# Genomic resources for canine cancer research

Jessica Alföldi
(for Kerstin Lindblad-Toh)
Broad Institute of MIT and Harvard

# Dog genome assembly – CanFam 3.1

## Comparison of most recent assembly versions

| | Sequence length (Gb) | Gaps as % of assembly | Scaffold N50 (Mb) | Contig N50 (kb) |
|---|---|---|---|---|
| **Human** | 3.23 | 0.5% | 59.4 | 56,413 |
| **Mouse** | 2.80 | 2.8% | 52.6 | 32,273 |
| **Dog** | 2.41 | 0.8% | 45.9 | 267 |
| **Rat** | 2.87 | 4.9% | 15.0 | 100 |
| **Rhesus** | 3.23 | 3.6% | 3.0 | 54.2 |

- Released in 2011 – 6 years of work
- 85 Mb added to genome assembly
    - – Went from 99.2 -> 99.6% euchromatic coverage
- Closed 1,044 gaps in promoters/first exons

# RNA-seq data for annotation

- 10 beagle tissues
  - blood, brain, heart, kidney, liver, lung, ovary, skeletal muscle, skin, and testis
  - Almost all single adult female beagle
- 3 sets of libraries
  - Strand-specific polyA isolated (mRNA)
  - DSN (mRNA + other transcripts)
  - microRNA

# Gene annotations - Ensembl

- Used our polyA and DSN RNA-seq for gene prediction/confirmation
- Very high quality – very conservative
- 19,856 coding genes, 3774 non-coding genes
- Orthology information: easily find equivalents in human and other genomes

