The hyperkinetic movement disorder of FOXG1-related epileptic–dyskinetic encephalopathy

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ON BEHALF OF THE FOXG1 SYNDROME STUDY GROUP*

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This article is commented on by Parker on page 15 of this issue.

AIM Forkhead Box G1 (FOXG1) syndrome is a developmental encephalopathy characterized by postnatal microcephaly, structural brain abnormalities, facial dysmorphisms, severe delay with absent language, defective social interactions, and epilepsy. Abnormal movements in FOXG1 syndrome have often been mentioned but not characterized.

METHOD We clinically assessed and analysed video recordings of eight patients with different mutations or copy number variations affecting the FOXG1 gene and describe the peculiar pattern of the associated movement disorder.

RESULTS The age of the patients in the study ranged from 2 to 17 years old (six females, two males). They had severe epilepsy and exhibited a complex motor disorder including various combinations of dyskinetic and hyperkinetic movements featuring dystonia, chorea, and athetosis. The onset of the movement disorder was apparent within the first year of life, reached its maximum expression within months, and then remained stable.

INTERPRETATION A hyperkinetic–dyskinetic movement disorder emerges as a distinctive feature of the FOXG1-related phenotype. FOXG1 syndrome is as an epileptic–dyskinetic encephalopathy whose clinical presentation bears similarities with ARX- and STXBP1-gene related encephalopathies.

Forkhead Box G1 (FOXG1), an evolutionarily conserved winged-helix transcriptional repressor, plays an important role in telencephalon development and is a crucial component of the transcription regulatory network that controls proliferation, differentiation, neurogenesis, and neurite outgrowth.

The core phenotype of the FOXG1 syndrome includes postnatal microcephaly, severe developmental delay with defective social interactions, and absence of language. Additional manifestations include behavioural disturbances, stereotypies, dyskinesia, and epilepsy. Agenesis of corpus callosum, simplified gyral pattern, and cortical thickening of the frontal lobes are commonly observed on brain magnetic resonance imaging. Characteristic facial dysmorphisms, including round face, flat midface and forehead, epicantal folds, depressed nasal bridge, upturned nose, and abnormally formed ears have also been described, most often in patients with large 14q12 deletions.

Abnormal movements in FOXG1 syndrome have been frequently mentioned, but a systematic description is lacking. We describe eight patients with different mutations or copy number variations affecting the FOXG1 gene and characterize the associated peculiar pattern of movement disorder.

CASE REPORT We obtained clinical data and brain magnetic resonance imaging findings from eight Italian patients (six females, two males) with FOXG1-related encephalopathy as diagnosed by clinical and genetic criteria. We also assessed all the patients with repeated video recordings in order to characterize their abnormal motor pattern. We followed them up with serial clinical evaluations in order to appreciate the movement disorder progression and any relevant clinical changes. We obtained blood or DNA samples from patients and their parents after written informed consent. Parents gave their consent to publish the results. The study was approved by the Institutional Review Board of the Tuscany Region, Italy.

Neurological examination revealed typical clinical and behavioural features of the FOXG1 syndrome (Table I).
including postnatal microcephaly (7/8 patients), facial dysmorphisms (8/8), axial hypotonia (6/8), absence of language (8/8), severe developmental delay (8/8), poor sleep pattern (6/8), hyperactivity (6/8), and crying or inappropriate laughing (5/8) (Table I). Brain magnetic resonance imaging revealed corpus callosum dysgenesis (6/8 patients) and a simplified gyral pattern in some patients (3/8) (Fig. 1). Most patients exhibited focal seizures (5/8), which had appeared from age 5 months to 6 years, often in association with epileptic spasms and requiring multiple antiepileptic medications. No significant period of remission of seizures was observed. Electroencephalography showed slow background, multifocal spikes, and sharp waves. Five patients exhibited a severe movement disorder (Patients 1, 2, 3, 5, and 7), manifested as continuous dyskinesia with a mixture of hyperkinetic, dystonic, choreic, and athetoid movements mainly involving the four limbs and face, and stereotypic movements that largely impaired normal hand use and fine motor skills. Dyskinesia of the mouth–tongue area was a prominent feature and was associated with hand-mouthing and sialorrhoea. There was, however, no evidence of dysphagia or swallowing difficulties. These patients had not reached independent walking and were overall severely disabled by the movement disorder. Two patients (Patients 6 and 8) exhibited only mild distal dyskinetic movements involving the arms and were able to walk with support. One patient (Patient 4), carrying a 14q12 duplication, had a distinctively less severe phenotype featuring mild hyperkinetic and perseverative stereotyped hand–mouth movements. Abnormal movements disappeared during sleep. The abnormal movements were first noticed between the fifth and twelfth month of life and had not changed in severity and semiology at last follow-up, between age 2 years and 17 years, as documented by video recordings (see Videos S1–S4, online supporting information).

