27 Jacquemont S, Reymond A, Zufferey F *et al*: Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. *Nature* 2011; **478**: 97–102.
28 Marshall CR, Noor A, Vincent JB *et al*: Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet* 2008; **82**: 477–488.
29 Sanders SJ, Ercan-Sencicek AG, Hus V *et al*: Multiple recurrent *de novo* CNVs, including duplications of the 7q11.23 williams syndrome region, are strongly associated with autism. *Neuron* 2011; **70**: 863–885.
30 Davis LK, Meyer KJ, Rudd DS *et al*: Novel copy number variants in children with autism and additional developmental anomalies. *J Neurodev Disord* 2009; **1**: 292–301.
31 Burnside RD, Pasion R, Mikhail FM *et al*: Microdeletion/microduplication of proximal 15q11.2 between BP1 and BP2: a susceptibility region for neurological dysfunction including developmental and language delay. *Hum Genet* 2011; **130**: 517–528.
32 Pinto D, Pagnamenta AT, Klei L *et al*: Functional impact of global rare copy number variation in autism spectrum disorders. *Nature* 2010; **466**: 368–372.
33 Wincent J, Bruno DL, van Bon BW *et al*: Sixteen New Cases Contributing to the Characterization of Patients with Distal 22q11.2 Microduplications. *Mol Syndromol* 2010; **1**: 246–254.
34 Roux-Buisson N, Cacheux M, Fourest-Lieuvin A *et al*: Absence of triadin, a protein of the calcium release complex, is responsible for cardiac arrhythmia with sudden death in human. *Hum Mol Genet* 2012; **21**: 2759–2767.
35 Eltit JM, Feng W, Lopez JR *et al*: Ablation of skeletal muscle triadin impairs FKBP12/RyR1 channel interactions essential for maintaining resting cytoplasmic Ca2+. *J Biol Chem* 2010; **285**: 38453–38462.
36 Gorokhova S, Bibert S, Geering K, Heintz N: A novel family of transmembrane proteins interacting with beta subunits of the Na,K-ATPase. *Hum Mol Genet* 2007; **16**: 2394–2410.
37 Xu B, Roos JL, Levy S, van Rensburg EJ, Gogos JA, Karayiorgou M: Strong association of *de novo* copy number mutations with sporadic schizophrenia. *Nat Genet* 2008; **40**: 880–885.
38 Elia J, Gai X, Xie HM *et al*: Rare structural variants found in attention-deficit hyperactivity disorder are preferentially associated with neurodevelopmental genes. *Mol Psychiatry* 2010; **15**: 637–646.
39 Calboli FC, Tozzi F, Galwey NW *et al*: A genome-wide association study of neuroticism in a population-based sample. *PLoS One* 2010; **5**: e11504.
40 Leblond CS, Jutta H, Delorme R *et al*: Genetic and functional analyses of SHANK2 mutations suggest a multiple hit model of autism spectrum disorders. *PLoS Genet* 2012; **8**: e1002521.
41 Girirajan S, Rosenfeld JA, Cooper GM *et al*: A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. *Nat Genet* 2010; **42**: 203–209.
42 Veltman JA, Brunner HG: Understanding variable expressivity in microdeletion syndromes. *Nat Genet* 2010; **42**: 192–193.
43 Shiow LR, Paris K, Akana MC, Cyster JG, Sorensen RU, Puck JM: Severe combined immunodeficiency (SCID) and attention deficit hyperactivity disorder (ADHD) associated with a Coronin-1 A mutation and a chromosome 16p11.2 deletion. *Clin Immunol* 2009; **131**: 24–30.
44 Vorstman JA, van Daalen E, Jalali GR *et al*: A double hit implicates DIAPH3 as an autism risk gene. *Mol Psychiatry* 2011; **16**: 442–451.
45 O'Roak BJ, Deriziotis P, Lee C *et al*: Exome sequencing in sporadic autism spectrum disorders identifies severe *de novo* mutations. *Nat Genet* 2011; **43**: 585–589.

Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)

Do regulatory regions matter in *FOXG1* duplications?

European Journal of Human Genetics (2013) **21,** 365–366; doi:10.1038/ejhg.2012.142; published online 4 July 2012

Duplications of *FOXG1* gene at 14q12 have been reported in patients with infantile spasms and developmental delay of variable severity.[1,2,3] *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.[4] Recently, Amor *et al*.[5] 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*,[6] 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.[7,8] 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,[9] 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*[5].

