

Short Report

Refining the phenotype associated with *MEF2C* haploinsufficiency

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Recently, submicroscopic deletions of the 5q14.3 region have been described in patients with severe mental retardation (MR), stereotypic movements, epilepsy and cerebral malformations. Further delineation of a critical region of overlap in these patients pointed to *MEF2C* as the responsible gene. This finding was further reinforced by the identification of a nonsense mutation in a patient with a similar phenotype. In brain, *MEF2C* is essential for early neurogenesis, neuronal migration and differentiation. Here we present two additional patients with severe MR, autism spectrum disorder and epilepsy, carrying a very small deletion encompassing the *MEF2C* gene. This finding strengthens the role of this gene in severe MR, and enables further delineation of the clinical phenotype.

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Recently, submicroscopic deletions of the 5q14.3 region have been described in patients featuring severe mental retardation (MR), stereotypic movements, epilepsy and cerebral malformations (1–3). Further delineation of a critical region of overlap in these patients pointed to the *MEF2C* gene as the responsible gene. This finding was further reinforced by the identification of a nonsense mutation in a patient with a similar phenotype. *MEF2C*, encoding transcription factor myocyte enhancer factor 2C, plays a crucial role during several embryological processes, including hematopoiesis, cardiogenesis and neurogenesis. In brain, members of the MEF2 family of MADS (MCM1, agamous, deficiens, serum response factor) box transcription

factors are expressed in overlapping but distinct regions of the central nervous system (CNS) that correlate with the withdrawal of neurons from the cell cycle and acquisition of a differentiated phenotype (4). In mouse, *Mef2c* is the first of four *Mef2* genes to be expressed in the CNS. In the adult brain, *Mef2c* is highly expressed in the frontal cortex, entorhinal cortex, dentate gyrus, and amygdala. Recently it was shown that the deletion of *Mef2c* transcription factor in the CNS of mice impairs hippocampal-dependent learning and memory by negative regulation of synapse numbers and function (5, 6).

Here we present two additional patients with severe MR, autism spectrum disorder and epilepsy,

carrying a very small deletion encompassing the *MEF2C* gene. This finding strengthens the role of this gene in severe MR, and enables further delineation of the clinical phenotype.

Methods

The protocol was approved by the appropriate Institutional Review Boards involved in the research (Universities of Leuven, Belgium and Pavia, Italy). Informed consent was obtained from the parents of the affected patients.

Cytogenetic analysis

Routine G-band karyotyping was performed according to routine protocol. Arrays were performed using the Agilent array 105 K according to the manufacturer's protocol.

Genotyping

Genotyping of polymorphic loci in patient 2 and his parents was performed by amplification with primers labeled with fluorescent probes (ABI 6-Fam and 8-Hex), followed by analysis on ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Primers were designed using the database tool Tandem Repeats Finder (<http://tandem.bu.edu/trf/trf.intermediate.submit.html>).

Real-time quantitative PCR

Specific target sequences were selected for Real-time quantitative PCR (qPCR) using Primer Express software (Applied Biosystems). A control amplicon was selected with the same parameters in the *MAPK1* gene on 22q11.2; size (approximately 60 bp) and T_m (59°C) were the same for all amplicons. Amplification and detection were performed on an ABI PRISM 7700 Sequence Detection System (Applied Biosystems) using SYBR Green PCR Master Mix (Applied Biosystems); thermal cycling conditions were 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min; all samples were amplified in duplicate. Relative quantification of the amount of DNA was obtained using the comparative CT method (described in Applied Biosystems User Bulletin #2, December 11, 1997: ABI PRISM 7700 Sequence Detection System).