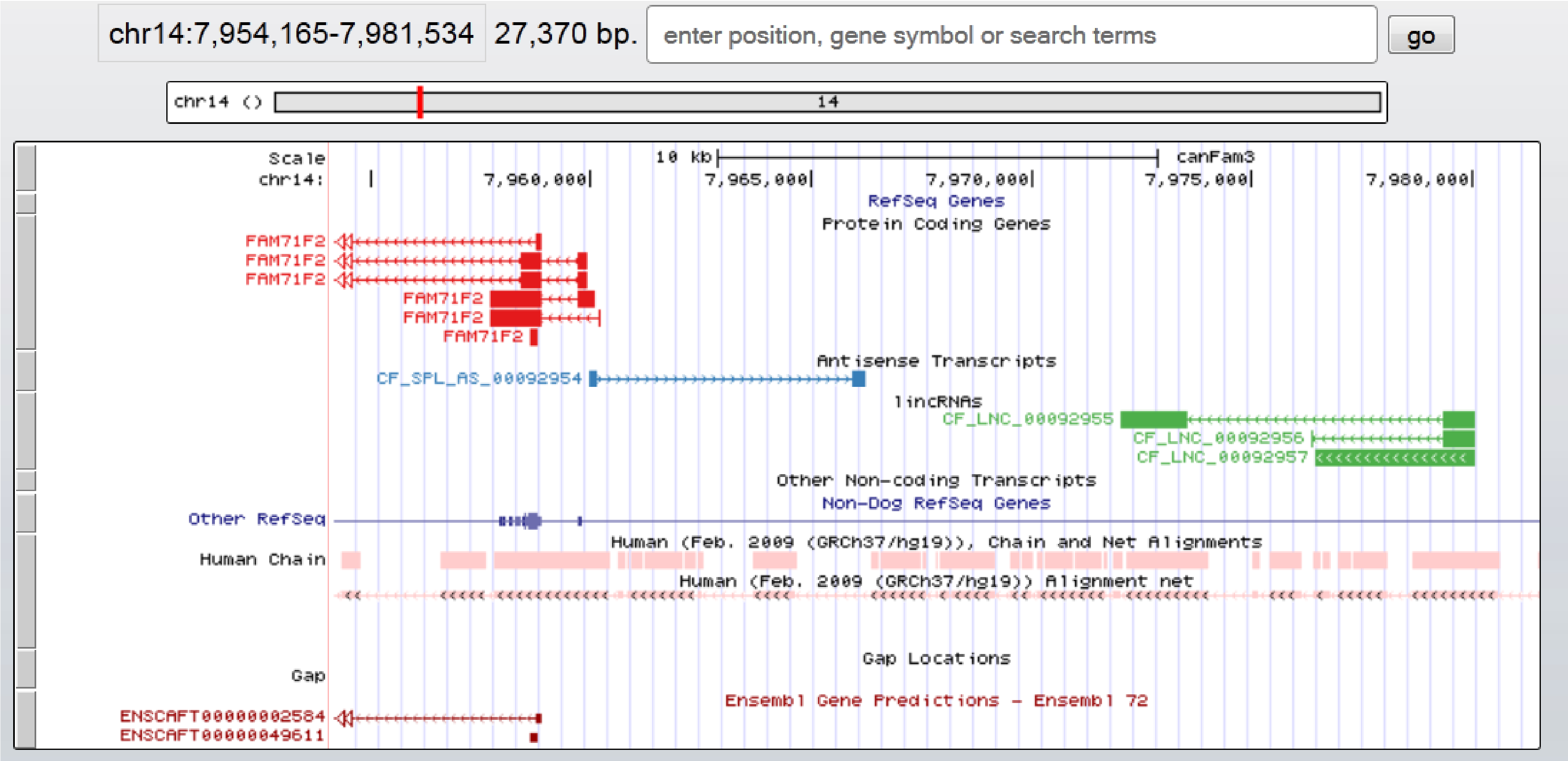
# Broad CanFam 3.1 annotation

- 20,657 high-confidence protein-coding genes
  - Based on Ensembl gene models + RNA-seq
  - 4.7 alternative transcripts/locus
- 2,141 low-confidence protein-coding genes
  - Ensembl (no RNA-seq confirmation)
  - Possible pseudogenes
  - Or only expressed in tissues we don’t have
    - Developmental
    - Specific brain regions

# New CanFam 3.1 annotation

- 5,301 Antisense transcripts (spliced + unspliced)
  - Overlap with protein-coding exon, on other strand
- 7,224 Long intergenic non-coding (lincRNAs)
  - No ORF, spliced, no overlap with pc genes
- >100K other transcripts (67% intronic)
  - 41,604 other intergenic non-coding transcripts

