Opportunities to improve genomic information and resources for marmosets

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Baylor College of Medicine
Houston, Texas
Fundamental Resources for Primate Genomics

• Whole Genome Reference Assembly
  ◆ Essentially complete (few gaps) with minimal sequence errors

• Accurate and “Complete” Annotation
  ◆ Protein coding genes
  ◆ Non-coding genes (lncRNA, miRNA, etc.)
  ◆ Regulatory sequences

• Extensive Data Describing Functionally Significant Variation

• Information about Population Genetic Structure of Research Colonies
Reference Genome Assembly for *Callithrix jacchus*

- **2010**: Initial genome assembly and annotation
  - Published by Worley et al., Nature Genetics (2014)
  - DNA sample for reference genome from Southwest NPRC colony
  - Contig N50: 29.3 kb

- **2015**: New assembly (Keio University)
  - Contig N50: 61.0 kb

- **2017**: Another improvement (Broad Inst.)
  - Contig N50: 155.3 kb
Current “Best” assembly for common marmoset

- Assembly ASM275486v1 submitted by Broad Institute
- DNA sample from marmoset from New England NPRC
- Total sequence length: 2.845 gigabases
- Contig N50: 155.3 kb
- Scaffold N50: 129.2 Mb
RNA sequencing to define tissue-specific transcriptome

- *Callithrix jacchus* – common marmoset
- 10 tissues (pituitary, spleen, lymph node, bone marrow, kidney, heart, skeletal muscle, liver, lung, colon)
- Illumina short read data produced by Baylor genome center
- Reads per tissue: 54.6 – 128.6 million
- Analysis: Chris Mason (Weill Cornell Med. Center)

Project Team: Nonhuman primate reference transcriptome project (Chris Mason, Michael Katze, Gary Schroth, Jeffrey Rogers)
Current “Best” assembly for common marmoset

- Assembly ASM275486v1 submitted by Broad Institute
- DNA sample from marmoset from New England NPRC

- Total sequence length: 2.845 gigabases
- Contig N50: 155.3 kb
- Scaffold N50: 129.2 Mb

Ensembl annotation
- Protein coding genes: 19,690
- Non-coding genes: 8,922
Coming soon (a few months?)

- New whole genome assembly for *Callithrix jacchus* in progress

- Evan Eichler (Univ. of Wash.) and Wes Warren (McDonnell Genome Institute, Wash. Univ.)

- De novo assembly using long read technologies, additional scaffolding

- Eichler-Warren gorilla assembly: 3.1 gigabases; Contig N50 9.6 Mb
Discovering Functionally Significant Genetic Variation in Marmosets

• Initial studies in 2010-2013: Whole genome sequences from seven animals

• Dr. Rosario: Whole genome sequences from >80 animals
• Several million single nucleotide variants identified

• The cost of whole genome sequencing continues to fall, approaching $1000 per genome
Why is discovering functional variation in marmosets important?

- Discovery of novel “damaging” mutations can lead directly to new naturally occurring models of human genetic disease.
- Knowledge of functional variation among marmosets can facilitate better selection of animals for specific experiments.
- Information about functional variation among marmosets allows for more thorough interpretation of research results.
Why is discovering functional variation in marmosets important?

• Discovery of novel “damaging” mutations can lead directly to new naturally occurring models of human genetic disease
• Knowledge of functional variation among marmosets can facilitate better selection of animals for specific experiments
• Information about functional variation among marmosets allows for more thorough interpretation of research results

• Selection of animals for gene editing
How much genetic variation is segregating among rhesus macaques?

How much of that variation is functionally significant?

