National Cancer Institute

Characterization of genetic variants in the *T* gene in familial and sporadic chordoma

4th International Chordoma Research Workshop

Session I: Genetics and Genomics of Chordoma

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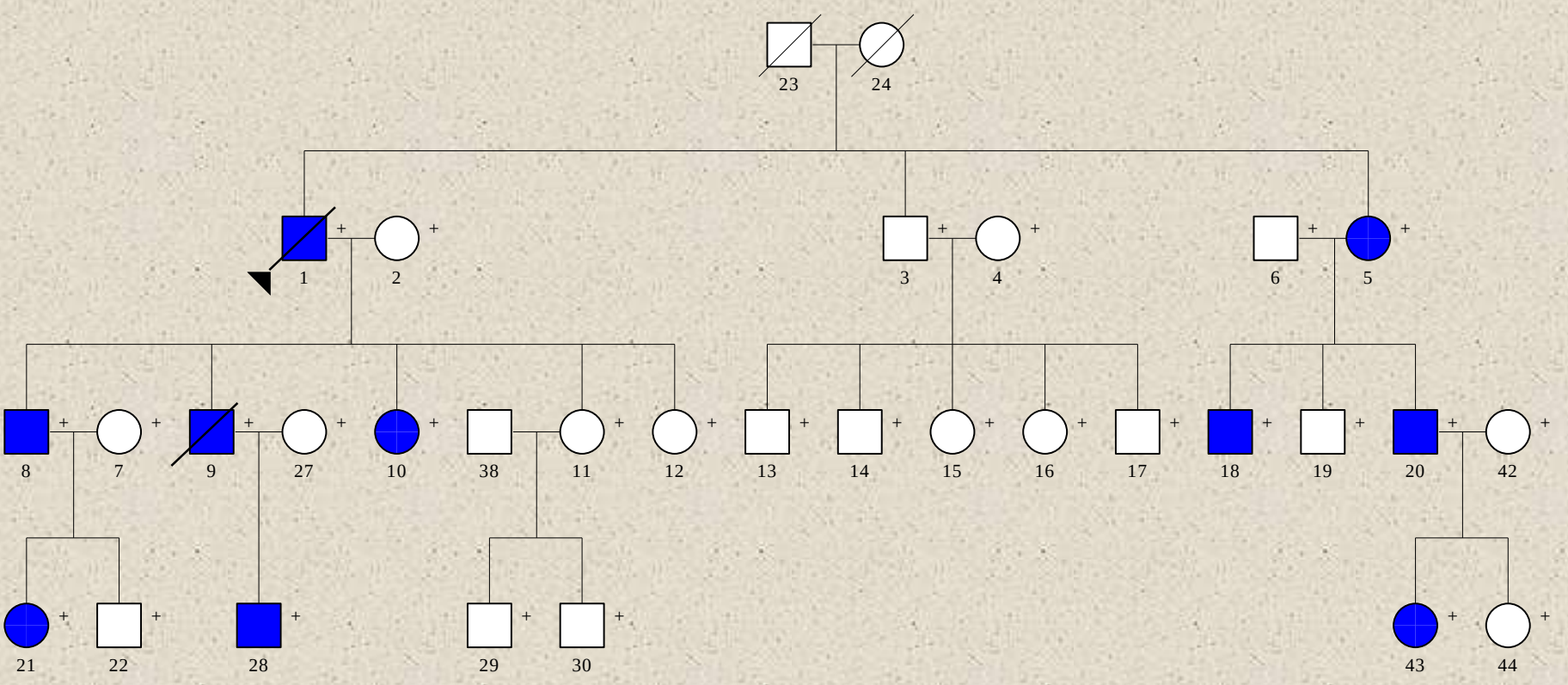
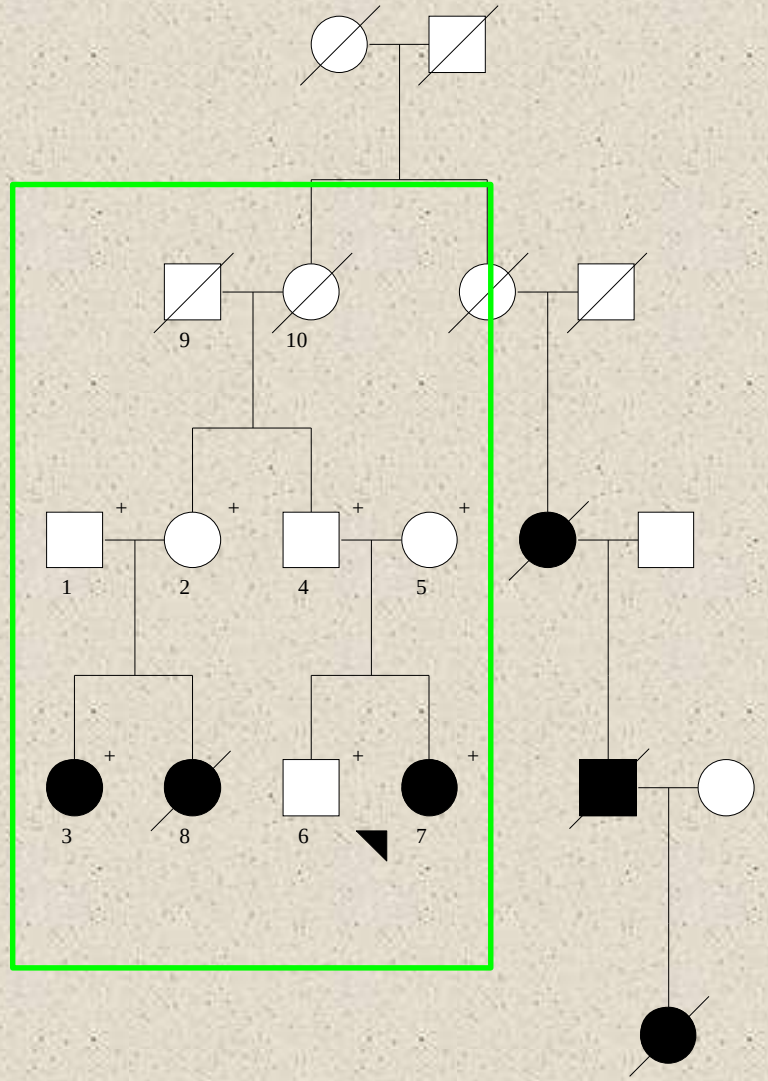
Identifying susceptibility genes for familial chordoma

