High-resolution whole genome analysis of skull base chordomas implicates FHIT loss in chordoma pathogenesis.

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Outline

- Background
- Hypothesis
- Methods
- Results
- Conclusions & Future directions

Background

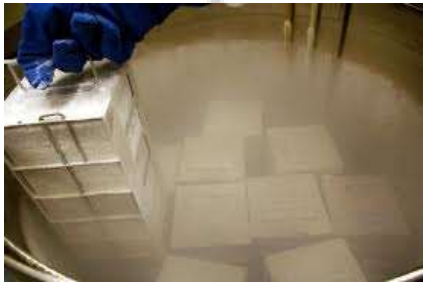
- Aberrations in chromosomes 2, 3, 4, 5, 6, 7, 9, 10, 12, 13, 14, 17, 20 have been documented in chordoma.
- Aberration of chromosome 3 by G-banding analysis has been observed in 62% of skull base chordomas. (Almefty et al, 2009)
- No specific tumor suppressor gene on chromosome 3 has yet been associated with skull base chordoma
- Only 9% of all chordomas analyzed for copy number alterations have been from the clivus or upper cervical spine even though ~35% of patients with chordoma have a skull base tumor.

Hypothesis

Characterization of tumor DNA gains and losses in skull base chordoma will give insight into the pathogenetic mechanisms of this disease

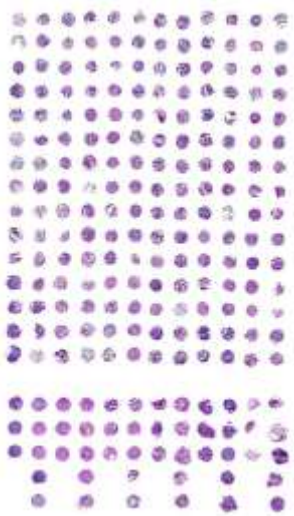
Methods

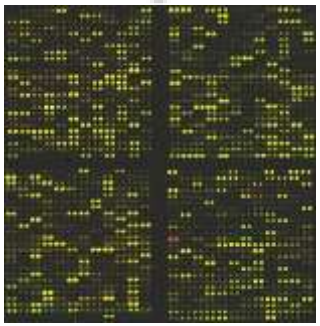
Skull base tumors excised
n=22 (Istanbul, Turkey)



Freezing in Liquid Nitrogen

IHC
on
Chordoma
TMA
(UK)





DNA isolation
& hybridization to
Affymetrix 500K SNP Array (Toronto)

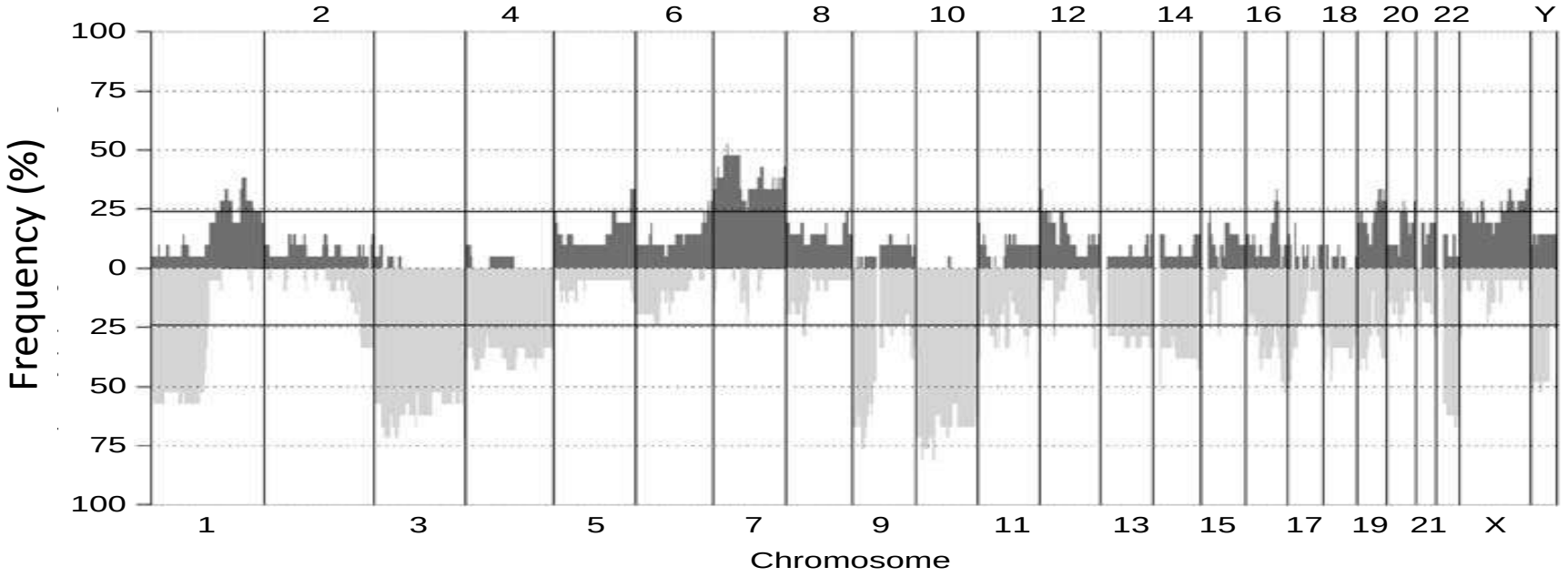
Patient Demographics and Tumor Pathology