Three patients were treated with antidyskinetic drugs (Patients 1, 5, and 7) (Table I). Limited benefit was obtained using pimozide in two patients (Patients 1 and 7).

Genetic analysis through multiplex ligation-dependent probe amplification (SALSA P075 version A1, MRC-Holland, Amsterdam, the Netherlands) revealed two de novo deletions of the FOXG1 gene in Patients 1 and 3. Array comparative genomic hybridization confirmed a 2.5 Mb deletion in Patient 1 (between nucleotides 27 154 000 and 29 743 000) and a 9.1 Mb deletion in Patient 3. This is the largest deletion reported so far at chromosome 14q12 (between nucleotides 25 168 212 and 34 247 857), which includes the FOXG1 and flanking genes. In Patient 2, array comparative genomic hybridization revealed a de novo 14q12 deletion of 2.8 Mb (minimal deleted interval 27 265 913–30 511 768). In Patient 4, multiplex ligation-dependent probe amplification identified a de novo duplication involving FOXG1, which was confirmed by array comparative genomic hybridization to be 7.3 Mb in length (minimal deleted interval 24 453 489–31 784 795) (Table I). Array comparative genomic hybridization was performed using the whole genome 180K Agilent platform (Agilent Technologies, Santa Clara, CA, USA). Physical positions correspond to the UCSC genome browser (genome assembly Feb 2009, hg19, http://genome.ucsc.edu).

Sanger sequencing detected four heterozygous FOXG1 intragenic point mutations, including a novel frameshift mutation c.298delC (p.Gln100Serfs*92) in Patient 5, a nonsense mutation c.136C>T (p.Q46X) in Patient 6, a recurrent frameshift mutation c.460dupG (p.Glu154Glyfs*301) in patient 7, and a frameshift mutation c.256delC (p.Gln86Argfs*106) in Patient 8 (FOXG1 Genbank Accession Number NM_005249).

All four point mutations were de novo and caused loss of all three downstream functional critical domains of the gene: the forkhead domain (FHD), which allows the binding to DNA, and the Groucho binding domain (GBD) and the JARID1B binding domain (JBD), which recruit transcriptional corepressor proteins. All mutations are predicted to cause haploinsufficiency.

**DISCUSSION**

Eighty-eight mutations involving the FOXG1 gene have been described in more than 90 patients and include 36 intragenic sequence changes (missense, nonsense, and frameshift), 27 deletions, 17 duplications, and 8 complex rearrangements.

Despite the relatively large number of identified mutations and reported patients, genotype–phenotype correlations are emerging slowly. Initial descriptions had ostensibly labelled FOXG1-related phenotypes as a ‘congenital variant’ of Rett syndrome. Only the subsequent identification of additional patients with FOXG1 mutations, having different ages and exhibiting sufficiently distinct clinical features, suggested the ‘FOXG1 syndrome’ to be a specific ‘developmental encephalopathy’. As is often the case with newly identified clinical entities, after the core phenotypic features have initially been characterized and a causative gene found, collection of a larger number of observations, including less typical cases, allows a proper characterization of the whole clinical spectrum.