Long-range PCR

Long-range PCRs were performed with Jump-Start Red ACCUTaq LA DNA polymerase (Sigma,

Milan, Italy) with the following protocol: 30 s at 96°C, 35 cycles of 15 s at 94°C/20 s at 58°C/10 min at 68°C, 10 min final elongation time. Primers were: for Patient 1, Del5-11F (5-CATCATTGCC CACATCAC-3) and Del5-13R (5-TGAAGGAG AGCTGGCTGTGA-3); for Patient 2, Del5-10F (5-TGTGGCTGAGCTGCTTCTAACA-3) and Del 5-11R (5-TTCCTGCCCTACCTTCATGTG-3). All sequencing reactions were performed with a Big Dye Terminator Cycle Sequencing kit 3.1 (Applied Biosystems) and run on an ABI Prism 3130xl Genetic Analyzer.

The UCSC Genome Browser (May 2004 assembly; <http://genome.ucsc.edu/>) maps and sequence were used as references. The primer sequences are available on request.

Patients

Patient 1 is the first child of healthy non-related parents, born at term with normal birth parameters after an uneventful pregnancy. Two younger siblings are normal. A brother and a cousin of the mother have a benign form of epilepsy, well responding to therapy and not interfering with daily functioning or cognition. The boy came to medical attention at the age of 3 months because of absent eye contact and social smile, hypotonia and irritable behavior. MRI scan at that age showed a cystic lesion and leucoencephalopathy in the left frontal region, probably due to a perinatal hemorrhage. Metabolic investigations including coagulation were all normal. Subsequently, his psychomotor development was severely delayed with sitting with little support at the age of 2 years. An MRI scan of the brain at that age showed signs of periventricular leucomalacia and atrophy of the frontal cortex at the left side. He never reached independent ambulation because of persistent severe axial hypotonia. Epilepsy started in the first year of life, initially as isolated myoclonic jerks, later evolving toward infantile spasms with continuous epileptic activity bi-posterior with no basic rhythm on EEG. Because of further deterioration of the drug-resistant epilepsy, he was admitted at a special epilepsy unit for intensive care at the age of 2 years. After this period there was a regression of his motor functions, necessitating a wheel chair and orthopedic orthoses. Speech was absent. Now at the age of 14 years, his growth parameters are within the normal range (OFC at 50th centile). He has a cerebral palsy with severe axial hypotonia and compensatory peripheral hypertonia. His eye contact is very poor with external strabismus of the right eye. He does not exhibit stereotypic movements. He has only mild dysmorphisms with