ID	AGE	SEX	LOCATION	PATHOLOGY
CHD1	32	M	Clivus	Classical
CHD2	42	F	Clivus	Classical
CHD3	65	M	Clivus	Classical
CHD6	12	F	Clivus	Classical
CHD7	13	F	Clivus	Classical
CHD8	22	F	Clivus	Classical
CHD9	36	M	C1-C2	Chondroid
CHD10	26	F	Clivus	Classical
CHD11	83	F	Clivus	Classical
CHD12	63	F	Clivus	Classical
CHD13	55	F	Clivus	Chondroid
CHD14	22	F	Clivus	Chondroid
CHD15	51	M	Clivus	Classical
CHD16	42	F	Clivus	Classical
CHD17	35	M	Clivus	Classical
CHD18	38	M	Clivus	Classical
CHD19	31	F	Clivus	Classical
CHD20	37	F	Clivus	Classical, Recurrence of CHD19
CHD21	43	M	Clivus	Classical
CHD22	35	F	Clivus	Classical
CHD23	65	M	Clivus	Classical
CHD26	50	M	Clivus	Classical

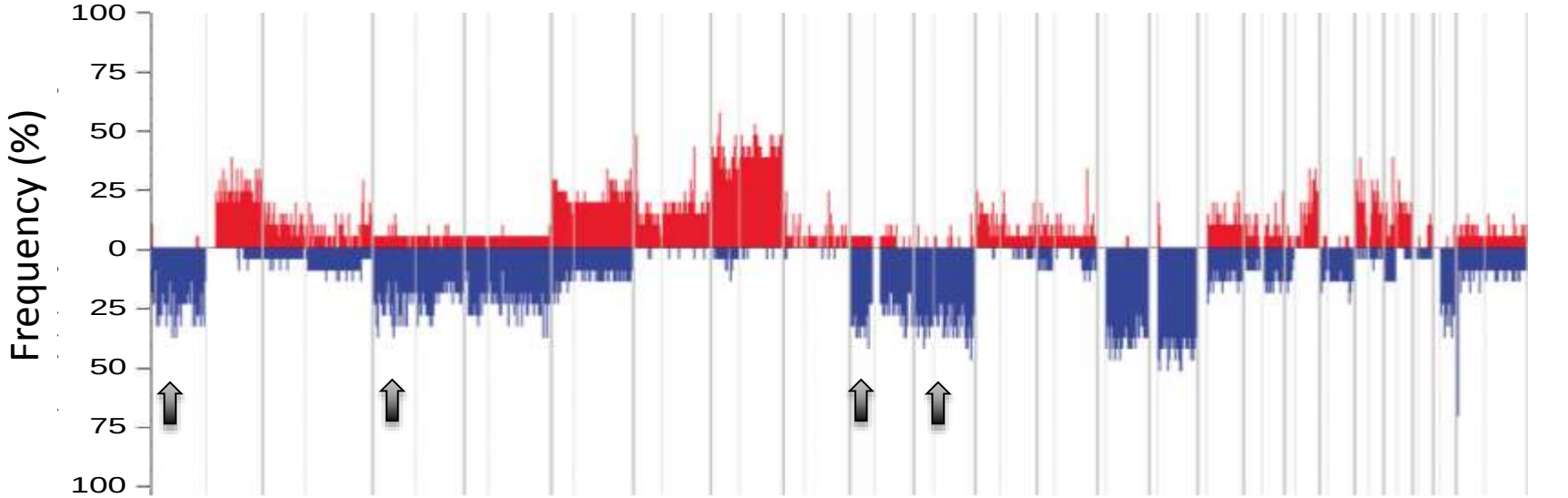
Comparison of tumor location, patient age, and recurrent copy number alterations in copy number studies of chordomas

Study	Scheil et al., 2001	Hallor et al., 2008	Le et al., 2011
Total Number of Tumors	16	26	20
Location			
Clival	5	0	2
Mobile Spine	1	2	7
Sacrum	10	24[b]	11
Primary samples	7	18	17
Median Age	61 years	60 years	61.5 years
Platform	CGH	BAC array CGH	Agilent 250K

