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## Do regulatory regions matter in *FOXG1* duplications?

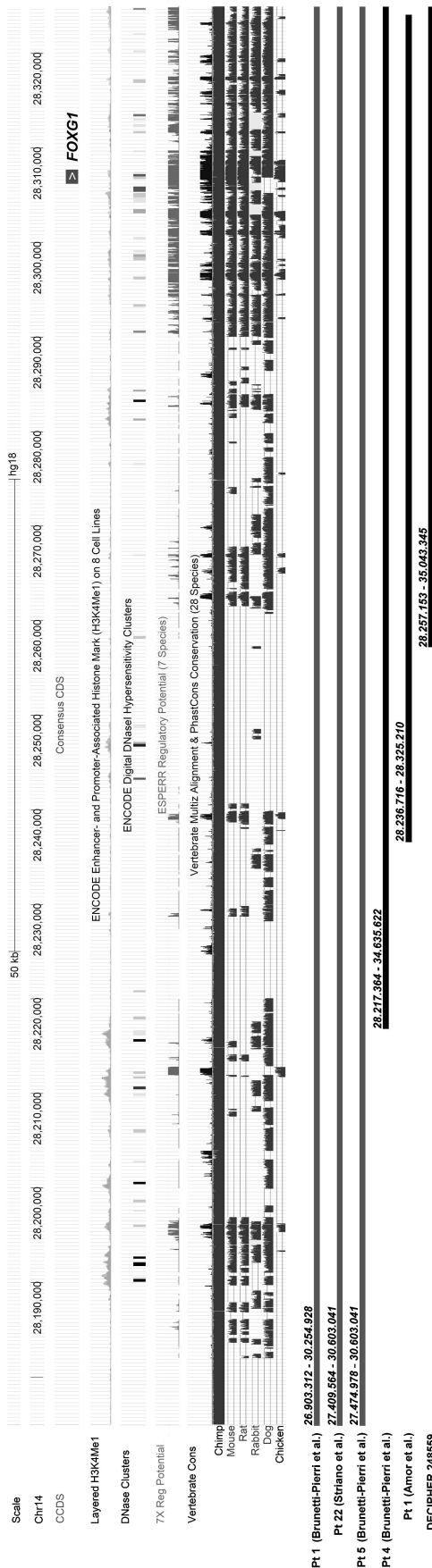
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Duplications of *FOXG1* gene at 14q12 have been reported in patients with infantile spasms and developmental delay of variable severity.<sup>1,2,3</sup> *FOXG1* encodes the forkhead protein G1, a brain-specific transcriptional repressor, regulating corticogenesis in the developing brain and neuronal stem cell self-renewal in the postnatal brain.<sup>4</sup> Recently, Amor *et al.*<sup>5</sup> reported on this journal an interstitial duplication of ~88 kb at 14q12 in a father–son pair with hemifacial microsomia and normal neurocognitive phenotype. The duplication contains only two polypeptide-encoding genes, *FOXG1* and *C14orf23*, suggesting that *FOXG1* duplication may be benign or at least incompletely penetrant. That makes the involvement of *FOXG1* duplication in the pathogenesis of the neurocognitive impairment and epilepsy controversial. As also discussed by Brunetti-Pierri *et al.*<sup>6</sup> we feel that this statement needs special caution.

Functional consequences of chromosomal microduplication and microdeletion rely on the final gene dosage, which is strongly influenced by the location of the breakpoint. In this context, the understanding of the contribution of regulatory sequences in gene transcription is critical to understand the relationship between CNVs and human diseases. With this purpose, the Encyclopedia of DNA Elements (ENCODE) project has recently performed a systematic analysis of transcriptional regulation in different human cell lines,

providing new understanding about transcription start sites, including their relationship with specific regulatory sequences and histone modification and features of chromatin accessibility.<sup>7,8</sup> Interestingly, analysis of histone modifications from the ENCODE project revealed the presence of a putative regulatory element upstream *FOXG1* gene between 28 188 and 28 217 kb (UCSC genome browser, NCBI Build 36/hg18) (Figure 1). This conserved region localizes about 130 kb upstream *FOXG1* gene and contains histone modifications typical of enhancers of gene transcription (eg, histone H3 and Lysine 4 monomethylation) in eight different human cells lines. Analysis of regulatory potential scores, comparing frequencies of short alignment patterns between known regulatory elements and neutral DNA,<sup>9</sup> also disclose two additional putative elements typical of *cis*-regulatory modules within this region (Figure 1). Moreover, it contains a DNaseI hypersensitive site (DHS). DHSs reflect genomic regions thought to be enriched for regulatory information and many DHSs reside at or near transcription start site. Notably, no other polypeptide-encoding genes or non-coding RNAs and pseudogenes are present in the region, suggesting that this regulatory element might regulate *FOXG1* transcription. Analysis of duplication breakpoints previously reported on 14q12 revealed that duplications associated with an epileptic phenotype localizes uniquely upstream this regulatory element, whereas downstream duplications were identified only in the cases without seizures (Figure 1). On the basis of this finding, we suggest that *FOXG1* duplication including this putative regulatory region allows the efficient transcription of the supernumerary copy of *FOXG1* gene, resulting in an effective increase in *FOXG1* expression and, thereby, in brain hyperexcitability. In contrast, duplications starting downstream this putative regulatory site do not allow efficient transcription of *FOXG1*, which may underlie the lack of neurological phenotype in the case reported by Amor *et al.*<sup>5</sup>

Even if the functional relevance of this putative long-range regulatory element on *FOXG1* transcription deserves to be experimentally verified, it provides an interesting clue to dissect



**Figure 1** UCSC genome browser (NCBI36/hg18) schematic view of reported 14q12 duplications encompassing *FOXG1* gene. Histone modifications from the ENCODE project<sup>7</sup> in eight cell lines (GM12878, H1-hESC, HMEC, HSMM, HUVEC, K562, NHEK and NHLF) indicate the presence of regulatory elements upstream *FOXG1* gene. Monomethylation of histone 3 at lysine1 (H3K4me1) is often found near regulatory elements, whereas the presence of DNaseI hypersensitive clusters reflect genomic regions containing regulatory informations. Potential regulatory elements identified with the ESPERR (Evolutionary and Sequence Pattern Extraction through Reduced Representations) computational method and evolutionary conservation are also shown. Grey bars represent duplications associated with an epileptic phenotype; black bars represent duplication identified in non-epileptic patients.

genotype–phenotype correlation in *FOXG1* microduplication and to uncover the real actual contribution of *FOXG1* in the neurodevelopmental phenotype associated with 14q12 duplication.

Notably, chromosome rearrangements disrupting or displacing putative *cis*-regulatory elements distal to *FOXG1* gene in patients with severe cognitive disabilities has been also reported,<sup>10,11</sup> pointing out the relevance of regulatory sequences in the expression of *FOXG1* gene.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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