In our series, all the patients exhibited the common clinical symptoms previously associated with FOXG1 syndrome. Almost all patients also exhibited a prominent movement disorder of variable severity. Five patients (1, 2, 3, 5, and 7) harbouring deletions or mutations exhibited a complex and more severe movement disorder initially noticed within first year of life and featuring hyperkinetic, dystonic, choreic, and athetoid movements, mainly involving the orobuccal area and extremities, with upper limb predominance severely interfering with voluntary movement (Video S1–S4). Two patients with truncating mutations exhibited only distal dyskinetic movements of the upper extremities (Patients 6 and 8), which in this series

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**What this paper adds**

- Assessment of movement disorder in patients with FOXG1 mutations or copy number variations.
- FOXG1 syndrome can be defined as an epileptic–dyskinetic encephalopathy.
### Table I: Clinical features and therapy of patients with *FOXG1* mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (y:mo)</th>
<th>FOXG1 mutation/CNV (Mb)</th>
<th>Possible effect of the FOXG1 mutation/CNV</th>
<th>Phenotype</th>
<th>Gene dosage effect</th>
<th>Epilepsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>9:0</td>
<td>Del (2.9Mb) on 14q12, involved genes: FOXG1, C14orf23, PKRD1 (de novo)</td>
<td>Haploinsufficiency</td>
<td>Microcephaly Postnatal (&lt; 4.5; 0; 3)</td>
<td>Loss of all the FOXG1 functional domains</td>
<td>Age at seizure onset (y:mo) 1:6</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>3:6</td>
<td>Del (2.8Mb) on 14q12, involved genes: FOXG1, C14orf23, PKRD1, SCFD1, G2E3, SCFD1, COCH, STRN3 (de novo)</td>
<td>Haploinsufficiency</td>
<td>Intellectual disability Postnatal (&lt; 4.5; 0; 3)</td>
<td>Loss of all the FOXG1 functional domains</td>
<td>Tonic, spasms</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>2:0</td>
<td>Del (9.1Mb) on 14q12, involved genes: STXBp6, NOVA1, FOXG1, C14orf23, PKRD1, SCFD1, G2E3, COCH, STRN3, AP451, HECTD1, DTD2, NUBPL, ARHGAP5, AKAP, NUBP3 (de novo)</td>
<td>Haploinsufficiency</td>
<td>Language development Postnatal (&lt; 4.5; 0; 3)</td>
<td>Loss of all the FOXG1 functional domains</td>
<td>Slow background activity, multifocal spikes</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>10:0</td>
<td>Dup (1.3Mb) on 14q12, involving FOXG1 and additional 50 genes (de novo)</td>
<td>Haploinsufficiency</td>
<td>Sleep disturbances Postnatal (&lt; 4.5; 0; 3)</td>
<td>Loss of all the FOXG1 functional domains</td>
<td>Spasms</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>10:0</td>
<td>c.298delC; p.Gln100Serfs*92 (de novo)</td>
<td>Haploinsufficiency</td>
<td>Epilepsy Postnatal (&lt; 4.5; 0; 3)</td>
<td>Loss of all the FOXG1 functional domains</td>
<td>Focal, spasms</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>17:0</td>
<td>c.186C&gt;T, p.046X (de novo)</td>
<td>Haploinsufficiency</td>
<td>Interictal EEG Postnatal (&lt; 4.5; 0; 3)</td>
<td>Loss of all the FOXG1 functional domains</td>
<td>Slow background activity, multifocal spikes</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>2:6</td>
<td>c.460dupG (de novo)</td>
<td>Haploinsufficiency</td>
<td>Seizure types Postnatal (&lt; 4.5; 0; 3)</td>
<td>Loss of all the FOXG1 functional domains</td>
<td>Focal</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>13:0</td>
<td>c.256delC (de novo)</td>
<td>Haploinsufficiency</td>
<td>Case Report Postnatal (&lt; 4.5; 0; 3)</td>
<td>Loss of all the FOXG1 functional domains</td>
<td>Tonic, spasms</td>
</tr>
</tbody>
</table>