- Chordoma is mostly sporadic; multiple families with ≥ 2 cases suggesting genetic predisposition
- The goal of our study: to identify susceptibility genes for familial chordoma
- Improve our understanding of biology underlying familial and sporadic chordoma

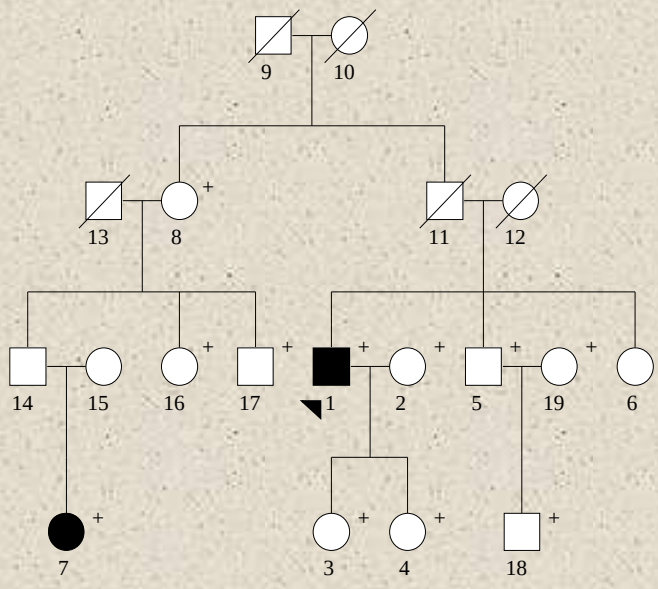
Chordoma Family 1

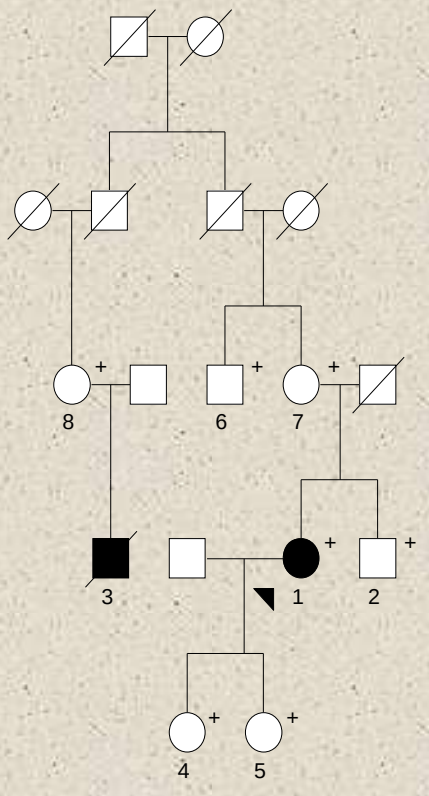


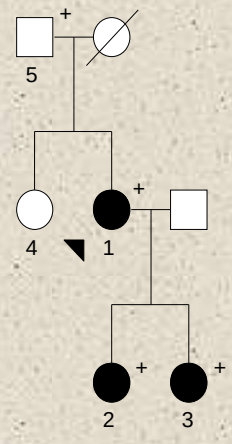
Chordoma Family 3

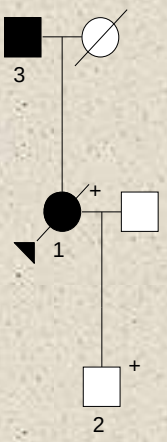


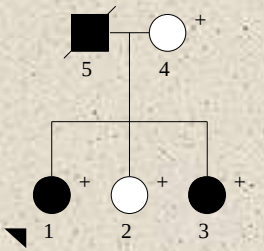
Other chordoma families



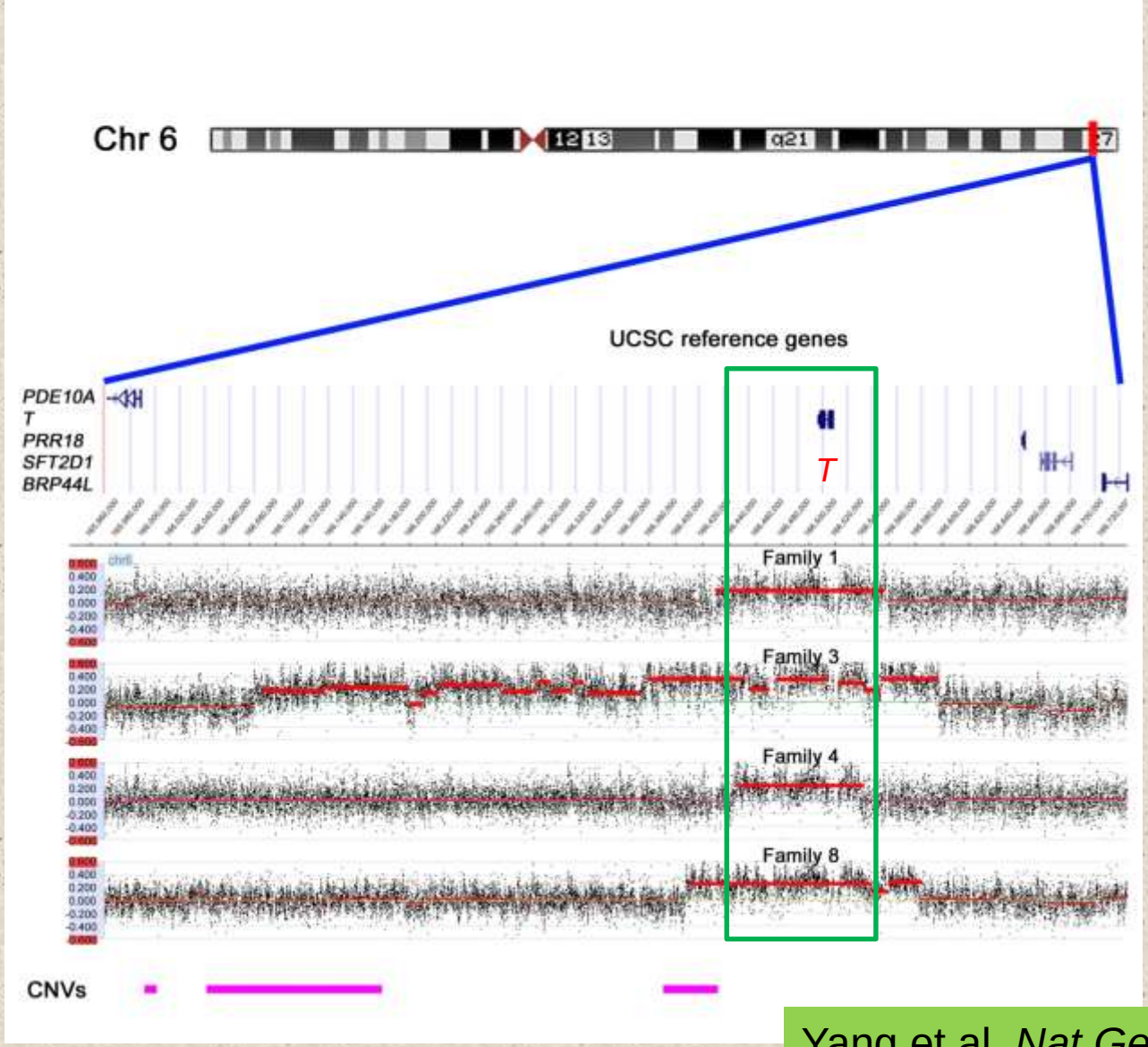








6q27 gain in 4 chordoma families



Yang et al. *Nat Genet*, 2009

T amplification in chordoma

- Presneau N et al. J Pathology, 2011
- Copy number change of the *T* locus is common in sporadic chordoma tumors (N=170).
  - ~50% showed gain of the *T* locus
  - ~7% with *T* amplification
- Knockdown of *T* reduced chordoma cell proliferation
- No germline CNVs among 40 patients

Common *T* variant associated with chordoma

- Pillay N et al. Nature Genet., 2012

- A common germline SNP in *T* (rs2305089) was associated with chordoma.
  - The only exonic NS variant in >1 chordoma case
  - Variant allele A present in all 23 cases
  - Common (~47% in HapMap CEU)
  - OR=6.1, $P=4.4 \times 10^{-9}$ (40 cases, 358 controls)
  - Validated in another case-control analysis (20 cases, 363 controls, combined OR=5.3, $P=4.6 \times 10^{-12}$)

- No *T* germline duplication in 22 sporadic cases

Finding additional susceptibility genes for familial chordoma

- Exome sequencing
  - Nimblegen v2/v3 exome capture, Illumina HighSeq, >80% coverage at 15x
- More than 800 subjects with cancers were sequenced using the same platform
- Sequenced germline DNA from 16 chordoma cases/carriers
  - 11 from 4 families; 5 young sporadic cases

Finding disease-related variants

- Non-synonymous
- Rare
  - <5 in Exome Variant Server (up to 4,300 Europeans)
  - <1% in dbSNP or 1k genome
  - <2 in internal controls (>800 subjects with other cancers)
- Same variant shared by all cases/carriers in one family and different variants in same genes shared by multiple cases across families

Finding disease-related variants

- Challenge: finding disease-causing variants
  - Database search: functional relevance, regulatory regions, somatic changes in tumors, etc.
- Technical validation/Co-segregation with disease
- Sequence genes in sporadic cases
- Functional study

