PHENOTYPE GENOTYPE ASSOCIATIONS: THE CASE OF PRIMATE HEARING AND VOCALIZATIONS

INTRODUCTION

Primates show remarkable variation in their hearing ranges, with species such as *Galago senegalensis* having an upper limit of 65kHz. This variability implies a large amount of evolution and gives an opportunity to answer the question : Are the candidate genes selected from knockout or mutational studies important for evolution of hearing?

In this project, I attempt at answering this question by analyzing whether there is a correlation between evolution of these candidate genes and changes in phenotypes of interest such as hearing frequencies and vocalization frequencies. Vocalization is a good proxy for hearing as an organism has to be able to hear the calls of other members of the species for intraspecific communication. However, it must be noted that in certain cases, hearing limits could have evolved due to the requirement to hear other species.

Four candidate genes were sequenced and analyzed: **GJB2, BRN4, TECTB** and **PRESTIN**.

METHODS

Genotype : Candidate genes sequenced with newly designed primers .

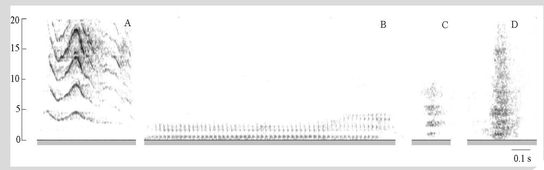
GJB2: 681 bps **BRN4:** 1086bps
TECTB: Exon 3 (153bps), 4 (73bps), 5 (104 bps), 6 (84bps), 7 (163 bps)
Prestin : Exon 3 (205bps), 4 (140bps)

Phenotype: Hearing data obtained from Coleman (2009) and Heffner (2008)
Vocalization data from meta analyses of literature
& current study on *Nasalis larvatus*

Vocalizations of *Nasalis larvatus*

Unique due to the boisterous nature and high frequency of calls.
Vocalizations of males are thought to be modified due to size of the nose
~23 hours of vocalizations from captive group of 2 adults and 2 infants)

Max f0 = 7200 Hz
Min f0= 180Hz



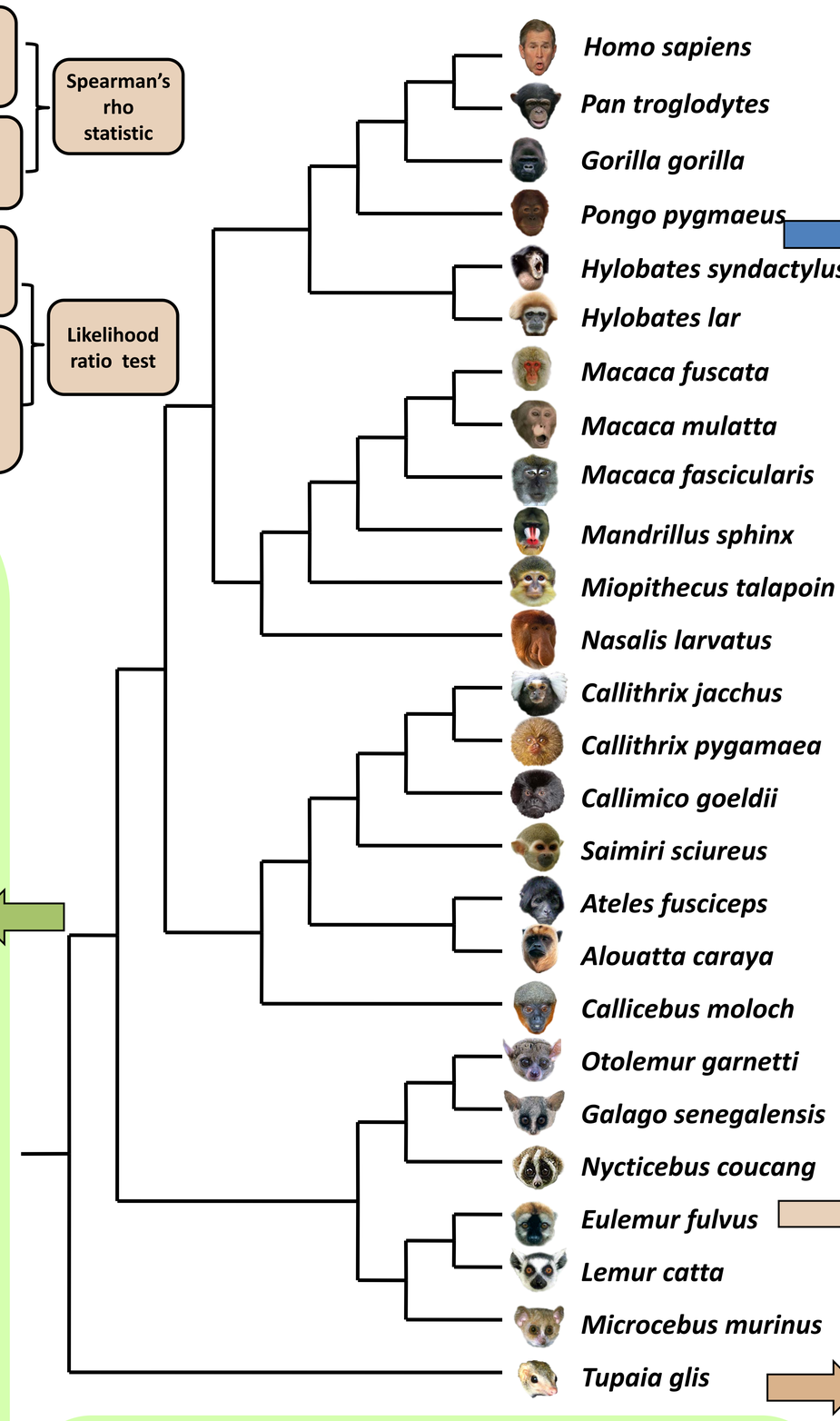
How to test for Phenotype Genotype Association