Can we use that variation to investigate questions related to either primate evolutionary adaptation or human health and disease?
The population genomics of rhesus macaques (Macaca mulatta) based on whole-genome sequences

Cheng Xue,1 Muthuswamy Raveendran,1 R. Alan Harris,1,2 Gloria L. Fawcett,1,19 Xiaoming Liu,3 Simon White,1 Mahmoud Dahdouli,1,20 David Rio Deiros,1 Jennifer E. Below,3 William Salerno,1 Laura Cox,4 Guoping Fan,5 Betsy Ferguson,6 Julie Horvath,7,8,9 Zach Johnson,10,21 Sree Kanthaswamy,11,12 H. Michael Kubisch,13 Dahai Liu,14 Michael Platt,15,16 David G. Smith,11 Binghua Sun,14 Eric J. Vallender,13,17,22 Feng Wang,2 Roger W. Wiseman,18 Rui Chen,1,2 Donna M. Muzny,1 Richard A. Gibbs,1,2 Fuli Yu,1,2 and Jeffrey Rogers1,2

Genome Research 26:1651–1662
Discovery of Single Nucleotide Polymorphism in Rhesus Macaques

- n = 133 rhesus macaque whole genome sequences
- 124 Indian-origin = 31.9 million SNVs
  13.66 million private alleles
- 9 Chinese-origin = 30.1 million SNVs
  11.81 million private alleles
- Total Rhesus SNVs = 43.77 million

Analysis: R.A. Harris
n = 133 rhesus macaque whole genome sequences

124 Indian-origin = 31.9 million SNVs
13.66 million private alleles

9 Chinese-origin = 30.1 million SNVs
11.81 million private alleles

Total Rhesus SNVs = 43.77 million

<table>
<thead>
<tr>
<th>VEP prediction</th>
<th>Number of rhesus variants observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missense</td>
<td>126,445</td>
</tr>
<tr>
<td>Splice region</td>
<td>42,054</td>
</tr>
<tr>
<td>Stop codon gained</td>
<td>2,642</td>
</tr>
<tr>
<td>Mature miRNA</td>
<td>650</td>
</tr>
</tbody>
</table>

Analysis: R.A. Harris
Rhesus vs. Human: Nonsynonymous / Synonymous ratio

Sample size n=133
<table>
<thead>
<tr>
<th>Primate Research Center</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tulane National Primate Research Center</td>
<td>143</td>
</tr>
<tr>
<td>California National Primate Research Center</td>
<td>126</td>
</tr>
<tr>
<td>Wisconsin National Primate Research Center</td>
<td>96</td>
</tr>
<tr>
<td>Oregon National Primate Research Center</td>
<td>78</td>
</tr>
<tr>
<td>Caribbean Primate Research Center (CPRC), Cayo Santiago</td>
<td>35</td>
</tr>
<tr>
<td>The University of Texas MD Anderson Cancer Center, Bastrop</td>
<td>16</td>
</tr>
<tr>
<td>New England Primate Research Center</td>
<td>14</td>
</tr>
<tr>
<td>Southwest National Primate Research Center</td>
<td>6</td>
</tr>
<tr>
<td>Yerkes National Primate Research Center</td>
<td>7</td>
</tr>
<tr>
<td>Wild caught Chinese</td>
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<tr>
<td>Hazleton--Texas Primate Center</td>
<td>1</td>
</tr>
<tr>
<td>Labs of Virginia</td>
<td>1</td>
</tr>
</tbody>
</table>

Total sample size n = 526
SNV results from 526 rhesus macaques

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Total number of variant SNV sites identified</td>
<td>72,746,387</td>
</tr>
<tr>
<td>Number of singletons</td>
<td>17,616,218</td>
</tr>
<tr>
<td>Average number of SNVs per individual</td>
<td>9,476,124</td>
</tr>
<tr>
<td>Average heterozygosity</td>
<td>0.0020</td>
</tr>
<tr>
<td>Number of missense variants</td>
<td>340,104</td>
</tr>
<tr>
<td>Number of genes affected by missense variants</td>
<td>19,924</td>
</tr>
<tr>
<td>Number of de novo stop codons gained</td>
<td>8,556</td>
</tr>
</tbody>
</table>

We have observed missense mutations in 19,924 different genes: 94.4% of protein coding genes annotated in the rhesus genome.
Lynch Syndrome
Hereditary Colon Cancer caused by mutations in DNA mis-match repair genes

Lynch Syndrome - autosomal dominant hereditary colorectal cancer

- Prevalence in humans of 1 in 440
- 2-7% of colorectal cancer cases

FDG PET-CT
FACE PET-CT

Beth Dray, DVM and Christian Abee, DVM
MD Anderson Keeling Center
for Comparative Medicine and Research
Bastrop, TX)
Lynch Syndrome
Hereditary Colon Cancer caused by mutations in DNA mis-match repair genes