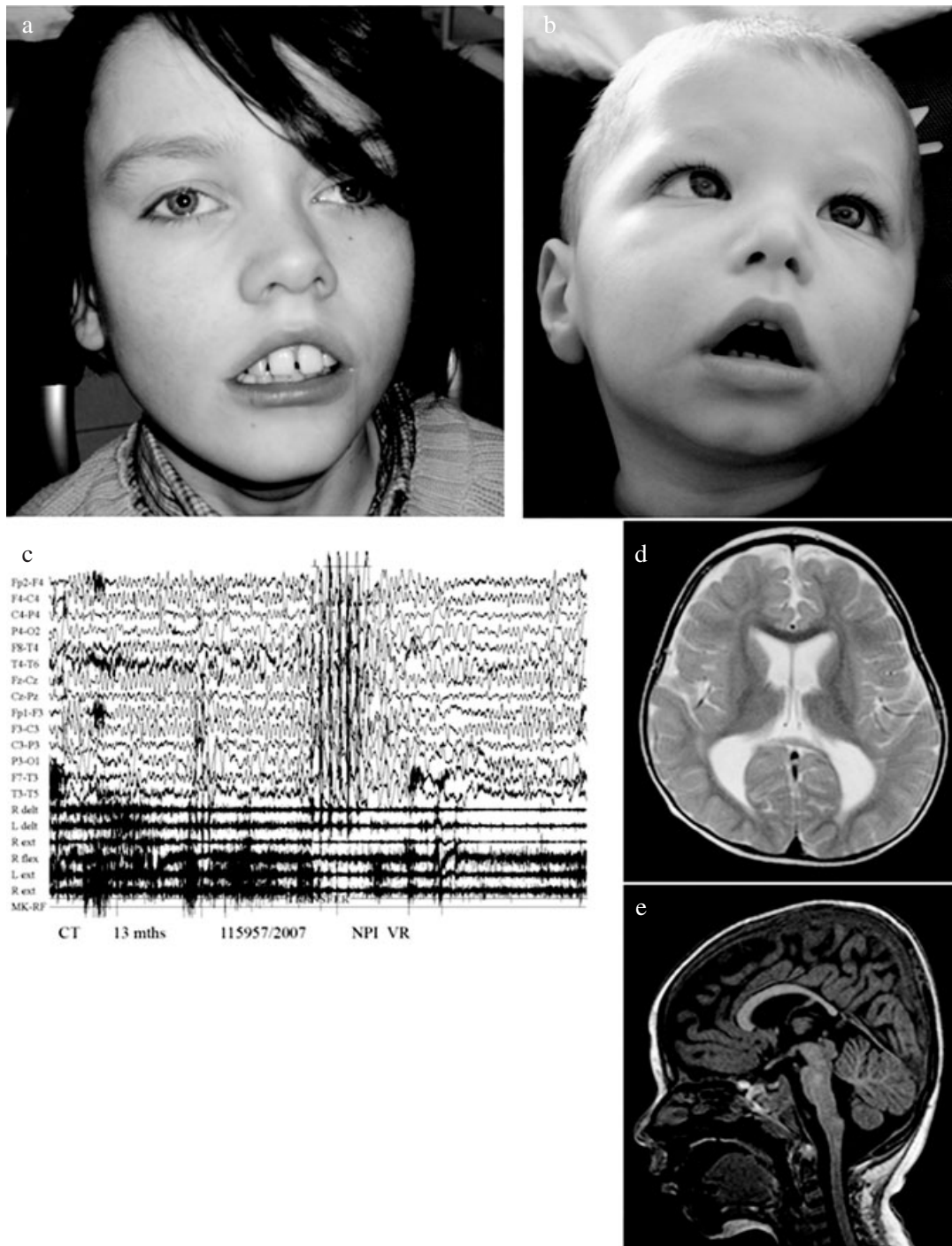


Fig. 1. Clinical picture of patient 1 at the age of 14 years (a) and patient 2 at the age of 3 years (b). Note the facial hypotonia, strabismus, prominent philtrum with cupid's bow in both boys. Patient 1 has in addition macrodontia. (c) EEG record of patient 2 at the age of 13 months showing slow background activity with theta waves degraded over the central regions of the two hemispheres and degraded diffuse discharges, together with rhythmic sharp wave activity; EEG velocity: 15 mm/s; amplitude: 50 μ V. (d,e) MRI images of patient 2: axial section showing dilatation of the lateral ventricles (d) and sagittal section showing hypoplasia of the distal part of the corpus callosum (e).

prominent ear lobes, short prominent philtrum with a cupid's bow and macrodontia (Fig. 1a).

Patient 2 was born at term with birth weight of 3600 g and length of 51 cm (both 75th centile).

His mother suffered from hypothyroidism and was treated with levothyroxin during pregnancy. At 4 months of age, lack of reactivity was observed in addition to severe hypotonia, dystonic motor

activity, absent head control and poor visual tracking. At the age of 5 months, psychomotor delay and myoclonus were observed. EEG showed slow background activity with theta waves degraded over the central regions of the two hemispheres and degraded diffuse discharges, sometimes with episodes of rhythmic sharp wave activity, associated with revulsion of eyes and myoclonus of the limbs (Fig. 1c). Clinical evaluation showed occipital plagiocephaly, hypertelorism, flattened nasal bridge, small and hooked nose, ogival palate, and low-set and dysmorphic ears (Fig. 1b). Marked myopia with alternating esotropia was also observed. Several MRI scans of the brain were performed at 5, 8 and 19 months of age, respectively; they showed moderate dilatation of lateral ventricles and hypoplasia of the corpus callosum with abnormal aspect of the splenius (Fig. 1d,e). The development quotient was calculated using the Griffith mental development scales at 13 and 19 months of age (35 and 30, respectively). At the last evaluation at 3 years and 10 months of age, he presented a severe cognitive deficit with numerous behavioral stereotypes. Head circumference was at the 50th centile. Neurological examination showed severe axial hypotonia with partial control of the head. In the lower limbs an increased muscular tonus was noticed with dystonic–dyskinetic movements. He was able to fix and follow objects and wore glasses for myopia. Language was absent. He still suffered from myoclonic and myoclonic–atonic seizures with falling of the head, not responding to any anti-epileptic therapy (Valproic Acid, Nitrazepam, Levetiracetam, Hydrocortison, Clobazam, and Ethosuximide).