# New Annotation

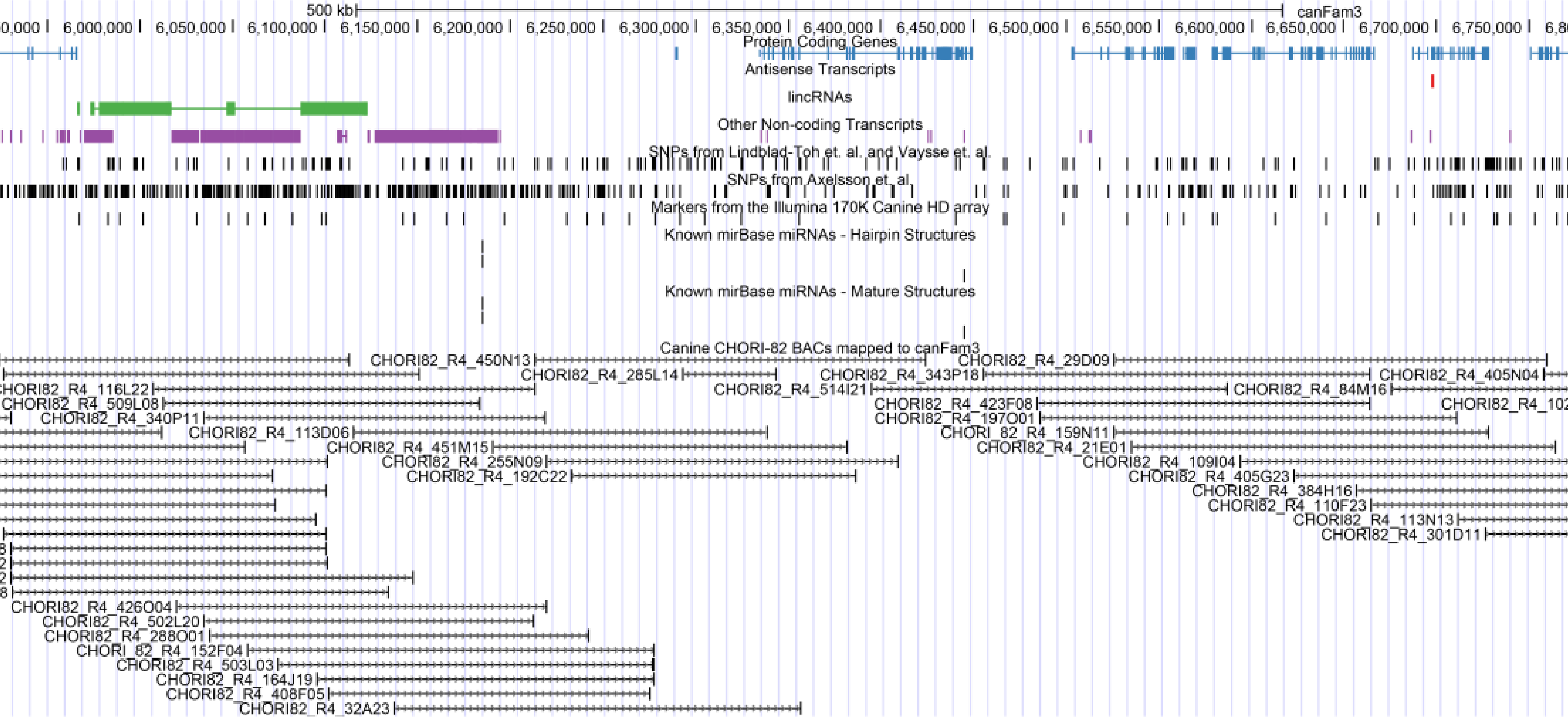


- Many spliceforms
- Spliced antisense transcripts and lincRNAs
- Antisense transcripts overlap protein-coding genes

# Broad – UCSC Canine Track Hub

- Data hosted by Broad integrated into the UCSC Genome Browser
- Annotation, RNA-seq data
- SNP sets including 170K chip
- CHORI-82 BAC ends
- Do you have data you'd like to have hosted?

# Broad-UCSC Canine Track Hub



# Illumina 170K Genotyping Chip

- Currently most high-density chip
- 175K SNPs evenly covered across genome
  - Including special efforts for regions >40kb without known SNPs
  - Wide variety of dog breeds + wolves
  - 99.2% of SNPs give reliable data
  - Mean spacing of 13 kb, only 21 gaps >200kb

# Osteosarcoma (OSA) in 3 breeds

- Most common bone cancer in dogs (~ 10,000 cases / year in USA )
- Highly metastatic, very high mortality
- Looks like human osteosarcoma (~ 1000 cases / year in USA)

