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ORIGINAL ARTICLE

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Mutations and novel polymorphisms in coding regions and UTRs of *CDK5R1* and *OMG* genes in patients with non-syndromic mental retardation

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Abstract Mental retardation (MR) is displayed by 57% of NF1 patients with microdeletion syndrome as a result of 17q11.2 region haploinsufficiency. We considered the cyclin-dependent kinase 5 regulatory subunit 1 (*CDK5R1*) and oligodendrocyte-myelin glycoprotein (*OMG*) genes, mapping in the NF1 microdeleted region, as candidate genes for MR susceptibility. *CDK5R1* encodes for a neurone-specific activator of cyclin-dependent kinase 5 (CDK5) involved in neuronal migration during central nervous system development. *OMG* encodes for an inhibitor of neurite outgrowth by the binding to the Nogo-66 receptor (RTN4R). *CDK5R1* and *OMG* genes are characterized by large 3′ and 5′ untranslated regions (UTRs), where we predict the presence of several transcription/translation regulatory elements. We screened 100 unrelated Italian patients affected by unspecific MR for mutations in *CDK5R1* and *OMG* coding regions and in their 3′ or 5′ UTRs. Four novel mutations and two novel polymorphisms for *CDK5R1* and three novel mutations for *OMG* were detected, including two missense changes (c.323C>T; A108V in *CDK5R1* and c.1222A>G; T408A in *OMG*), one synonymous codon variant (c.532C>T; L178L in *CDK5R1*), four variants in *CDK5R1* 3′UTR and two changes in *OMG* 5′UTR. All the mutations were absent in 370 chromosomes from normal subjects. The allelic frequencies of the two novel polymorphisms in *CDK5R1* 3′UTR were established in both 185 normal and 100 mentally retarded subjects. Prediction of mRNA and protein secondary structures revealed that two changes lead to putative structural alterations in the mutated c.2254C>G *CDK5R1* 3′UTR and in *OMG* T408A gene product.

Keywords Non-syndromic mental retardation · 17q11.2 · *CDK5R1* and *OMG* · NF1 microdeletion syndrome · UTR regulatory elements

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Introduction

Mental retardation (MR) is a condition affecting 2–3% of the general population [1, 2]. Non-syndromic MR, without other clinical manifestations and with apparently normal brain development, is the most common cognitive dysfunction, and its molecular basis is still poorly understood. Most of the known genes found to be involved in non-syndromic MR map to the X chromosome, and only recently, a mutation in the neuronal serine protease neurotrypsin gene (*PRSS12*) and a mutation in the cereblon gene (*CRBN*) have been associated with autosomal recessive non-syndromic MR [1, 2]. These genes, mapped to chromosomes 4 and 3, respectively, result the only autosomal genes so far demonstrated to be involved in MR.

Due to the complexity of the central nervous system and to the fact that the pathogenesis of MR can be only partially explained by the known genes, additional genes are expected to have a role in MR.

We have recently reported evidence that MR is significantly more frequent in NF1 patients with microdeletion syndrome than in those with classic NF1 (57 vs 4–8%) [3]. This evidence suggests that hemizygosity of one or more genes within the deleted region might be involved in

the onset of MR and points to 17q11.2 as a further autosomal candidate region for MR.

Among the genes within the 17q11.2 deleted region, which are known to be involved in the development or in the correct function of central nervous system, we selected the cyclin-dependent kinase 5 regulatory subunit 1 (*CDK5R1*) and oligodendrocyte-myelin glycoprotein (*OMG*) genes as potential candidates for MR.

Cyclin-dependent kinase 5 regulatory subunit 1 (*CDK5R1*) encodes for p35, a neurone-specific activator of cyclin-dependent kinase 5 (CDK5), whose activity plays a central role in neuronal migration during central nervous system development [4]. Cdk5r KO mice have severe cortical lamination defects and suffer from adult mortality and seizures [5–7]. The active CDK5–p35 complex is involved in several processes required for central nervous system development and function such as axonal regeneration [8], cellular differentiation, neuronal apoptosis [9], learning and memory processes [10], synaptic transmission [11] and membrane trafficking during the outgrowth of neuronal processes [12]. Moreover, increased CDK5 activation by p25, a proteolytic fragment containing the C-terminal portion of p35, has been implicated in the pathogenesis of several neurodegenerative disorders, such as Alzheimer's disease [13], Parkinson's disease [14] and amyotrophic lateral sclerosis [15].

In addition, the *CDK5R1* gene displays a large 3′ untranslated region (UTR). Extended UTR length provides significant potential for transcript-specific regulation originating in this region [16]. UTR sequences are known to play crucial roles in the post-transcriptional regulation of gene expression, including modulation of mRNA transport out of the nucleus, and of translational efficiency [17], subcellular localization [18] and stability [19]. Moreover, several regulatory elements have already been identified in 3′UTR and are considered as hot spots for pathology [20, 21].

