3. Bluestone CD. Humans are born too soon: impact on pediatric otolaryngology. Int J Pediatr Otorhinolaryngol 2005;69:1–8.
4. Bluestone CD. Galapagos: Darwin, evolution, and ENT. Laryngoscope 2009;119:1902–5.
4. Lovejoy CO. Reexamining human origins in light of *Ardipithecus ramidus*. Science 2009;326:741– 8.
5. Martin RD. Primate Origins and Evolution: A Phylogenetic Reconstruction. Princeton, NJ: Princeton University Press; 1990.
6. Tague RG, Lovejoy CO. The obstetric pelvis of A.L. 288-1 (Lucy). J Human Evol 1986;15:237–55.
7. Bluestone CD. Humans are born too soon: impact on pediatric otolaryngology. Int J Pediatr Otorhinolaryngol 2005;69:1–8.
8. Bentdel YE, Haberg SE, Karevold G, *et al*. Birth characteristics and acute otitis media in early life. Int J Pediatr Otorhinolaryngol 2010; 74:168 –72
9. Nishimura T, Mikami A, Suzuki J, *et al*. Descent of the larynx in chimpanzee infants. Proc Natl Acad Sci 2003;100:6930–3.
10. Schumacher K-U, Koppe T, Fanghanel J, *et al*. Morphometric studies on the facial skeleton of humans and Pongids based on CT-scans. Acta Anat Nippon 1994;69:636 – 44
11. Wrangham R. Catching Fire: How Cooking Made Us Human. New York: Basic Books; 2009.
12. Bluestone CD. Galapagos: Darwin, evolution, and ENT. Laryngoscope 2009;119:1902–5.
13. Stern-Bertholtz W, Sjostrom L, Hakanson NW. Primary secretory otitis media in the Cavalier King Charles spaniel: a review of 61 cases. J Small Anim Pract 2003;44:253–6.
14. Corfield GS, Burrows AK, Imani P, *et al*. The method of application and short term results of tympanostomy tubes for treatment of primary secretory otitis media in three Cavalier King Charles Spaniel dogs. Aust Vet J 2008;86:88–94.
15. Dawkins R. The greatest show on earth: the evidence for evolution. New York: Free Press; 2009. p.36.
16. Davidson TM, Sedgh J, Tran D, *et al*. The anatomic basis for the acquisition of speech and obstructive sleep apnea: evidence from cephalometric analysis supports The Great Leap Forward Hypothesis. Sleep Med 2005;6:497–505.
17. Hayes GM, Friend EJ, Jeffrey ND. Relationship between pharyngeal conformation and otitis media with effusion in Cavalier King Charles spaniels. Vet Rec 2010;167:55–58.
18. Swarts JD, Ghadiali S. Interspecies comparisons of eustachian tube flow patterns during swallowing in the forced-response test (Abstract). Presented at the 9th International Symposium on Recent Advances in Otitis Media, St. Petersburg Beach, Florida, June 3–7, 2007:124.
19. Doyle WJ, Rood SR. Comparison of the anatomy of the eustachian tube in the rhesus monkey (*Macaca mulatta*) and man: implications for physiologic modeling. Ann Otol Rhinol Laryngol. 1980 Jan-Feb;89(1 Pt 1):49-57

20. Bluestone CD. Studies in otitis media: Children's Hospital of Pittsburgh & University of Pittsburgh Progress Report 2004. Laryngoscope 2004;114(Suppl 105):1–26.
21. Swarts JD, Bluestone CD. Eustachian tube function in older children and adults with persistent otitis media. Int J Pediatr Otorhinolaryngol 2003;67:853–9.
22. Cantekin EI, Doyle WJ, Reichert TJ, *et al*. Dilation of the eustachian tube by electrical stimulation of the mandibular nerve. Ann Otol Rhinol Laryngol 1979;88:40 –51.
23. Bluestone CD. Eustachian tube obstruction in the infant with cleft palate. Ann Otol Rhinol Laryngol 1971;80:1–30.
24. Doyle WJ, Reilly JS, Stool SE, *et al*. Eustachian tube function in children with unrepaired cleft palates. In: Lim DJ, Bluestone CD, Klein JO, *et al*, eds. Recent Advances in Otitis Media: Proceedings of the Third International Symposium. Philadelphia: BC Decker; 1984. p. 59–61.
25. Doyle WJ, Cantekin EI, Bluestone CD, *et al*. A nonhuman primate model of cleft palate and its implications to middle ear pathology. Ann Otol Rhinol Laryngol 1980;89(Suppl 68):41–6.
26. Buchman CA, Doyle WJ, Skoner D, *et al*. Otologic manifestations of experimental rhinovirus infection. Laryngoscope 1994;104: 1295–9.