Finding disease-related variants

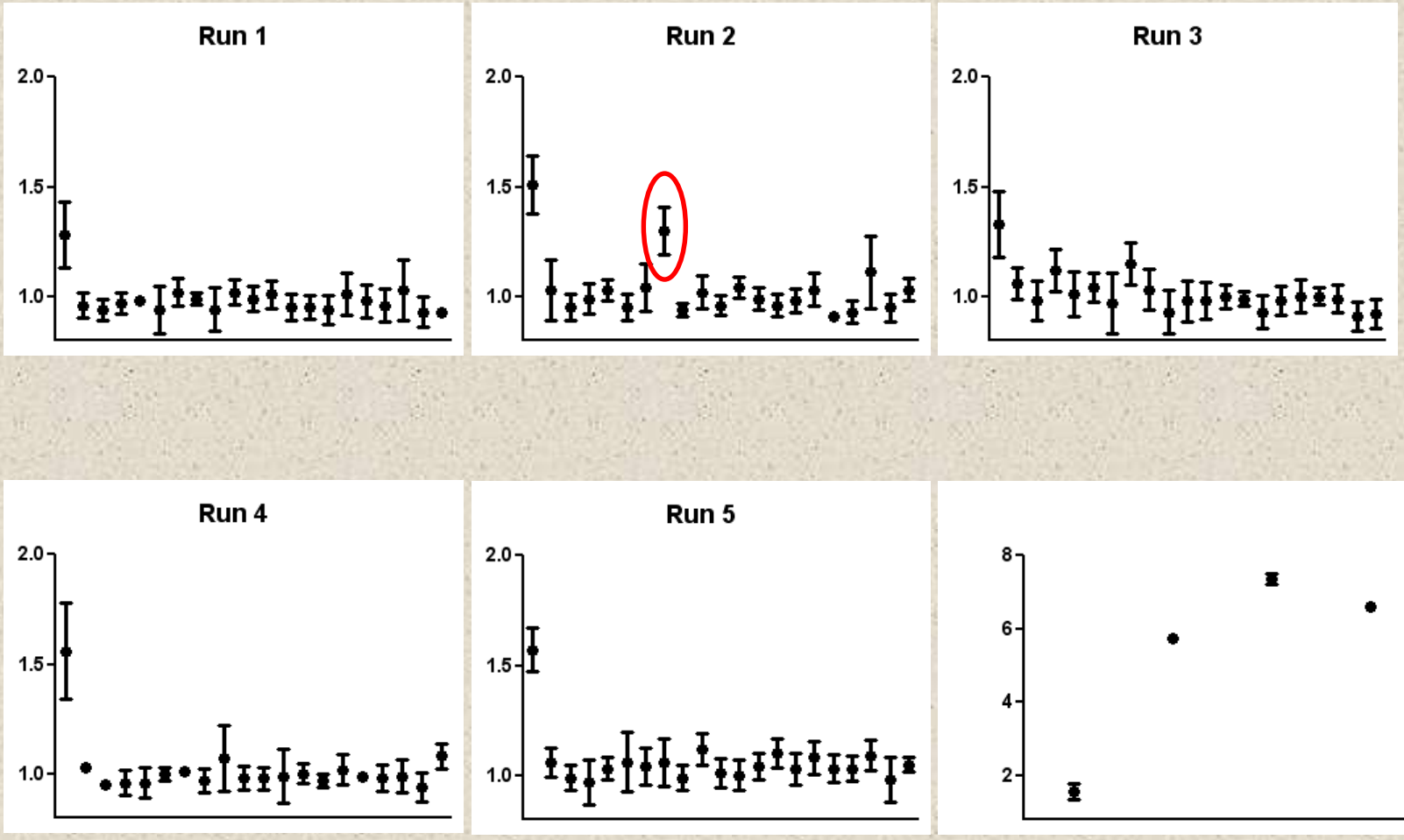
- Found one rare variant in *T* in a single case
  - In a young sporadic case with multi-centric chordoma
  - Observed once in ESP, not in other databases or internal controls
  - Located in a potential splice site
  - Validated by Sanger sequencing
  - Found in one parent and one of two unaffected siblings
- Function analysis, the variant did not increase the transcription activity by *T*

Characterization of *T* variants

- Germline *T* duplication
  - One new family with 2 chordoma cases
  - ~100 sporadic chordoma cases (any age, any primary site, identified in US and Canada)
  - qPCR (exon 6 of *T*)