Copy number variation across the Chordoma genome



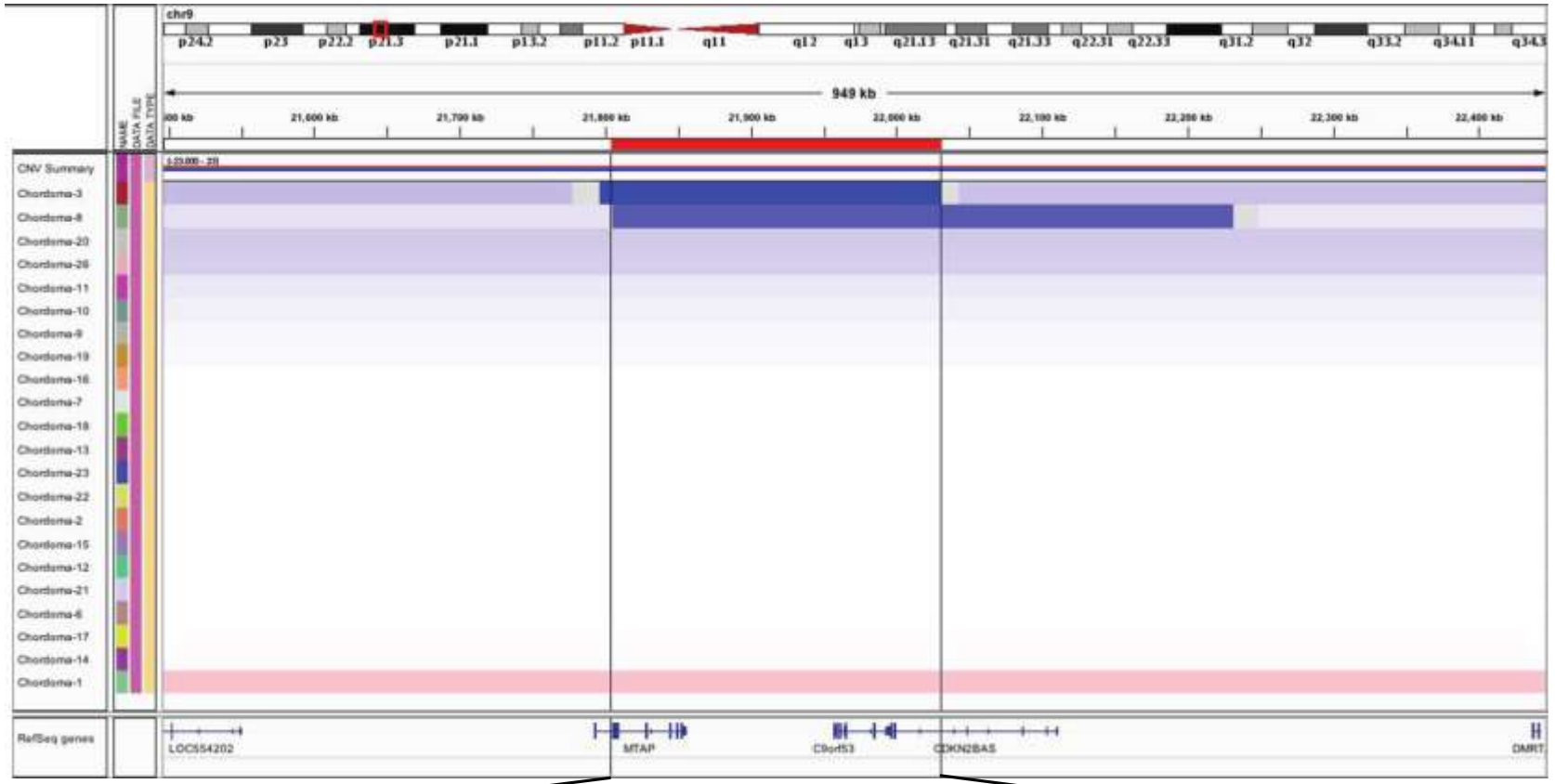
Sacral Chordoma (Hallor et al, 2008)