Greyhounds

*~25% OSA*

153 cases

114 controls

*(Very little OSA in show Greyhounds)*

Irish Wolfhounds

*~20% OSA*

28 cases

62 controls

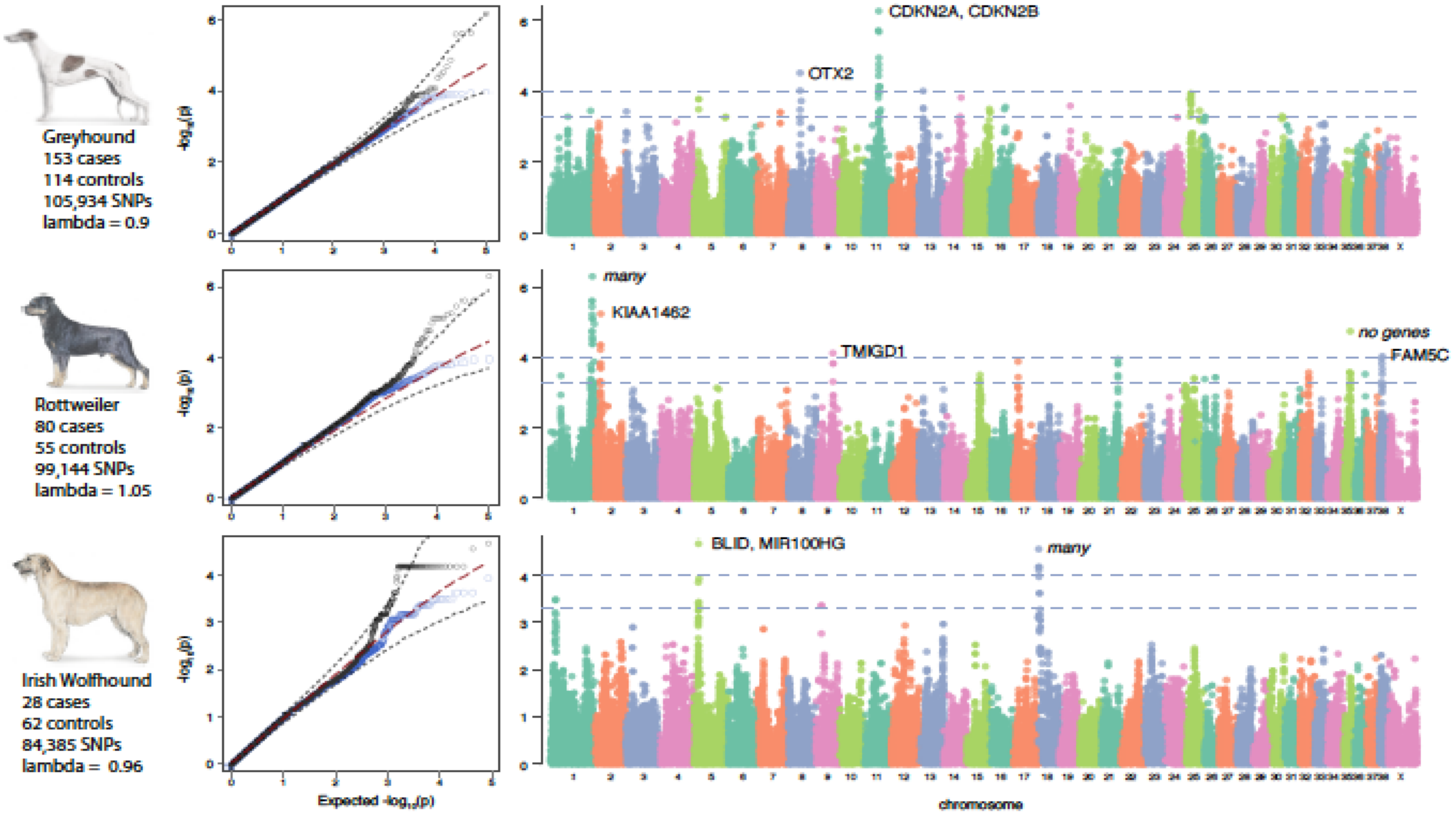
Rottweilers

*~15% OSA*

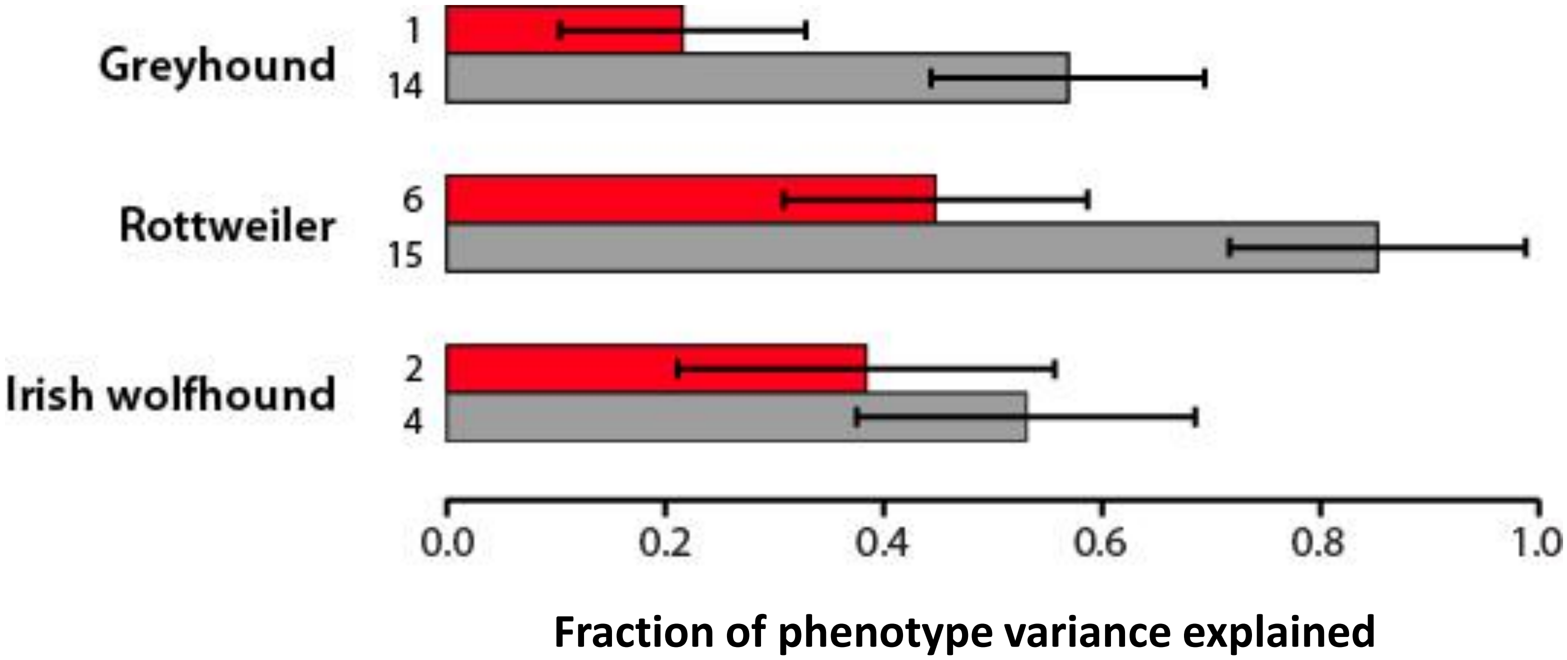
80 cases

55 controls

# Lots of loci (33) – but none shared



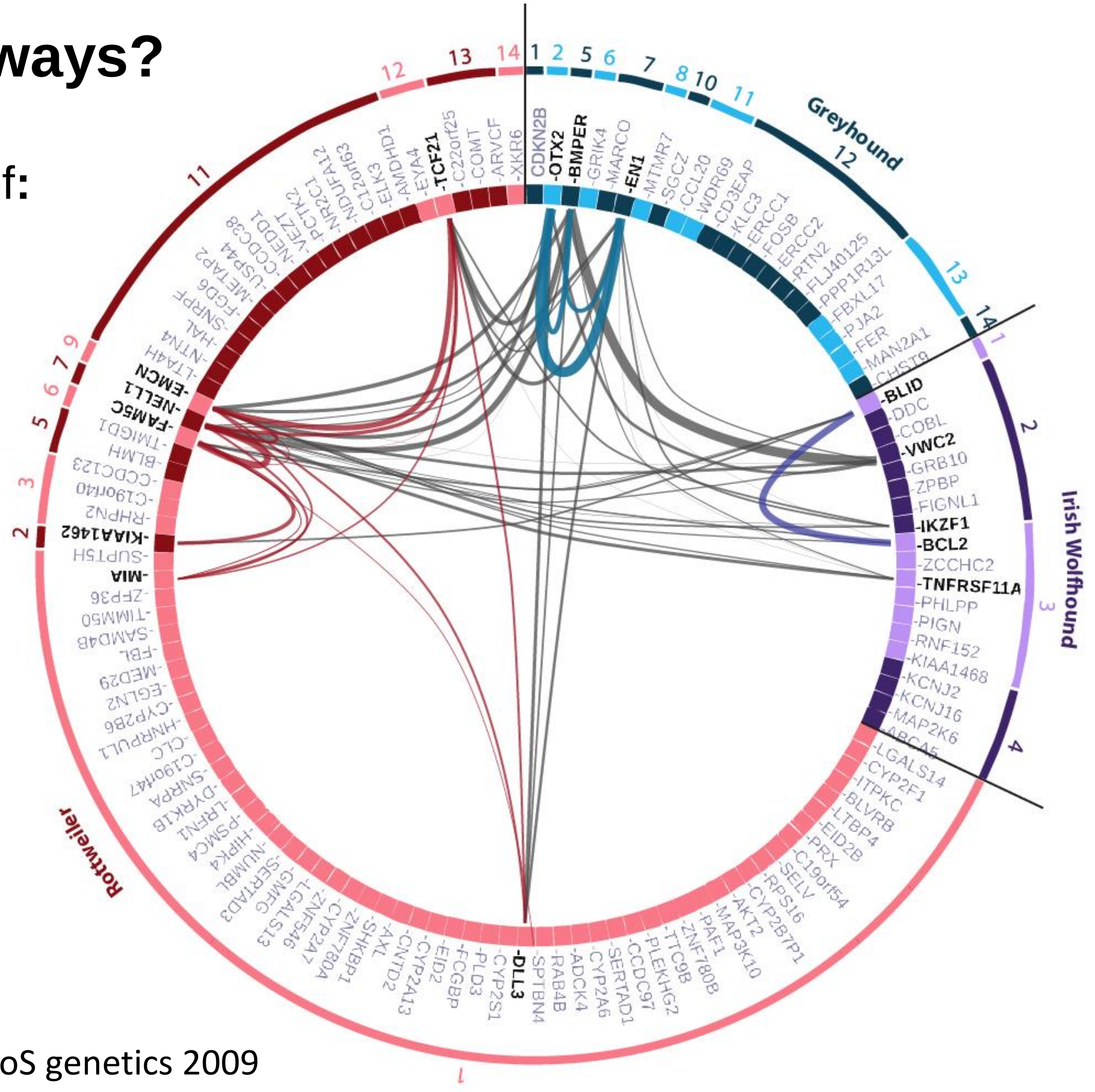
# Phenotype variance explained: high



# Shared pathways?

found regulators of**:**

- osteoblast differentiation, proliferation
- skeletal growth
- mesenchymal-epithelial cell transitions



GRAIL Raychaudhuri PLoS genetics 2009

# Osteosarcoma conclusions

- Need to separate breeds for analysis
- 100 cases/controls yield high heritability
- Risk factors are entirely independent
  - Although in same pathways
- Non-coding mutations predominate
- Pathways align with somatic mutations
  - Breen CGH tumor data

# Canine exome designs

- Several Roche/Nimblegen designs available
- Ensembl based CanFam3.1 currently in use:
    - 49 Mb – protein-coding exons only
- Two new designs to be published shortly