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

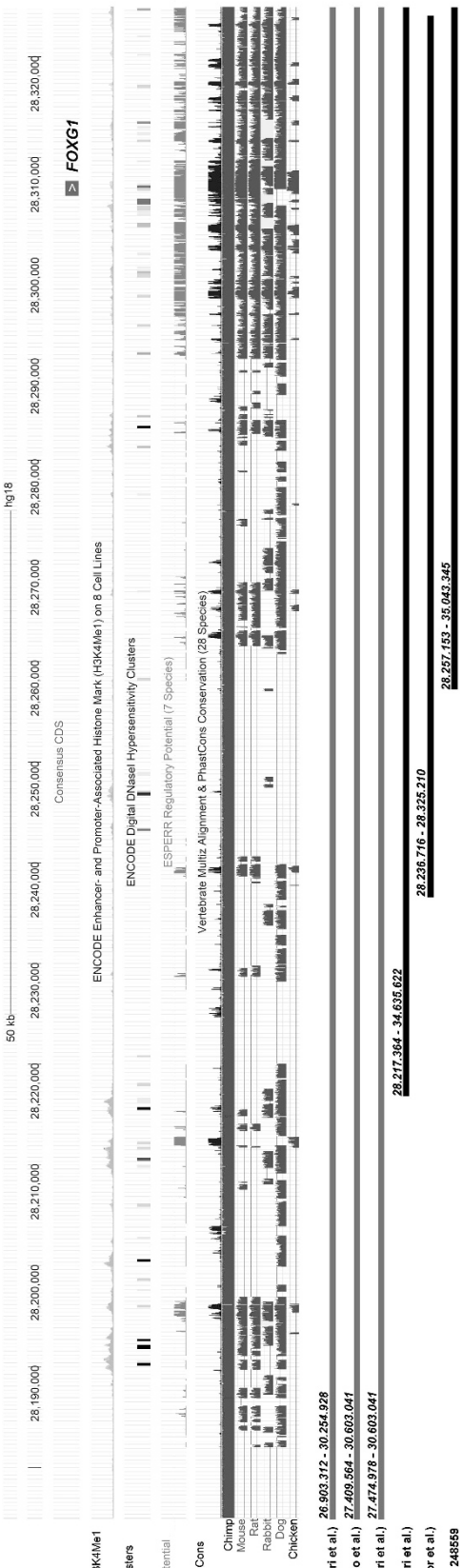

Figure 1 UCSC genome browser (NCBI36/hg18) schematic view of reported 14q12 duplications encompassing *FOXG1* gene. Histone modifications from the ENCODE project[7] in eight cells lines (GM12878, H1-hESC, HMEC, HSMM, HUVEC, K562, NHEK and NHLF) indicate the presence of regulatory elements upstream *FOXG1* 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,[10,11] pointing out the relevance of regulatory sequences in the expression of *FOXG1* gene.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Antonio Falace[1], Nicola Vanni[2], Antonello Mallamaci[3], Pasquale Striano[2] and Federico Zara[2]

[1]*Department of Experimental Medicine, Section of Physiology and National Institute of Neuroscience, University of Genova, Genova, Italy;*
[2]*Laboratory of Neurogenetics, Department of Neuroscience, Institute G. Gaslini, Genova, Italy;*
[3]*Laboratory of Cerebral Cortex Development, SISSA, Neurobiology Sector, Trieste, Italy*
E-mail: federicozara@ospedale-gaslini.ge.it

1 Yeung A, Bruno D, Scheffer IE *et al*: 4.45 Mb microduplication in chromosome band 14q12 including *FOXG1* in a girl with refractory epilepsy and intellectual impairment. *Eur J Med Genet* 2009; **52**: 440–442.
2 Striano P, Paravidino R, Sicca F *et al*: West syndrome associated with 14q12 duplications harboring *FOXG1*. *Neurology* 2011; **76**: 1600–1602.
3 Brunetti-Pierri N, Paciorkowski AR, Ciccone R *et al*: Duplications of *FOXG1* in 14q12 are associated with developmental epilepsy, mental retardation, and severe speech impairment. *Eur J Hum Genet* 2011; **19**: 102–107.
4 Brancaccio M, Pivetta C, Granzotto M *et al*: Emx2 and Foxg1 inhibit gliogenesis and promote neuronogenesis. *Stem Cells* 2010; **28**: 1206–1218.
5 Amor DJ, Burgess T, Tan TY *et al*: Questionable pathogenicity of *FOXG1* duplication. *Eur J Hum Genet* 2012; **20**: 595–596.
6 Brunetti-Pierri N, Cheung SW, Stankiewicz P: Reply to Amor *et al*. *Eur J Hum Genet* 2012; **20**: 597.
7 ENCODE Project Consortium. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* 2007; **447**: 799–816.
8 ENCODE Project Consortium. The ENCODE (ENCyclopedia Of DNA Elements) Project. *Science* 2004; **306**: 636–640.
9 Kolbe D, Taylor J, Elnitski L *et al*: Regulatory potential scores from genome-wide three-way alignments of human, mouse, and rat. *Genome Res* 2004; **14**: 700–707.
10 Shoichet SA, Kunde SA, Viertel P *et al*: Haploinsufficiency of novel *FOXG1B* variants in a patient with severe mental retardation, brain malformations and microcephaly. *Hum Genet* 2005; **117**: 536–544.
11 Kortüm F, Das S, Flindt M *et al*: The core *FOXG1* syndrome phenotype consists of postnatal microcephaly, severe mental retardation, absent language, dyskinesia, and corpus callosum hypogenesis. *J Med Genet* 2011; **48**: 396–406.