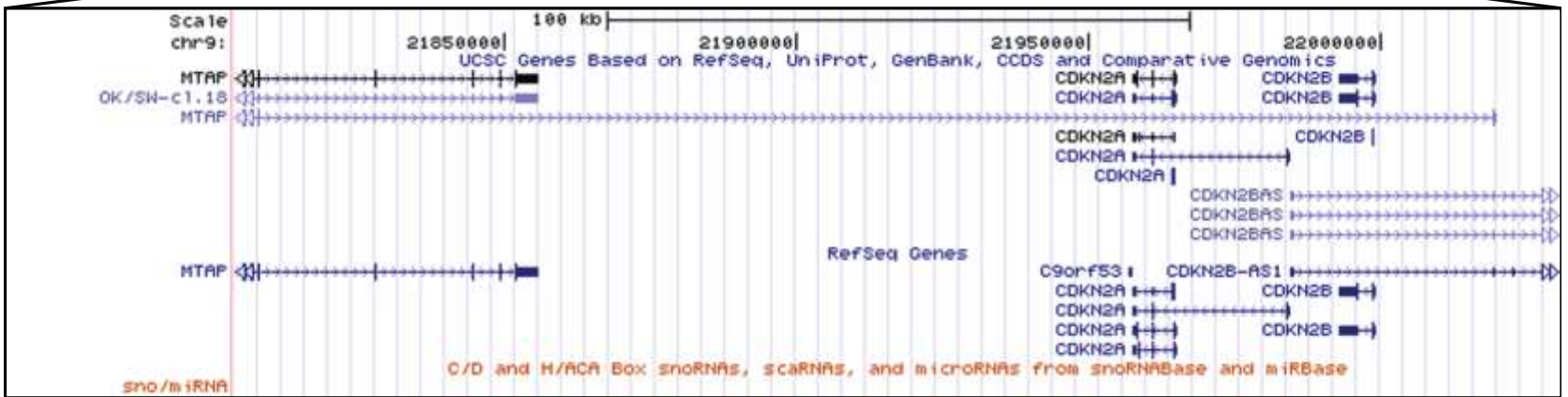


Skull Base Chordoma (Diaz et al, 2012)