    - Exome plus (based on Broad annotation)
        - 152 Mb – protein-coding and non-coding transcribed
    - Exome CDS (based on Broad annotation)
        - 71 Mb – Exome plus without UTRs

# Somatic mutations in canine lymphoma - methods

| Breed | B-cell pairs | T-cell pairs |
|---|---|---|
| Boxer | - | 16 |
| Cocker | 10 | - |
| Golden | 54 | 25 |

Exome Capture (Roche/Nimblegen)

↓

Illumina Sequencing
N > 60x, T > 100x

↓

Alignment to CanFam3.1 (bwa)

↓

Somatic Variant calling
(muTect, indeLocator)

↓

Variant Filtration

↓

Identifying significantly mutated genes
(MuSiC)

Tools chosen for canine compatibility

*UNPUBLISHED*

# Key genes in B-cell lymphomas

*Fraction of individuals with at least one mutation in a gene/pathway*

| Pathways/genes | All B | Cocker B | Golden B | All T | Boxer T | Golden T | Human Cancer |
|---|---|---|---|---|---|---|---|
| FBXW7 | **0.25** | 0.10 | 0.28 | **0.05** | 0 | 0.08 | Lymphoma |
| FBXW7-Myc | **0.28** | 0.30 | 0.28 | **0.05** | 0 | 0.08 | Lymphoma |
| TRAF3-TRAF2-cIAP1-cIAP2-MAP3K14 | **0.30** | 0.50 | 0.26 | **0** | 0 | 0 | Lymphoma |
| POT1 | **0.17** | 0.20 | 0.17 | **0** | 0 | 0 | Lymphoma |
| DDX3X | **0.11** | 0.30 | 0.07 | **0** | 0 | 0 | Breast |

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# Key genes in T-cell lymphomas

*Fraction of individuals with at least one mutation in a gene/pathway*

| Pathways/genes | All B | Cocker B | Golden B | All T | Boxer T | Golden T | Human Cancer |
|---|---|---|---|---|---|---|---|
| SATB1 | **0** | 0 | 0 | **0.17** | 0.25 | 0.12 | Lymphoma |
| PTEN | **0** | 0 | 0 | **0.10** | 0.25 | 0 | Lymphoma |
| PTEN-PI3K-Akt-mTOR | **0** | 0 | 0 | **0.22** | 0.44 | 0.08 | Lymphoma |
| LTA4H | **0.02** | 0 | 0.02 | **0.10** | 0 | 0.27 | Carcinoma |

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# Lymphoma somatic mutations - lessons

- Separating by breed is essential
- Underlying risk factors likely result in greater tumor homogeneity within breeds
- Thorough phenotyping of tumors increases power
- Dog breeds can act as models for subtypes of human cancers

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