Results

In the absence of an etiological diagnosis in both patients, array CGH analysis using the 105 K array (Agilent) was performed. In both patients, a small deletion in 5q14.3 was detected (Fig. 2). All breakpoint locations were refined by qPCR, and then both junctions were amplified by long-range PCR and sequenced. Patient 1 has a deletion of 318357 bp (87,978,527–88,296,884) harboring only *MEF2C*, while patient 2 has a slightly larger deletion of 1140131 bp (87,234,127–88,374,258). Besides *MEF2C*, the latter deletion also includes the *TMEM161B* gene. The mother of patient 1 does not carry the deletion, while his father could not be tested. In patient 2 the deletion occurred *de novo*. Breakpoint junctions are outside low copy repeats and have no particular identity. They are consistent with microhomology-mediated (patient 1) or non-homologous end-joining (NHEJ)

(patient 2) mechanisms. (Table S1, Supporting information). Parental origin was not investigated in patient 1. In patient 2, the chromosomal imbalance originated at the paternal meiosis. Three out of four microsatellites analyzed in the deleted region showed the presence of the maternal allele only (data not shown).

Discussion

Recently, *MEF2C* haploinsufficiency has been described in five patients presenting a severe and syndromic form of MR and carrying a submicroscopic deletion on chromosome 5q14.3 of variable size ranging from 216 kb to 8.8 Mb (1). Moreover, the same authors detected a nonsense mutation in *MEF2C* in an additional patient exhibiting a similar phenotype and thus underscoring the clinical relevance of this new autosomal dominant MR gene. *MEF2C* encodes myocyte enhancer factor 2 that functions as a transcription factor, with MEF2C as the predominant isoform in the brain. The role of MEF2C during brain development and functioning, including neurogenesis, neuronal migration and synaptic plasticity, has been well established in murine models and *in vitro* functional studies (4–7). Its role in learning and memory as well as maintaining the critical balance between inhibitory and excitatory synapses is consistent with the human haploinsufficiency phenotype we and others have observed. Both patients we describe here carry a very small deletion, involving only the *MEF2C* in patient 1. The deletion in patient 2 also affects the *TMEM161B* gene, a gene of unknown function that is predicted to encode a transmembrane protein. We can not exclude an involvement of haploinsufficiency of this gene in the clinical phenotype of patient 2.

When we compare the clinical phenotype in our patients with the reported patients, the phenotype remains very consistent (Table 1). All patients present with severe primary developmental delay reflected by early hypotonia, delayed motor development and no speech development. Thus far, our patients and previously reported patients who are hemizygous for the gene did not reach independent walking and remain very hypotonic, while the patient with a reported point mutation started to walk at 3 years of age without hypotonia (1) (Table 1). This might point toward a more severe effect of the deletion compared to the intragenic mutation, but more cases are needed to make any genotype–phenotype correlation. Stereotypic movements and poor eye contact are present in many patients, suggesting the diagnosis of autism spectrum disorder (ASD). Interestingly, a role for