Focal CDKN2A/2B loss is infrequent in skull base chordoma

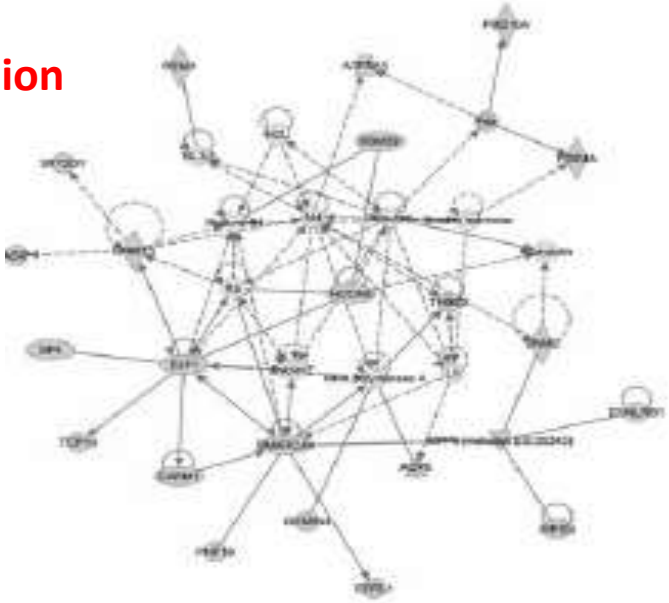
22%

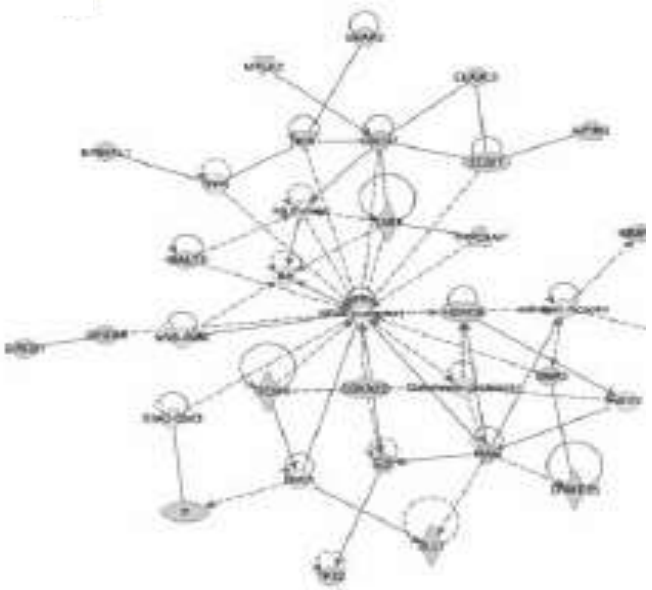




Pathway analysis of genes within focal amplifications or deletions

DNA methylation & Stem cell signaling

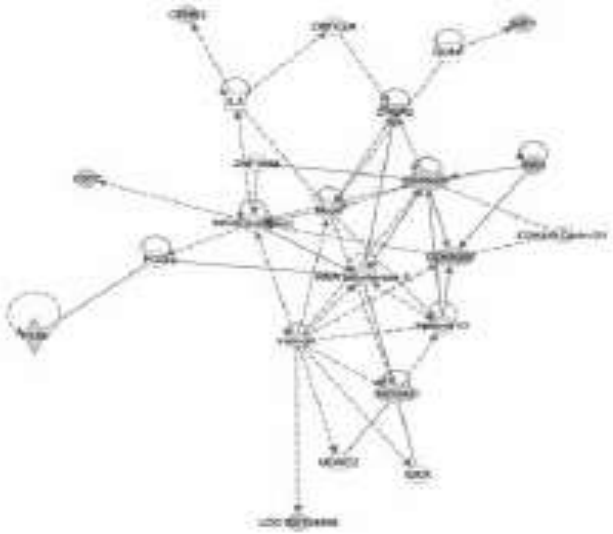




FOCAL AMPLIFICATIONS

49 events, 370 genes

Cell cycle regulation



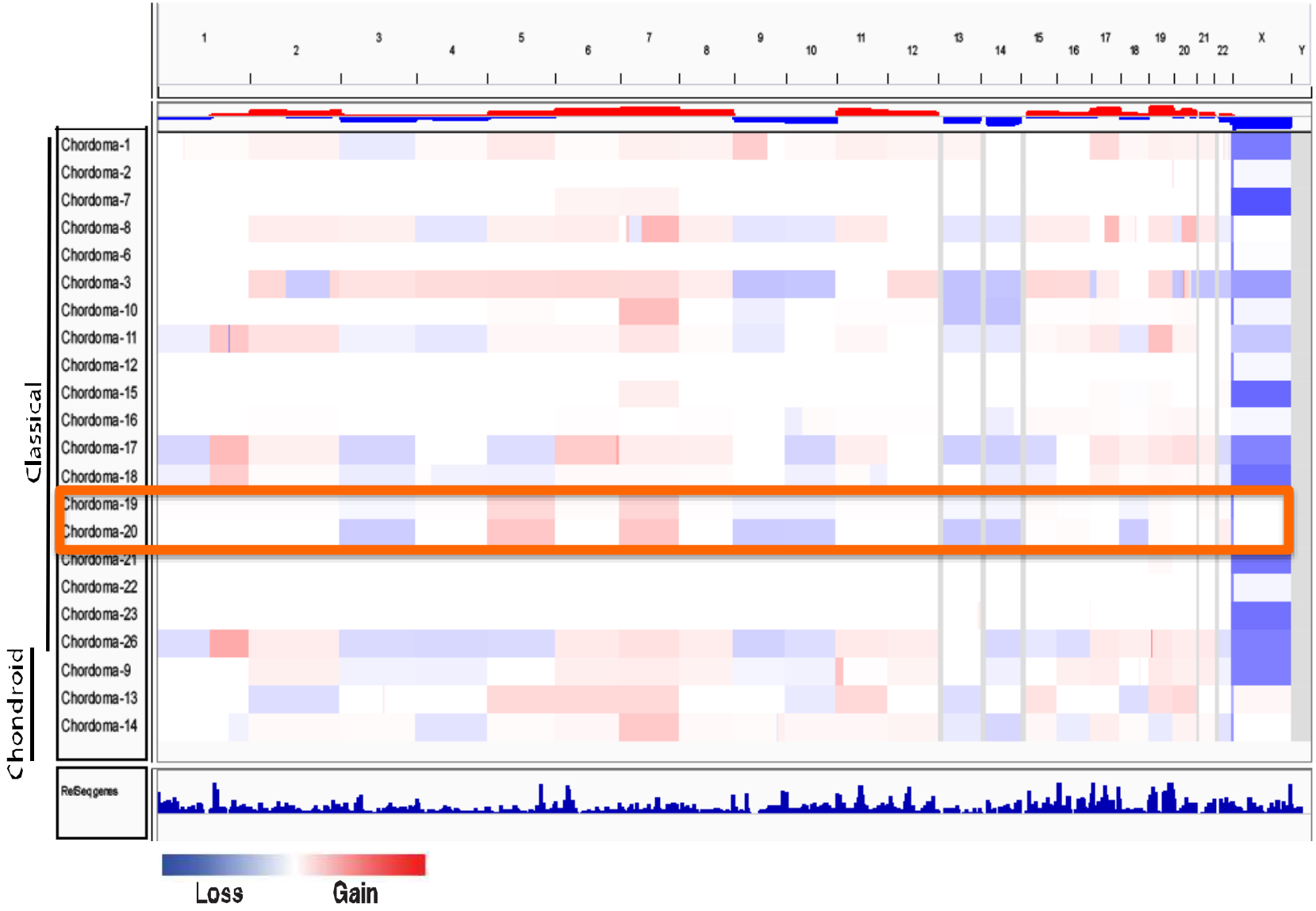
FOCAL DELETIONS

25 events, 98 genes

Loss in Chromosome 3 is a frequent finding in all chordomas

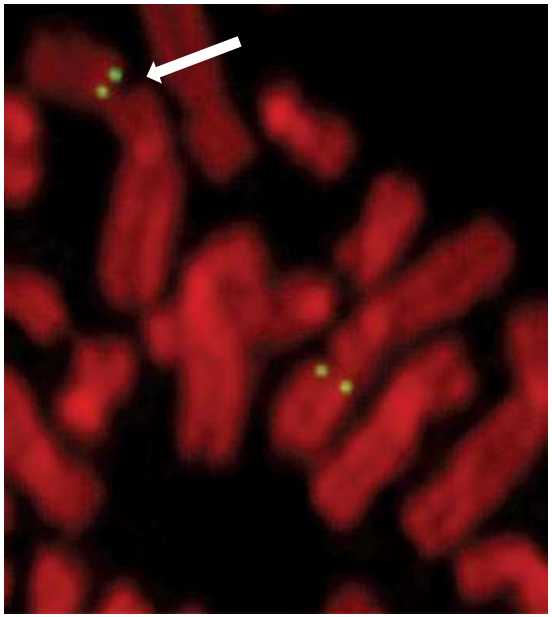
Broad significance Copy number changes[a] (frequency)	Diaz et al., 2012	Le et al., 2011	Hallor et al., 2008	Scheil et al., 2001