I Test for Direct correlation between amino acid and phenotype changes: Overall number of changes across the gene with the phenotypic changes
- **Phenotype reconstruction**
  (1) Square root parsimony
  (2) Farris Optimization for recoded additive characters
- **Amino acid changes**
  Fitch optimizations
  ACCTRAN & DELTRAN

→ **Spearman's rho statistic**

II Test for selection signature for lineages with large phenotypic changes (foreground branches): measures site specific dN/dS (ω) to identify potentially functionally relevant sites under selection.
- **Foreground branch designation** using additive phenotype character states
- **Codon substitution models** for branches with major (i) changes, (ii) decreases (iii) increases. **Null models:** random selection of foreground branches

→ **Likelihood ratio test**



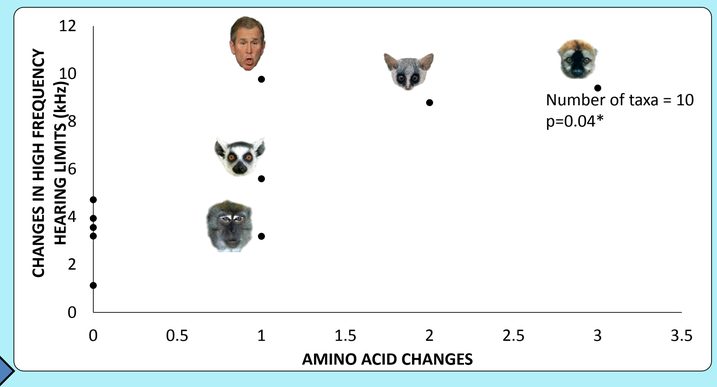
BRN4 evolution is associated with high frequency hearing limit

BRN4: POU family transcription factor involved in inner ear development

Why?
- Mutations associated with reduced cochlear coiling and cochlear size in mice
- Mutations in humans lead to both high and low frequency hearing loss

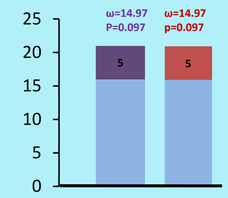
HYPOTHESIS 1: Change at genotypic level of BRN4 is correlated with changes in high frequency limit of hearing (No. of taxa = 10) **.**

I Direct correlation approach:
Square root parsimony : **Significant correlation** (See below)



Farris Optimization (1 optimization): **correlation is not significant**

II Selection signature approach
Foreground branches: lineages with >10kHz change in high frequency limit



Null Models
ω=1 or p>>0.05

All foreground branches were branches with major decreases

Correlation is **not significant at 0.05 level**.
- TYPE II Error due to small sample size
- Only 1 identified site with significantly large ω.

HYPOTHESIS 2: Change at genotypic level of BRN4 is correlated with changes in highest frequency of vocalizations (No of taxa =20)**.**

Selection signature approach : ω > 1, p>0.05 for major decreases.
Null models showed no correlations.

BRN4 evolution showed association with no low frequency limits of vocalizations (No.of taxa =22)

POSSIBLE EXPLANATIONS:
- BRN4's role in cochlear development and coiling: cochlear volume negatively correlated with high frequency hearing
- BRN4 is directly involved in high-frequency hearing

GJB2 evolution is associated with highest frequency of vocalizations

Connexin 26 (encoded by GJB2): a transmembrane protein in the inner ear gap junction

Why?
- Mutations correlated with **high frequency hearing loss**
- Role in K+ transport in the inner ear, transport of second messenger IP3 and maturation of hair cells.

