

ABSTRACT 11398

DISTINCT AND OVERLAPPING GENETIC CHANGES IN OSTESARCOMA ACROSS SPECIES

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Background: While osteosarcoma is uncommon in humans, it affects 50,000 dogs per year. The similarities between human and canine osteosarcomas have been well-described. Cross-species comparisons using exome sequencing have not been conducted. It is hypothesized that common genetic changes can be identified across species and that such changes that are common to more than one species are particularly significant.

Questions/Purposes: 1) Can deep exome sequencing reveal genetic changes in osteosarcoma that are common across multiple species? 2) Can a syntenic map be generated to describe the physical co-localization of genetic loci between dog and mouse? 3) Will common genetic variations reflect potential driver mutations in osteosarcoma or will they reflect merely the chance overlap of random derangements to the genome.

Patients and Methods: Paired tumor and normal tissues were collected from four mice and two dogs with osteosarcoma. The canine osteosarcomas were naturally occurring and were obtained from dogs presenting for treatment of their disease. The murine osteosarcomas had been generated by Cre-recombinase mediated deletion of floxed p53 restricted to the ng2 lineage (pericytes). Exome sequencing, syntenic mapping, and somatic copy number variation analysis were performed as follows: Sequence alignment was performed using Burrows-Wheeler Aligner (BWA-MEM), and data was processed using the Genome Analysis Toolkit (GATK). Somatic mutations were identified by comparing tumor to normal tissue using MuTect, and SnpEff was used to annotate the variants. Somatic copy number variations (CNVs) were analyzed using Varscan2. Comparative analysis of similarly altered chromosomal regions across species was performed using Cinteny. CNVs were compared across mouse and dog on the gene-level using Ensembl, and genes with recurrent amplifications or deletions across these species were identified. These CNVs were then compared to human osteosarcoma genetic variations published by Perry et al. (PNAS 2014) from 59 tumor/normal tissue pairs using whole-exome, whole-genome, and RNA-sequencing.

Results: A syntenic map between dog (38 chromosomes, 16,000 genes) and mouse (19 chromosomes, 38,000 genes) was successfully generated. Comparison across all 3 species identified 5 genes as consistent mutations in OS: deletions of p53, PTEN, and PIK3R1 and amplifications in ATRX and MYC. All species showed mutations in genes associated with the PI3K/mTOR pathway. The human and canine samples also shared consistent mutations in TSC2, PIK3CA, NF2, and CDKN2A/B. The human OS samples showed consistent hypermutation as did one canine tumor. Human and canine tumors had many CNVs, both broadly and focally. The mouse tumors showed fewer CNVs overall.

Conclusions: Comparative genomic analysis can identify distinct and overlapping genetic changes across species. Whereas the common finding of PTEN, p53, MYC, and PIK3R1 mutations are not surprising, the occurrence of ATRX mutations across species represents a novel target for study.