Losses			**1p36.33 –p11.1**	**1p (0.44)**
			2q34 – q37.3	
	3 (0.45)	**3p29-p26.3 (0.75)**	**3**	**3p (0.5)**
		4p16.3-q35.2 (0.40)	**4**	
		6q21-q22.33 (0.25)	6p21.1	
			7q11.22 – q11.23	
			8p12 – p11.1	
		9p24.3-q34.3 (0.25)	**9p24.3 –q31.3; 9q33.3 –q34.3**	
	10p (0.61); 10q (0.57)	**10p15.3-q26.3 (0.65)**	**10**	

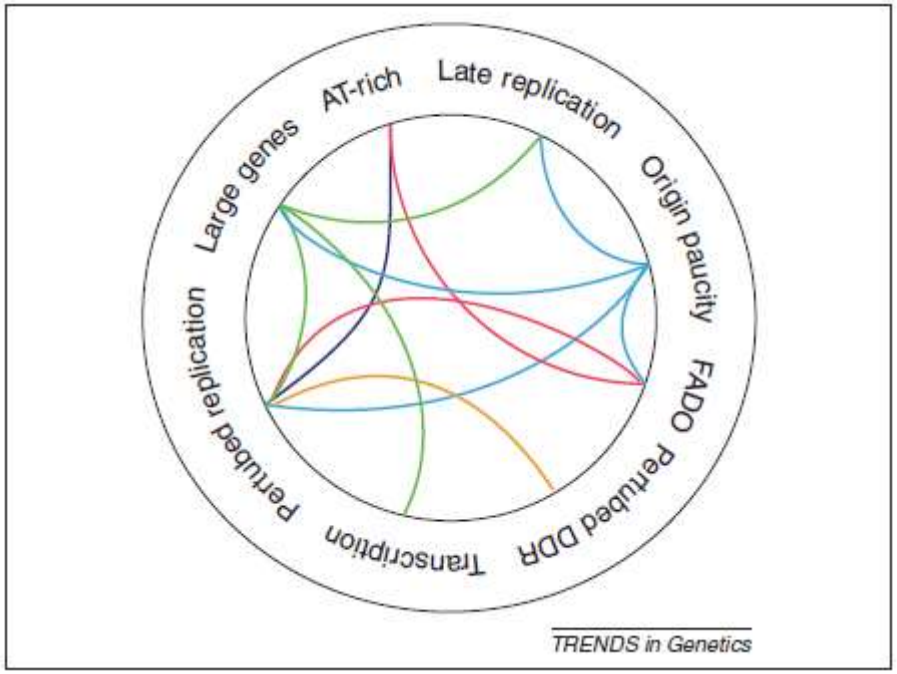
Loss of Chromosome 3 observed in a recurrent clival chordoma



3/18 primary + 1 recurrent tumor had chr 3 loss

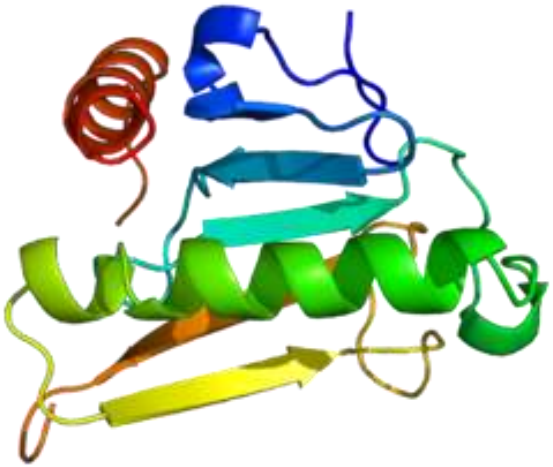
FRA3B, fragile site, Aphidicolin type, contains FHIT gene (~1.5Mb), region of instability is 4 Mb