OMG encodes for a protein that has been shown to be a potent inhibitor of neurite outgrowth by the binding to the Nogo-66 receptor (RTN4R), a protein associated with myelin [22], recently suggested as one of the genetic causes predisposing to schizophrenia [23]. The *OMG* gene was found to be highly expressed during the late stages of myelination in the rat central nervous system, indicating that it could be important for a proper myelination process [24]. The *OMG* 5′UTR is very large, raising the possibility, as for *CDK5R1*, that this region could bear UTR regulatory elements or, alternatively, upstream AUG codons (uAUG) and upstream open reading frame (uORF), which have been recently shown to act in the control of the translation process [25].

We performed mutation analysis of *CDK5R1* and *OMG* genes in 100 patients with non-syndromic MR to verify the presence of rare alleles with a possible functional significance in MR condition. Following the prediction of several regulatory elements in the *CDK5R1* and *OMG* UTRs, we carried out the mutation study in both the coding regions and the predicted UTR regulatory elements. Here, we report four novel mutations and two novel polymorphisms for *CDK5R1* and three novel mutations for *OMG*.

Materials and methods

Subjects

The patient panel used for the mutation screening consisted of 100 subjects with unexplained MR, as the most common genetic causes of MR (chromosome rearrangements, abnormalities in blood and urine amino acids, urine organic acids, Fragile X, Angelman and Rett syndromes) were previously excluded.

The group of patients analysed was made up of 42 males and 58 females: 30 were familial cases, whereas 70 were apparently sporadic cases.

With few exceptions, participants were younger than 18 years (2–53 years), most of them between 5 and 10 and all were Caucasian.

Genomic DNA samples were provided by the Laboratory of Medical Cytogenetics and Molecular Genetics of the Istituto Auxologico Italiano (Milan), to which the patients were referred by internal or external clinicians in the period from 1995 to 2004.

We used the definition of MR provided by the American Association on Mental Retardation [26]; severity was categorized according to the World Health Organization classification (1968) and the Diagnostic and Statistical Manual (4th rev.) (DSM-IV) [27]. Children with severe ($35 < IQ < 20$) and profound ($IQ \leq 20$) MR were grouped together in a single category.

Formal IQ values were accessible and previously obtained by administering one of the Wechsler scales or the Leiter International Performance Scale in non-verbal subjects or the Griffiths Developmental Scales in younger children.

One third of patients were previously referred for Angelman syndrome genetic screening due to absence of speech, severe to profound MR ($IQ < 35$) and seizures or abnormal EEG. As for the remaining two thirds of patients, severity of MR ranged from mild ($70 < IQ < 55$) to moderate ($54 < IQ < 36$), and they were referred for Fragile X molecular testing. All the patients, regardless of their cognitive impairment, had a history of speech and language delay. Most of them also had a history of psychomotor delay and displayed short attention span and behavioural abnormalities suggesting, or fitting, the autism spectrum of disorders.

Fragile X testing was subsequently carried out on the whole patient panel, regardless of the reason for sample referral. Mecp2 was further investigated in the six females referred for Angelman syndrome methylation testing [28], and all of them had a history of psychomotor delay and severe language disabilities. The above two tests resulted negative in all these patients.

Subtelomeric rearrangements were ruled out in subjects with family history for MR and in those with dysmorphic

facial features. A few patients displayed, beside MR, additional isolated dysmorphic facial features, which however did not fit a specific syndromic pattern.

All the control subjects were Caucasian.

Mutation analysis

CDK5R1 coding sequence (consisting of a single exon) was amplified in two overlapping PCR fragments termed CDK5R1-CDS.1 and CDK5R1-CDS.2. *OMG* coding sequence (a single exon also) was amplified in three overlapping PCR fragments termed OMG-CDS.1, OMG-CDS.2 and OMG-CDS.3. Three additional fragments were designed to cover the AU-rich and GY-box elements in the 3′UTR of *CDK5R1* (named CDK5R1-3′UTR.1 and CDK5R1-3′UTR.2) and exon 1 in the 5′UTR of *OMG* (named OMG-5′UTR) (Table 1). Primers used in this study were designed using the Oligo 4.0 software and are listed in Table 1.

PCR was carried out in 50 μl reaction volumes containing 100 ng of genomic DNA, 0.2 μM primers, 100 μM dNTPs, 5 μl reaction buffer, 50 mM $MgCl_2$ and 2.5 U BIOTAQ DNA Polymerase (Bioline) with the following cycling profile: 4 min initial denaturation at 95°C, 35 cycles as follows: denaturation at 95°C for 30 s, specific annealing temperature for 30 s (Table 1), extension at 72°C for 30 s. The specificity of the amplified PCR products was checked by 1.5% agarose gel electrophoresis.