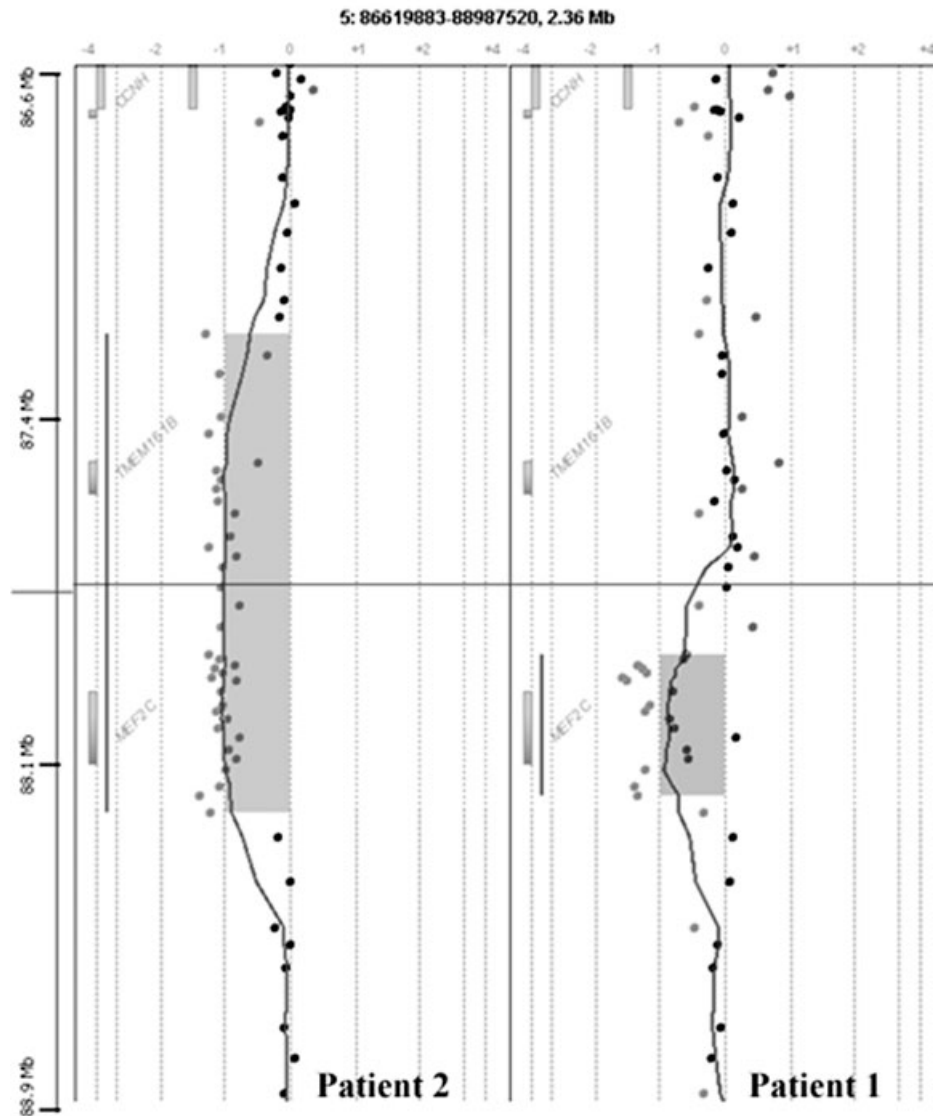


Fig. 2. Detailed view of the chromosome 5q14.3 aCGH profile showing the deletion in patient 1 (right) and patient 2 (left).

MEF2C in ASD was already shown in classical and conditional knock-out mouse models (4, 8). Moreover, Morrow et al. identified many *MEF2* target genes in their screen for autism genes by means of homozygosity mapping in pedigrees with shared ancestry (9). The recent findings in humans further reinforce the role of *MEF2C* during neurogenesis and synaptogenesis. Epilepsy is another frequent feature of *MEF2C* haploinsufficiency, although the type (myoclonic, tonic-clonic, infantile spasms and febrile seizures) and age at onset may vary considerably. In both patients the severe drug-resistant infantile spasms even necessitated admission at an epilepsy care center. Patient 1 has two male relatives with a benign form of epilepsy, not affecting cognition or daily functioning. As the deletion is *de novo*, and the phenotype in patient 1

is very distinct in severity, we believe that another genetic trait is responsible for their epilepsy.