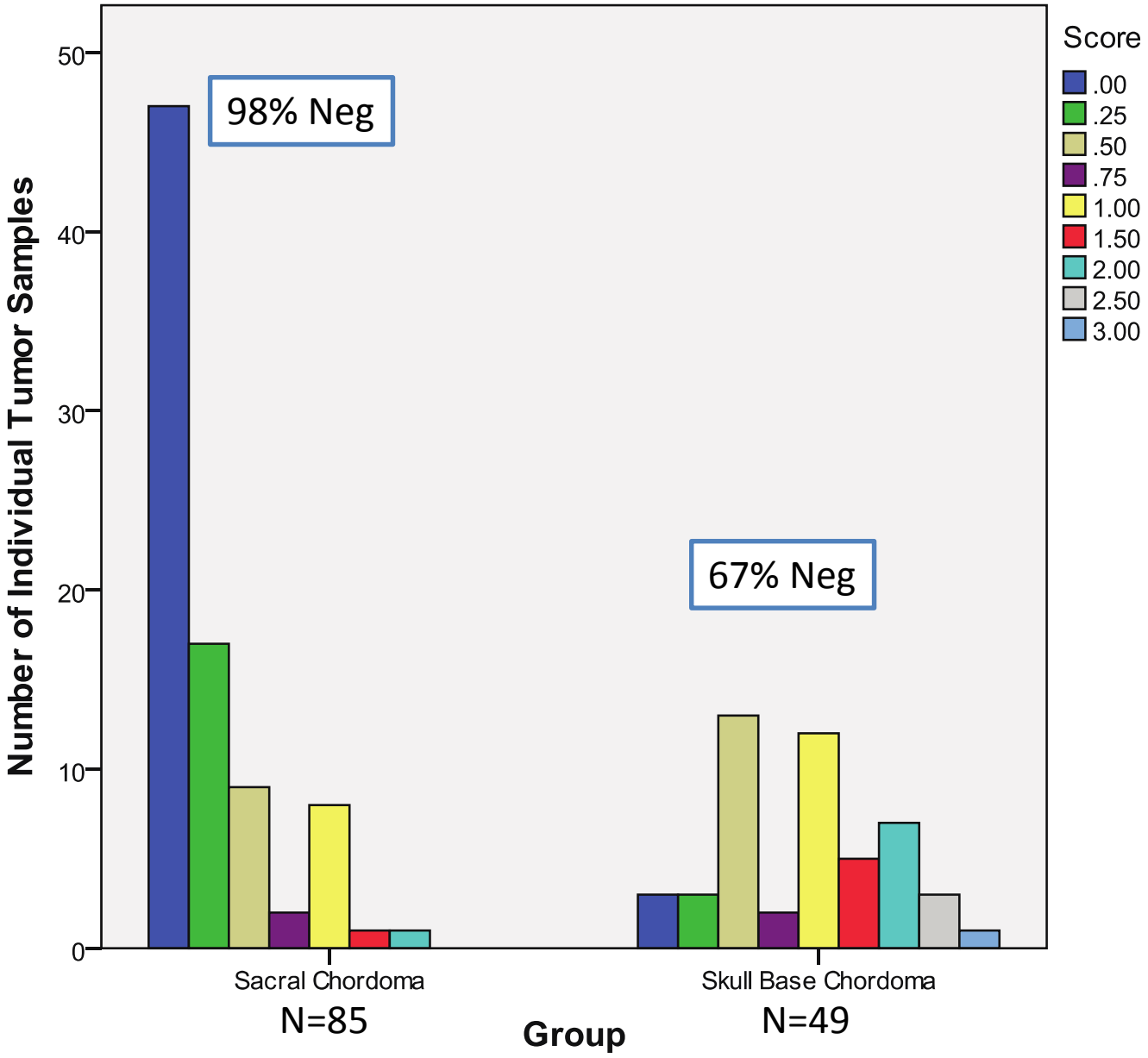
Heubner, Nature 2011

FHIT

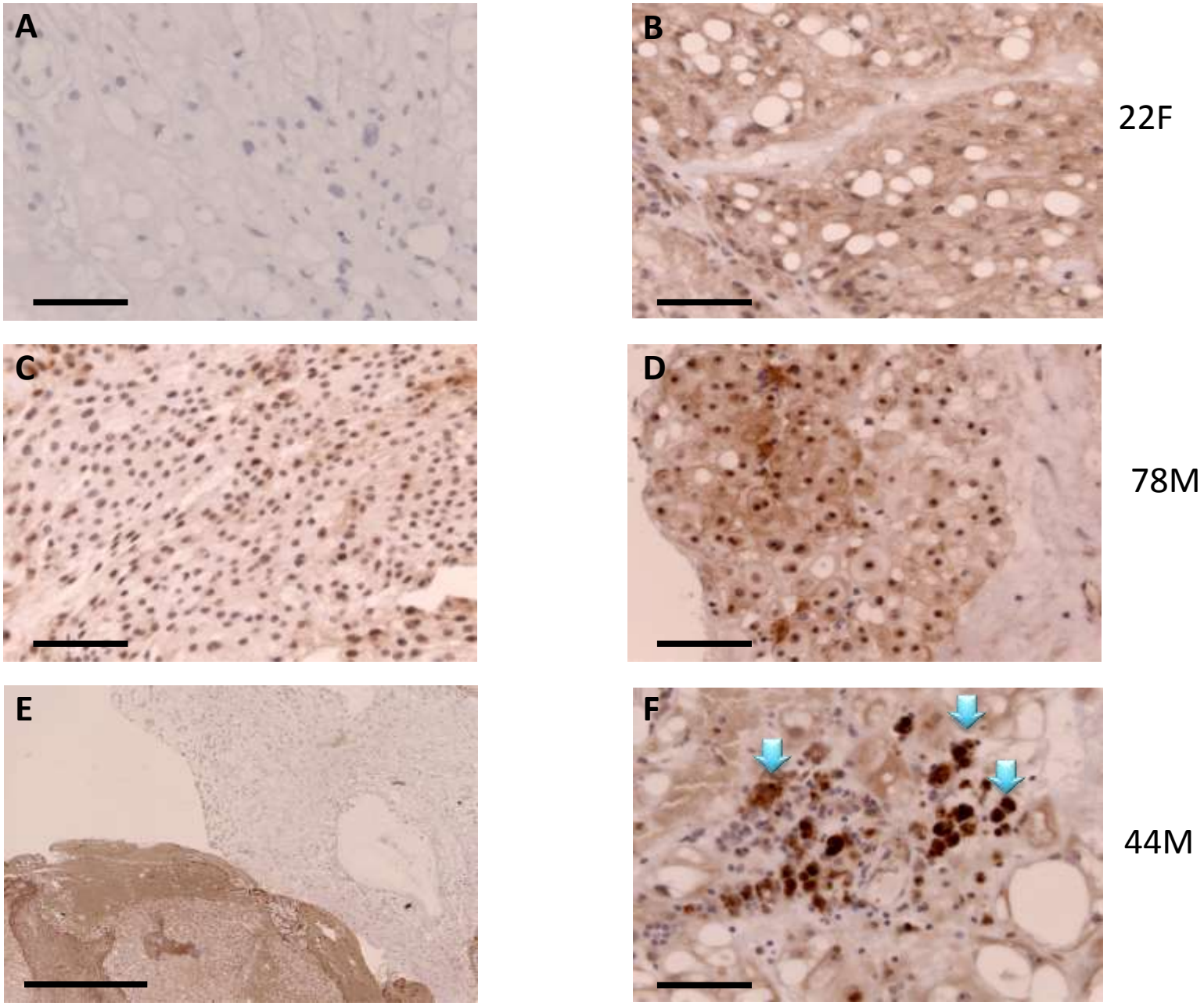


- Tumor suppressor (FHIT +/- mice develop tumors when exposed to carcinogen)
- Cytoplasmic expression
- Involved in purine metabolism and DNA damage response (Chk1-pathway)
- Epigenetic regulation by CpG methylation
- Loss or aberrant transcripts seen in multiple tumor types

FHIT protein expression profile in skull base and sacral chordomas (UK)



Investigation of FHIT expression in skull base chordomas (Toronto)



Conclusions

- Unique and shared copy number alterations in skull base chordomas versus those observed for sacral chordoma
- Focal deletions affect cell-cycle checkpoints
- Focal amplifications affect Stem cell signaling and DNA methylation
- Loss of chromosome 3 and FHIT protein expression is frequent in both clival and sacral chordomas. FHIT expression is almost always lost in sacral chordoma.

Hypotheses Generated

1. Epigenetic and genetic mechanisms may contribute to frequent loss of FHIT gene expression in chordoma

2. Loss of FHIT gene function may be a factor in the radio- and chemo-resistance observed in chordoma

Future Directions

- Further characterization of FHIT gene loss – recurrent versus primary tumors (genotyping/promoter methylation/mRNA/protein). Relation to survival, radiation, and tumor location.

- Analysis of re-introduction of FHIT in UCH-1 cell line which has 3p loss - GENE THERAPY

- Brachyury overexpression in FHIT -/- mice ---
  ? Animal model of chordoma