PCR products of both patients' and controls' DNA were directly sequenced in both directions using the Big Dye Terminator kit (Applied Biosystem) and resolved on a 3100 ABI Prism Genetic Analyzer (Applied Biosystem, Foster City, CA). All the variations detected were confirmed on a second PCR product.

In silico analysis

Reference sequences of the *CDK5R1* and *OMG* genes were retrieved from the NCBI Refseq Project (http://www.ncbi.nlm.nih.gov/RefSeq/).

Functional regulatory elements in *CDK5R1* and *OMG* UTRs were searched by means of UTRScan, a program that looks for UTR functional elements by searching for the patterns defined in the UTRsite collection (http://bighost.area.ba.cnr.it/BIG/UTRHome/).

Multiple alignment of protein sequences was performed with Clustal X, a windows interface for the ClustalW multiple sequence alignment program (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/).

For protein secondary structure prediction, we used the PSIPRED Protein Structure Prediction Server (http://bioinf.cs.ucl.ac.uk/PSIPRED/psiform.html), while the GlobPlot web service was used to determine the propensity to order/globularity and disorder of the proteins and to explore globular domains (http://globplot.embl.de/).

CDK5R1 3′UTR secondary structure was calculated by means of Mfold, a web server for nucleic acid folding prediction (http://www.bioinfo.rpi.edu/applications/mfold/old/rna/). The reference secondary structure of the *CDK5R1* 3′UTR was retrieved in the UCSC Genome Browser (http://genome.ucsc.edu/).

The splice site prediction was performed with Splice View (http://l25.itba.mi.cnr.it/~webgene/wwwspliceview.html) and with NNSPLICE0.9 (http://www.fruitfly.org/seq_tools/splice.html).

Results and discussion

One hundred MR patients were analysed for mutations in *CDK5R1* and *OMG* genes using direct sequencing. The sequence analysis was carried out for the coding regions and for parts of the UTRs, selected for the presence of predicted UTR regulatory elements. This study allowed us to identify four novel mutations and two novel polymorphisms in *CDK5R1* and three novel mutations in *OMG*. All the mutations were heterozygous, and none was found in a sample of 370 healthy chromosomes. The frequencies of the novel and known polymorphisms were estimated in both MR patients and 185 controls.

Table 2 summarizes novel and known mutations and polymorphic changes identified in this study. Mutation nomenclature refers to the NCBI RefSeq cDNAs NM_003885 (*CDK5R1*) and NM_002544 (*OMG*).

Two novel mutations, c.323C>T (p.A108V), c.532C>T (p.L178L) and one known c.904C>A (p.L302I), were

Table 1 Primer sequences, amplicon size and PCR annealing temperature for *CDK5R1* and *OMG* fragments

Primer name	Forward primer, reverse primer	Size (bp)	PCR annealing (°C)
CDK5R1-CDS.1	5′-TCGGTGAGCGGTTTTATCCC-3′, 5′-ACAGGTGCTTCAGGCGGTAG-3′	587	63
CDK5R1-CDS.2	5′-GTCATCGTCCAGGCGTCC-3′, 5′-GCTCGTGAGTTCAAAAGTGC-3′	681	63
CDK5R1-3′UTR.1	5′-GTTTCCACCTTACCCTACTG-3′, 5′-GTGCTGTGTGAAGTCTGTG-3′	521	60
CDK5R1-3′UTR.2	5′-CCACAGGAATAATAGTTCAGG-3′ 5′-CCCCAATAACACAAATCCAAT-3′	527	56
OMG-5′UTR	5′-GAAAACTATCCATGAGGAAGG-3′, 5′-TATCCCTCAACTGACACTGC-3′	613	58
OMG-CDS.1	5′-GGAGTGCTTTCATTAGTTTCA-3′, 5′-CACACCTTCTGGATTTACCT-3′	555	57
OMG-CDS.2	5′-GTTTGGGTATTTTGGTCACTT-3′, 5′-TCTCAACCTCAGTAGTAACAA-3′	467	57
OMG-CDS.3	5′-ATGTACCTGGAAGAATTTGTG-3′, 5′-TTTGCCTAATGCTAACTGACC-3′	646	57

