# Multiple new susceptibility loci identified in genome-wide association study of Ewing sarcoma

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**Abstract**

Ewing sarcoma (EWS) is a rare pediatric tumor predominantly occurring in children of European ancestry and is characterized by the pathognomonic *EWSR1-FLI1* fusion oncogene. To identify germline susceptibility loci associated with EWS risk, we performed a genome-wide association study (GWAS) meta-analysis of 749 EWS cases and 1,378 unaffected individuals of European ancestry from sample collections within the Institut Curie, National Cancer Institute and the Childhood Cancer Survivor Study. Our study replicated previously reported susceptibility loci at 1p36.22, 10q21.3 and 15q15.1 as well as identified novel loci at 6p25.1, 8q24.23, 20p11.22 and 20p11.23 (P-values$<5\times10^{-8}$). These seven EWS susceptibility loci discovered in only 749 cases make EWS one of the most productive GWAS studied cancers when considering a locus to case discovery ratio. All estimated effect estimates were high for cancer GWAS with odds ratios (ORs) in excess of 1.7 observed. These high per allele effects among relatively common germline variants are striking in light of the rarity of EWS cases and lack of evidence of EWS as part of a familial cancer syndrome and therefore suggest a distinctive genetic architecture for EWS. Interestingly, *in silico* bioinformatics analysis identified that most EWS susceptibility loci reside near GGAA nucleotide repeat sequences where binding of the *EWSR1-FLI1* fusion protein occurs. ChIP-seq analyses confirmed *in vivo* binding of *EWSR1-FLI1*, suggesting germline variation in these regions could alter *EWSR1-FLI1* binding and potentially deregulate neighboring genes. To identify genes with allele specific expression differences, we carried out expression quantitative trait locus (eQTL) analyses at each identified EWS susceptibility locus. We identified eQTLs for plausible candidate genes at 6p25.1 with *RREB1*, a *RAS*-responsive element, and at 20p11.23 with *KIZ*, a centrosomal stabilization protein. We also noted the 20p11.22 locus is near *NKX2-2*, a highly overexpressed gene in EWS, although no eQTL was

observed in our expression data. Furthermore, knockdown of *EWSR1-FLI1* in EWS cell lines indicated a more than 2-fold difference in expression of *RREB1* and *NKX2-2*, further supporting the role of specific regulation of these genes by *EWSR1-FLI1* and suggesting *RREB1* and *NKX2-2* may be transcription factors involved in core regulatory circuitries of EWS. Overall, our study suggests a distinctive underlying genetic architecture for EWS in which moderate risk common germline variants interact with *EWSR1-FLI1* binding to alter expression of nearby target genes.