Possible effect and Phenotype:
- **Microcephaly**: Postnatal (< 4.5; 0; 3)
- **Developmental delay**: +
- **Intellectual disability**: +
- **Language development**: –
- **Walking**: –
- **Hypotonia**: +
- **Behaviour**:
  - **Social Interactions**: –
  - **Sleep disturbances**: +
  - **Irritability**: +
  - **Hyperactivity**: +
  - **Crying**: +
  - **Laughing**: +

Facial dysmorphisms:
- **Flat forehead and midface, bilateral epicanthal folds, thin upper lip, large abnormally formed ears**
- **Midface hypoplasia, thin upper lip, bulbous nasal tip, anteverted nares**
- **Flat forehead, long eyelashes, tented upper lip, bulbous nasal tip**
- **Round face, exotropia, small nose, simple shaped ears**
- **Flat forehead and midface, slight upslanting palpebral fissures, bulbous nasal tip and anteverted nares, prognathism, diastasis of teeth, thick and everted lower lip**
- **Flat forehead, bulbous nasal tip and anteverted nares, everted lower lip**
- **Flat forehead, thick eyebrows with mild synophrys, long eyelashes, tented upper lip, prominent incisors**
represents the mild end of the movement disorder spectrum. No significant worsening or attenuation of the abnormal movements was apparent in any of the patients over time.

Assessment of our patients and the review of previous reports in which the movement disorder was mentioned, suggest its presence in most cases, with a variable combination of dyskinetic and hyperkinetic movements and, with few exceptions, an age at onset within the first years of life.

We could not find a genotype–phenotype correlation between the type of genetic defect affecting FOXG1 and the severity of the movement disorder since all the truncating mutations and deletions we observed (Patients 1–3, 5–8) were predicted to cause haploinsufficiency. The duplica-
tion at 14q12 in Patient 4 was associated with hand–mouth stereotypies and less prominent hyperkinetic movements. Clinical features in patients with 14q12 duplications have been consistently reported to differ from those observed in association with deletions and inactivating mutations, suggesting that the phenotype is markedly influenced by FOXG1 gene dosage.

Overall, the severe movement disorder observed in most patients in this series is also remarkably different from that described in Rett syndrome, in which hyperkinetic choreoathetoid movements are unusual and not as prominent.11

Unfortunately, no effective antidyskinetic treatment for these patients has been reported to date and our experience does not allow us to draw any conclusion with respect to treatment issues. However, we noticed that among the three patients who performed antidyskinetic therapy (Table I), the two who were treated with pimozide, exhibited some improvement (Patients 1 and 7). This preliminary observation might encourage further therapeutic trials against the hyperkinetic–dyskinetic movements. We did not observe any (beneficial or worsening) effect of the different antiepileptic medications on the movement disorder either.

The FOXG1-related phenotype can be classified as an epileptic–dyskinetic encephalopathy as are ARX-12–14 and STXBP1-related15 encephalopathies. We recommend the consideration of FOXG1 mutational analysis in the diagnostic assessment of patients with developmental delay, epilepsy, and prominent dyskinetic movements.

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SUPPORTING INFORMATION
The following additional material may be found online:

Video S1: Patient 1: Severe hyperkinetic–dyskinetic movement disorder affecting all four limbs, with prominent hand–mouth–tongue movements. This patient is severely affected, has a very poor control of her posture, and is non-ambulatory.

Video S2: Patient 5: Hyperkinetic–dyskinetic movements affecting the upper limbs. Finalized hand movements are impaired. This patient is severely affected, has a very poor control of her posture, and is non-ambulatory.

Video S3: Patient 7: Severe generalized hyperkinetic–dyskinetic movement disorder affecting the four limbs and the face, markedly impairing fine motor skills. The male’s motor development is severely delayed.

Video S4: Patient 8: Mild dyskinetic movements involving the upper extremities. There are no dyskinetic movements in the lower extremities. This female is able to walk with support.

REFERENCES