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# Noncoding Regulatory Mutations as a Driving Event for the Oncogenic Core Regulatory Circuitries of Neuroblastoma †

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## 1. Introduction

Neuroblastoma (NB) is a pediatric tumor composed of adrenergic (ADRN) and mesenchymal-like (MES) cells which derive from the dysregulation of normal cell differentiation imposed by NB Core Regulatory Circuitries (CRCs). We hypothesize that somatic single-nucleotide variants (SNVs) in active CRCs transcription factor binding sites (aTFBSs) may underlie such perturbation, promoting NB tumorigenesis. We aim to investigate such patterns of regulatory elements and identify putative driver SNVs, exploring their role in NB.

## 2. Methods

MES and ADRN aTFBSs were identified by integrating 42 ChIP-seq and 12 ATAC-seq experiments in 7 ADRN and 2 MES NB cell lines. Using the Fisher test, we tested these regions for an enrichment of somatic SNVs obtained from the WGS data of 397 NB patients. SNVs were selected based on their impact on CRC TF binding through the FABIAN-variant tool. Next, aTFBS target genes were identified by analyzing the promoter capture HiC (CHiC) in 2 ADRN and 2 MES NB cell lines and their expression values were correlated with clinical and survival data of a second cohort of 498 NBs.

## 3. Results

We found a significant enrichment of SNVs (FDR $\leq 0.1$) in six aTFBS sets bound by 5 ADRN (GATA3, HAND2, ISL1, MYCN, and TBX2) and 1 MES (FOSL2) TFs. 689 mutations impacting the binding of CRC TFs (Fabian $\neq 0$) were localized in aTFBSs interacting with genes of neuronal differentiation and MES proliferation pathways, suggesting the potential impact of SNVs on NB cell identities. By focusing on genes of developmental and differentiation processes interacting with aTFBSs carrying SNVs with the highest (cut-off $\geq 0.1$)

or the lowest (cut-off $\leq -0.1$) Fabian score, we found targets (*ROBO2*, *CACNB1*, *PIK3R1*, *MDGA1*, *HES6*, *LDLRAD4*, *DGUOK*, *IRX1*, and *SPOCK2*) whose expression significantly correlated with worse NB outcomes (FDR $\leq 0.05$).

## 4. Conclusions

These results demonstrated that somatic noncoding SNVs may act synergistically to affect NB CRCs and thus contribute to tumorigenesis.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the Ospedale Bambino Gesù of Rome (protocol no. 20757 of the 9 April 2019).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** Public WGS data presented in the study are openly available in the the European Genome-phenome Archive (EGA) under accessions EGAD00001001687, EGAD00001006739, EGAD00001006626 and in the database of Genotypes and Phenotypes (dbGaP) under accession no.: phs000218.v21.p7; project ID: #14831. Public ChIP-seq data are available in the NCBI Gene Expression Omnibus (GEO) under accession nos.: GSE94824, GSE169616, GSE80151, GSE94782, GSE120074, GSE65664, GSE138315. Public ATAC-seq data are available in the NCBI Gene Expression Omnibus (GEO) under accessions nos.: GSE94824, GSE80152, GSE138315. Public RNA-seq data are available in the NCBI Gene Expression Omnibus (GEO) under accession no.: GSE62564. The in-house generated raw WGS, ChIP-seq, ATAC-seq and CHiC data supporting the conclusions of this article will be made available by the corresponding author on request.

**Conflicts of Interest:** The authors declare no conflict of interest.