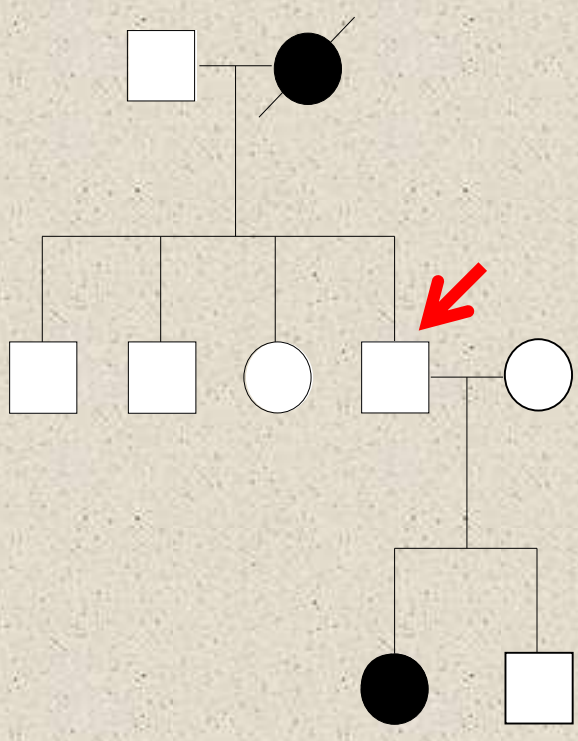
- Exonic single nucleotide variants in *T*
  - All exons in *T* sequenced using Ion Torrent
  - 115 subjects in chordoma families (39 cases/carriers, 76 unaffected family members and spouses)
  - ~100 sporadic chordoma cases

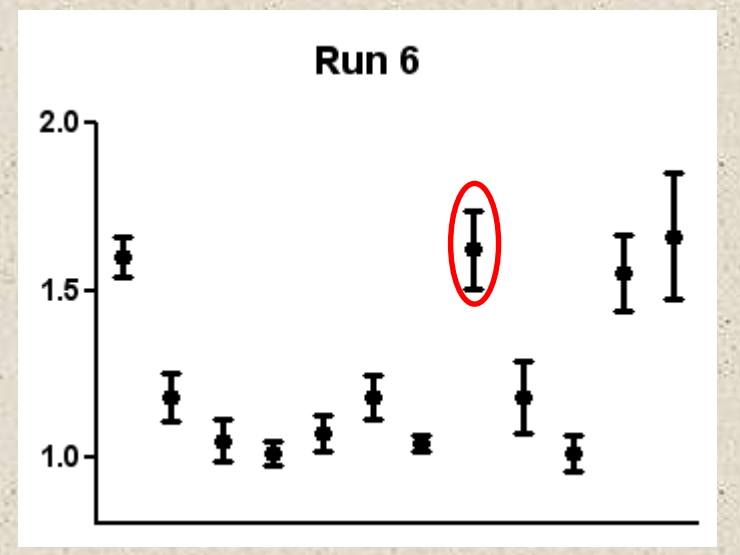
Germline *T* amplification in sporadic chordoma



Germline *T* duplication in a new family

Family 9





Germline *T* dup/amp: 2% (2/100) in sporadic cases

~50% (5/9) in chordoma families

rs2305089 in familial and sporadic chordoma

Reference allele: C Variant allele: T

Population	Freq of T	P value
ESP European	0.51	
Controls in chordoma families	0.59	
Cases/carriers in chordoma families	0.72	P=0.0005 vs. controls
Sporadic cases	0.77	P<0.0001 vs. ESP

OR=2.76 (1.15, 6.63) comparing familial cases to controls using conditional logistic regression;
OR=3.17 (2.24, 4.49) comparing sporadic cases to ESP.

Summary of *T* variants

- The variant allele in rs2305089 was associated with chordoma, however, it is very common
  - Limited value in predicting risk
- The variant identified in a single case by exome sequencing was not observed in any other cases
- 3 additional novel variants were identified that occurred only in one or two cases
  - Unknown significance
  - Analyses in process to evaluate these novel and other common variants

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