Lynch Syndrome - autosomal dominant hereditary colorectal cancer

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency in affected patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSH2</td>
<td>60%</td>
</tr>
<tr>
<td>MLH1</td>
<td>30%</td>
</tr>
<tr>
<td>MSH6</td>
<td>7-10%</td>
</tr>
<tr>
<td>PSM2</td>
<td>Infrequent</td>
</tr>
<tr>
<td>PSM1</td>
<td>Case Report</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>Case Report</td>
</tr>
</tbody>
</table>

- Prevalence of 1 in 440
- 2-7% of colorectal cancer cases
## Lynch Syndrome: Candidate mutations in rhesus macaques

<table>
<thead>
<tr>
<th>SNV</th>
<th>FaST-LMM p value</th>
<th>Variant Count (Het’s)</th>
<th>Case MAF</th>
<th>Control MAF</th>
<th>VEP Consequence</th>
<th>Gene Symbol</th>
<th>CADD PHRED Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr13:48556451</td>
<td>1.9 x 10^{-25}</td>
<td>6 / 20</td>
<td>0.15</td>
<td>0</td>
<td>missense</td>
<td>MSH6</td>
<td>22.8</td>
</tr>
<tr>
<td>chr13:48564908</td>
<td>2.2 x 10^{-12}</td>
<td>6 / 20</td>
<td>0.15</td>
<td>0</td>
<td>downstream</td>
<td>MSH6</td>
<td>5.033</td>
</tr>
<tr>
<td>chr2:105789825</td>
<td>3.4 x 10^{-16}</td>
<td>4 / 20</td>
<td>0.10</td>
<td>0</td>
<td>stop gained</td>
<td>MLH1</td>
<td>36</td>
</tr>
</tbody>
</table>

- **N=20** rhesus macaques diagnosed with colorectal cancer
- All rhesus SNVs lifted over to human coordinates
  - same reference base
  - same consequence except for chr13:48564908 in *MSH6* isoform 3’ UTR
- CADD Score >= 20 means 1% most functionally significant SNPs in human genome

*Dray et al. (2018)*
*Genes and Cancer*  
Vol 9: 142-152
Rhesus as models for human eye disease
Rui Chen, Jeffrey Rogers, Timothy Stout

Do macaques carry damaging mutations in genes known to cause retinal diseases in humans?
Discovery of new model of cone dystrophy
Sara Thomasy, Ala Moshiri, Jeff Roberts, Rui Chen, Tim Stout, Jeff Rogers

• Behavioral observations at California National Primate Res. Center suggested that two juvenile rhesus macaques had partial visual impairment
• Ophthalmic examination by Drs. Thomasy and Moshiri revealed near complete loss of cone photoreceptor function with normal rod photoreceptors

Moshiri et al. (in revision)
Discovery of new model of cone dystrophy

- Whole genome sequencing identified a missense mutation in *PDE6C* that is homozygous in both affected animals
- This gene codes for an enzyme that is expressed in cone photoreceptors and is critical to the phototransduction cascade. The enzyme hydrolyzes cGMP causing gated channels to close.
- *In vitro* functional assay shows this mutation essentially eliminates enzymatic function (N.O. Artemyev)

Moshiri et al. (in revision)
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Expectations for functional variation in marmosets

• There is no reason to believe marmosets will have significantly lower levels of functionally significant genetic variation than rhesus macaques

• Functional variation can be the subject of investigation on its own and lead to new genetic models

• Functional variation can serve as modifying or compensatory variants when outbred animals are used for gene-editing experiments
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Acknowledgments

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• Christian Abbe

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• Sara Thomasy
• Ala Moshiri
• Jeff Roberts

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• NIH Office of Research Infrastructure Programs
• NIH National Human Genome Institute
C) CRHRI SNPs predict AT and Hippocampal Metabolism

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CRHRI genotypes, neural circuits and the diathesis for anxiety and depression


Molecular Psychiatry (2013) 18, 700–707