In most patients brain imaging is reported to be abnormal, including anomalies of the corpus callosum, enlarged ventricles, periventricular white matter hyperintensities and cortical atrophy (Table 1). None of these anomalies seems to be specific and some might be secondary to the severe epileptic activity. Interestingly, Cardoso et al. reported periventricular heterotopia in a patient with a larger deletion including *MEF2C* (2). For the moment it is unclear whether *MEF2C* haploinsufficiency is responsible for this variable spectrum of features or whether other genes within larger deletions exert an additional effect. The same holds true for the facial dysmorphisms that seem to be more pronounced in the patients with larger

Table 1. Clinical features of 10 patients with 5q14.3 deletions involving MEF2C and 1 patient with MEF2C mutation (reported in Refs 1–3)

Patient number Nature of haploinsufficiency	This paper							Le Meur et al.			Engels et al.			Cardoso et al.
	Patient 1 del	Patient 2 del	Case 1 del	Case 2 del	Case 3 del	Case 4 del	Case 5 del	Case 7 c.683C>G	Patient 1 del	Patient 2 del	Patient 2 del	Patient 2 del	Patient 2 del	
Size deletion	318 kb	1.1 Mb	2.68 Mb	3.5 Mb	8.8 Mb	1.57 Mb	216 kb	–	5.69 Mb	3.93 Mb	3.93 Mb	8.4 Mb	8.4 Mb	
Age at last examination	14 y	3 y, 10 m	4 y, 9 m	9 m	18 m	3 y	8 y	7 y	6 y	7 y	7 y	5 y	5 y	
Hypotonia	+	+	+	+	+	+	+	–	+	+	+	+	+	
Independent walking (age)	–	–	–	–	–	–	–	– (3 y)	–	–	–	–	NA	
Absent speech	+	+	+	+	+	+	+	+	+	+	+	+	+	
Poor eye contact	+	+	+	+	+	+	+	+	+	+	+	+	+	
Stereotypic movements	–	+	+	–	–	+	+	+	NA	+	+	+	+	
Epilepsy type/ onset	Myoclonic + Infantile spasms/1 y	Myoclonic/5 m	Myoclonic/4 m	Tonicoclonic/birth	–	–	Febrile seizures/3 y	Tonicoclonic/9 m	Infantile spasms/birth	Febrile seizures	Febrile seizures	Infantile spasms/9 m	Infantile spasms/9 m	
Dysmorphic features	+	+	+	+	+	+	+	–	–	+	+	+	+	
Microcephaly (<2 SD)	–	–	–	+	–	–	–	–	–	–	–	–	–	
Brain imaging	–	–	–	–	–	–	–	–	–	–	–	–	–	
Abnormal corpus callosum	–	+	–	+	+	–	+	–	+	–	–	–	–	
Enlarged ventricles	–	+	–	+	+	+	–	+	+	–	–	–	–	
Other	Periventricular leucomalacia Atrophy frontal cortex	Myopia	Fronto-parietal atrophy	Reduced cortical gyration edema hands and feet	Vertical tent of cerebellum	Left 2–3 toe syndactyly Agnesis toes 2–5	Episodic hyperventilation	Periventricular hypertense white matter Feeding difficulties	Aplasia cerebellar vermis Plexus cysts	Prominent arachnoidal spaces Feeding difficulties	Bilateral periventricular heterotopia Bilateral pes talus	Bilateral periventricular heterotopia Bilateral pes talus	Bilateral periventricular heterotopia Bilateral pes talus	

m, months; y, years; del, deletion; NA, not assessed.

deletions (1). We did not observe any effect of *MEF2C* hemizyosity on growth and head circumference (Table 1).

In summary, we present two new patients with severe MR, epilepsy and ASD associated with deletion of *MEF2C*.

Supporting Information

The following Supporting information is available for this article: Table S1. Cloning of the deletion breakpoints in patients 1 and 2. Additional Supporting information may be found in the online version of this article.

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Conflict of interest

The authors do not have any affiliation with any group with a direct financial interest in the subject matter or materials discussed in the manuscript.

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