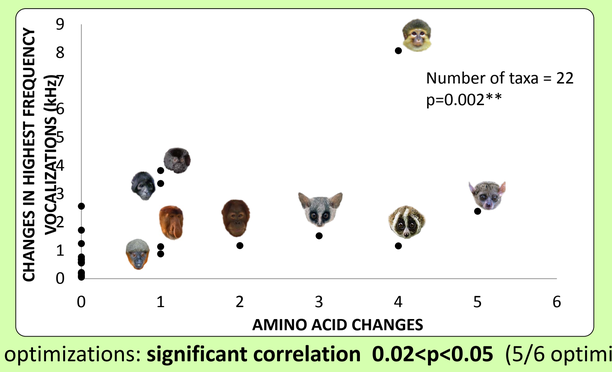
HYPOTHESIS 1: Changes at the genotypic level of GJB2 is correlated with changes in high frequency limit of hearing (No. of taxa = 10)**:** No significant correlations found

HYPOTHESIS 2: Changes at the genotypic level of GJB2 is correlated with changes in highest frequency of vocalizations (No. of taxa= 22)

RESULTS

I Direct correlation approach

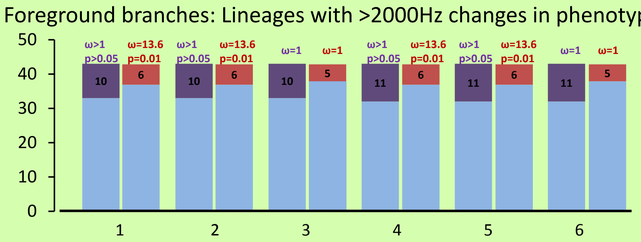
(1) Square root parsimony: **Significant correlation**



(2) Farris optimizations: **significant correlation 0.02<p<0.05** (5/6 optimizations)

II Selection Signature Approach:

Foreground branches: Lineages with >2000Hz changes in phenotype.



Null models : ω=1 or p>>0.05

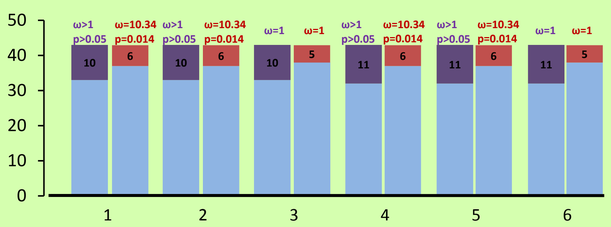
Results for major increases were not significant

4/6 Optimizations: **significant correlation** for major decreases

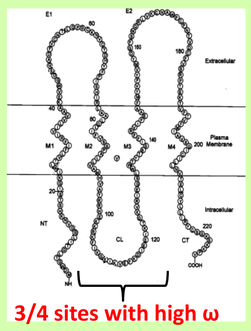
POSSIBLE EXPLANATIONS:
Connexin 26 density low at cochlear apex: limiting factor for high frequency hearing?
Significantly higher ω's for branches with major decreases suggests **relaxed selection**: No selection pressure due to lack of high frequency vocalizations

TRANSMEMBRANE vs. NONTRANSMEMBRANE REGIONS

Non transmembrane regions



4/6 Optimizations: **significant correlation** for major decreases
Null models : ω=1 or p>>0.05
Transmembrane regions: non-significant results.



3/4 sites with high ω

TECTB : Lack of correlation

TECTB: found in the tectorial membrane of the inner ear and is involved in the tuning of cochlea to lower frequencies.

No correlation between TECTB evolution and low frequency limits of vocalizations using any method.

Due to lack of data for low frequency hearing limit, correlation with the hearing phenotype could not be tested

PRESTIN: Lack of variability

PRESTIN: an outer hair cell protein found to be involved in high frequency selectivity and sensitivity of inner ear.

2 exons were amplified and sequenced for the study; however very low variability was found at nucleotide as well as amino acid levels.

Not likely to be involved in the evolution phenotypes studied in primates

CONCLUSION

1. Two candidate genes, GJB2 and BRN4 identified from knockout studies and population screening of mutations, are potentially involved in evolution of hearing in primates
2. Vocalizations of primates is a key phenotypic feature associated with the selection of GJB2, a hearing gene
3. Evolution of BRN4, a gene involved in cochlear morphogenesis is correlated with high frequency hearing limits
4. As such correlations are not a proof of function, further characterization of gene of interest by mutagenesis studies at selected sites should be performed