Level of Evidence: Level IVb

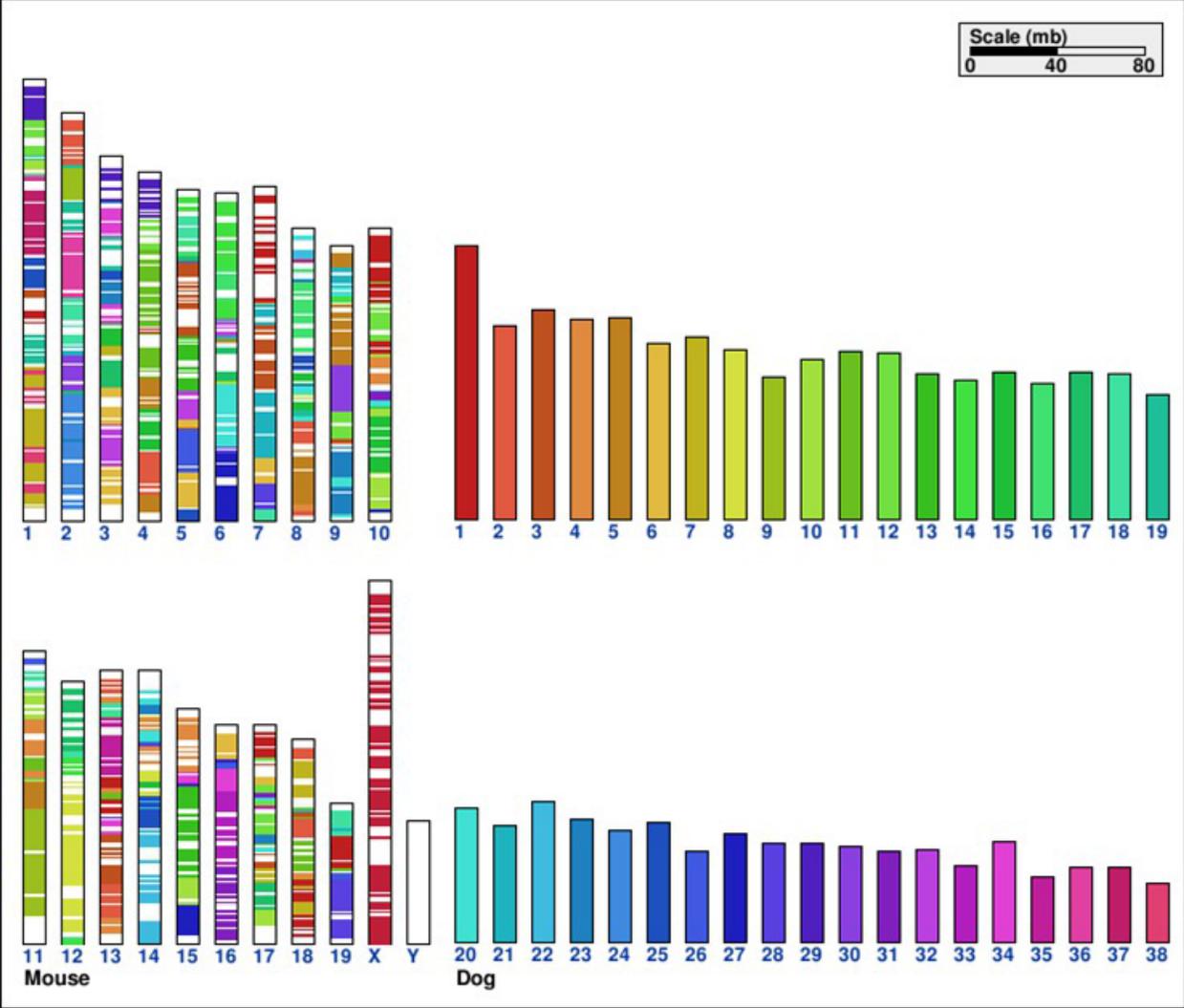
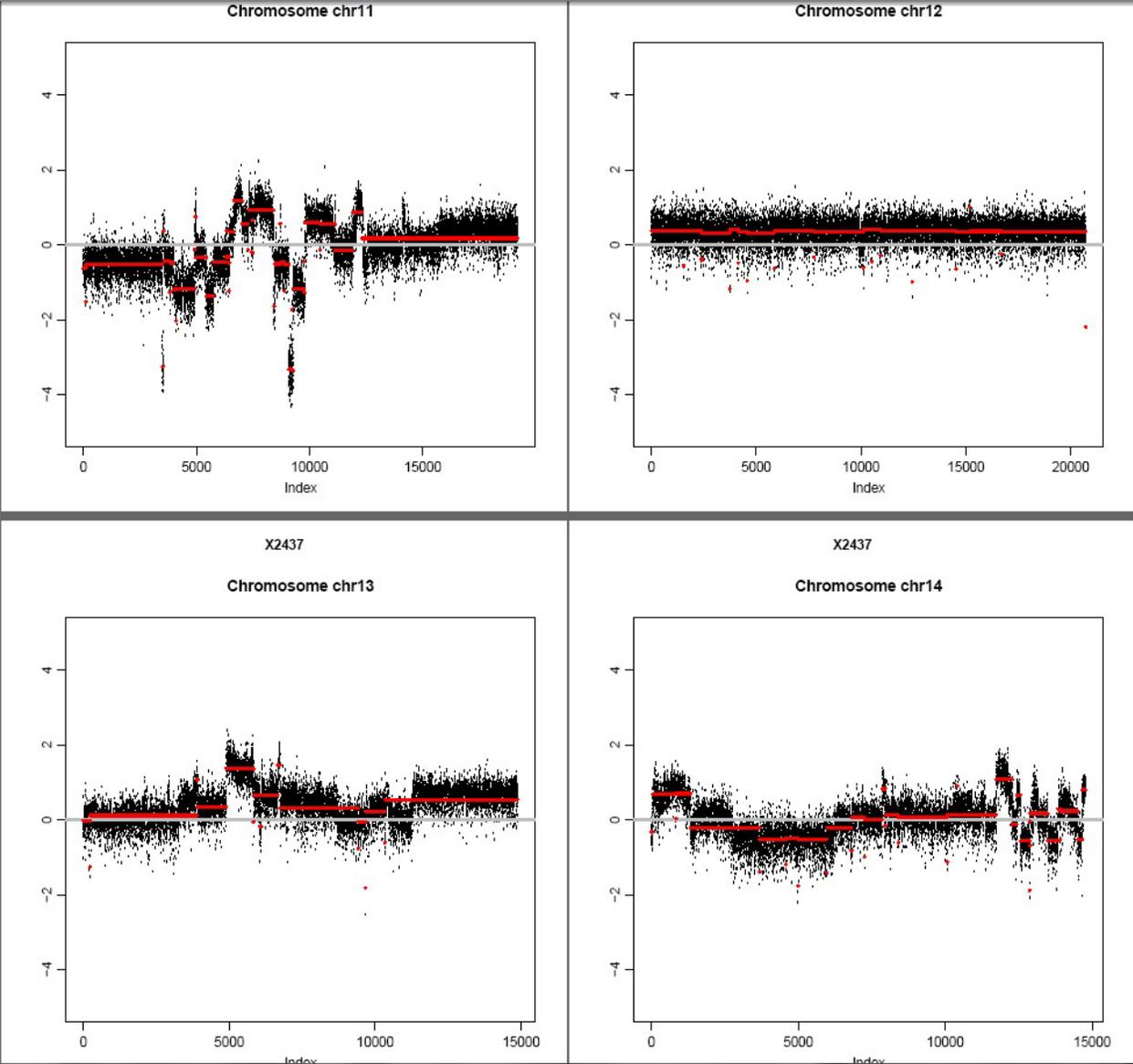
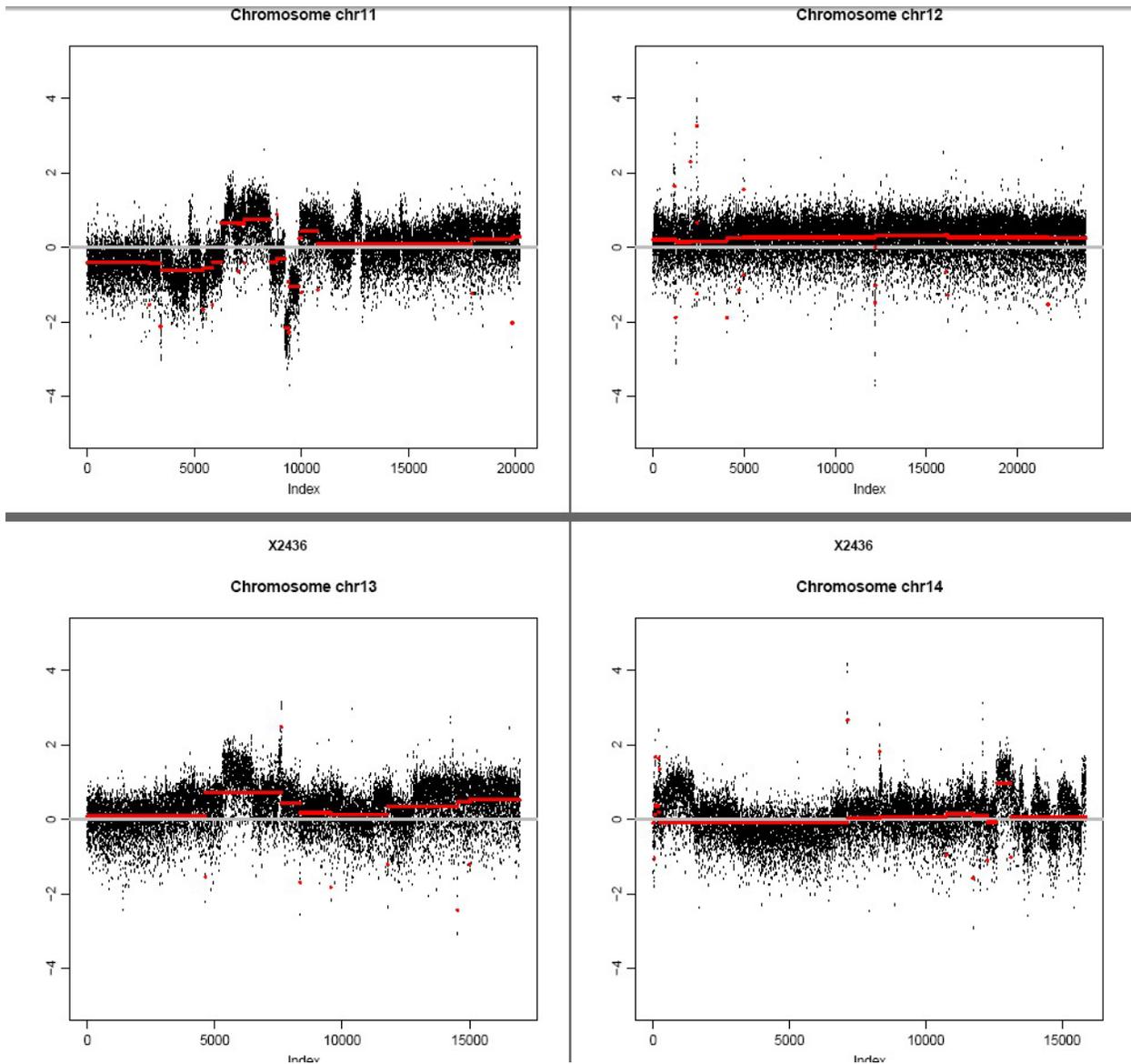


Fig 1: A synteny map was constructed to show the physical co-localization of shared genetic loci between dogs (38 autosomes with solid color bars) and mice (19 autosomes with multicolored bars) with osteosarcoma.



A



B

Fig 2: Graphs of genetic tumor-vs-normal copy number variation on chromosomes 11, 12, 13, and 14 between two dogs with osteosarcoma (A and B). Positive variance above the horizontal line (denoting average normal copy number for that individual) represents gene amplification within the tumor while negative variance below the horizontal line represents gene deletion. Note the similar patterns of relative amplification and deletion between individuals.