Table 2 Summary of mutations in *CDK5R1* and *OMG* identified in this study

Gene	Region	Nucleotide change	Protein level	Allelic frequencies	Familial/ sporadic	Reference
CDK5R1	**Coding**	**c.323C>T**	**p.A108V**	–	**Sporadic**	**This study**
CDK5R1	**Coding**	**c.532C>T**	**p.L178L**	–	**Sporadic**	**This study**
CDK5R1	Coding	c.904C>A	p.L302I	–	Sporadic	(1) and this study
CDK5R1	**3′UTR**	**c.1005G>A**	**–**	**pts: fr(G)=0.995, fr(A)=0.005; c: fr(G)=0.995, fr(A)=0.005**	**Sporadic**	**This study**
CDK5R1	3′UTR	c.1043G>A	–	pts: fr(G)=0.690, fr(A)=0.310; c: fr(G)=0.725, fr(A)=0.275	Sporadic	(2) and this study
CDK5R1	**3′UTR**	**c.2160C>T**	**–**	**pts: fr(C)=0.975; fr(T)=0.025; c: fr(C)=0.960, fr(T)=0.040**	**Sporadic**	**This study**
CDK5R1	**3′UTR**	**c.2254C>G**	**–**	–	**Familial**	**This study**
CDK5R1	**3′UTR**	**c.3452G>A**	**–**	–	**Sporadic**	**This study**
OMG	**5′UTR**	**c.−1028A>C**	**–**	–	**Sporadic**	**This study**
OMG	**5′UTR**	**c.−734A>G**	**–**	–	**Sporadic**	**This study**
OMG	Coding	c.62G>A	p.G21D	pts: fr(G)=0.910, fr(A)=0.090; c: fr(G)=0.875, fr(A)=0.125	Sporadic	(3) and this study
OMG	**Coding**	**c.1222A>G**	**p.T408A**	–	**Familial**	**This study**

Novel variants are in bold. Nucleotide positions refer to *CDK5R1* cDNA (accession no. NM_003885) and *OMG* cDNA (accession no. NM_002544)
(1) Kam et al. [30], (2) refSNP ID: rs8192474 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Snp), (3) Vourc'h et al. [36]

identified in the *CDK5R1* coding sequence. The first variation (c.323C>T), found in patient MR-82, results in the substitution of alanine 108 by valine (p.A108V). This amino acid is positioned within a proline-rich region of p35 which is perfectly conserved in mammals, while displaying marked differences in other organisms, as shown by the multiple alignment performed with the ClustalX program (Fig. 1a). No 3-D structure data are available for this region, which is likely to serve as link between two functional domains of the protein, as suggested by the analysis with the GlobPlot and PSIPRED software (data not shown), as well as by the alignment itself. The substitution from alanine to valine is conservative; however, the PSIPRED analysis predicts the lack of two short helices 46 amino acids upstream and 2 amino acids downstream the A108V mutation (data not shown).

The A108V change involves a residue situated in position +10 with respect to the cleavage site of the calpain protease, which generates the p35 proteolytic fragment p25. In a recent work, a consensus sequence was identified around the scissible bond from the position −4 to the position + 7 [29]. Even if the mutation falls outside the critical region, a potential effect on the calpain cleavage efficiency cannot be ruled out, taking into account the vicinity of the mutated residue to the cleavage site. In addition, the high conservation of the affected region is indicative of a possible role in the regulation of CDK5/*CDK5R1* activity, thus suggesting to further investigate a possible effect of the identified variation.

The second sequence change (c.532C>T) is a synonymous substitution of leucine 178 (p.L178L) in the domain of binding to the CDK5 kinase. Interestingly, both c.323C>T (p.A108V) and c.532C>T (p.L178L) mutations were found in the same patient (MR-82), who displays MR and cognitive dysfunction.

Another change, c.904C>A, was found in the coding region of *CDK5R1*. This causes the substitution of leucine 302 with isoleucine (p.L302I) at the C terminus of the protein. Leucine 302 is a conserved residue in *CDK5R1* vertebrate orthologs, but the PSIPRED Server does not predict any change in the protein secondary structure. This mutation was found in patient MR-9, who displays psychomotor and development delay, MR, speech and language impairment and microcephalia. This mutation was previously reported by Kam et al. [30], who observed the variation in the constitutional DNA of a patient who developed ganglioglioma.

Because of the exceptional extent of *CDK5R1* 3′UTR (>2.5 kb), the presence of UTR regulatory elements is expected. Sequence and structural elements located in the UTRs of mRNA are known to play significant roles in translational regulation. These regions are defined as *cis*-regulatory elements, and they regulate mRNA stability and translational efficiency by acting as binding sites for *trans*-acting cytoplasmic factors [31]. In addition, several regulatory elements have been described in 3′UTR for most of which a pathogenetic role has been demonstrated [20, 21].

We thus performed a bioinformatic analysis of *CDK5R1* 3′UTR by means of UTRScan and predicted a GY-box motif (GUCUUCC) at position 2,264–2,270 on the cDNA and a region in the last 200 nt of the transcript showing three putative AU-rich elements (Fig. 2).

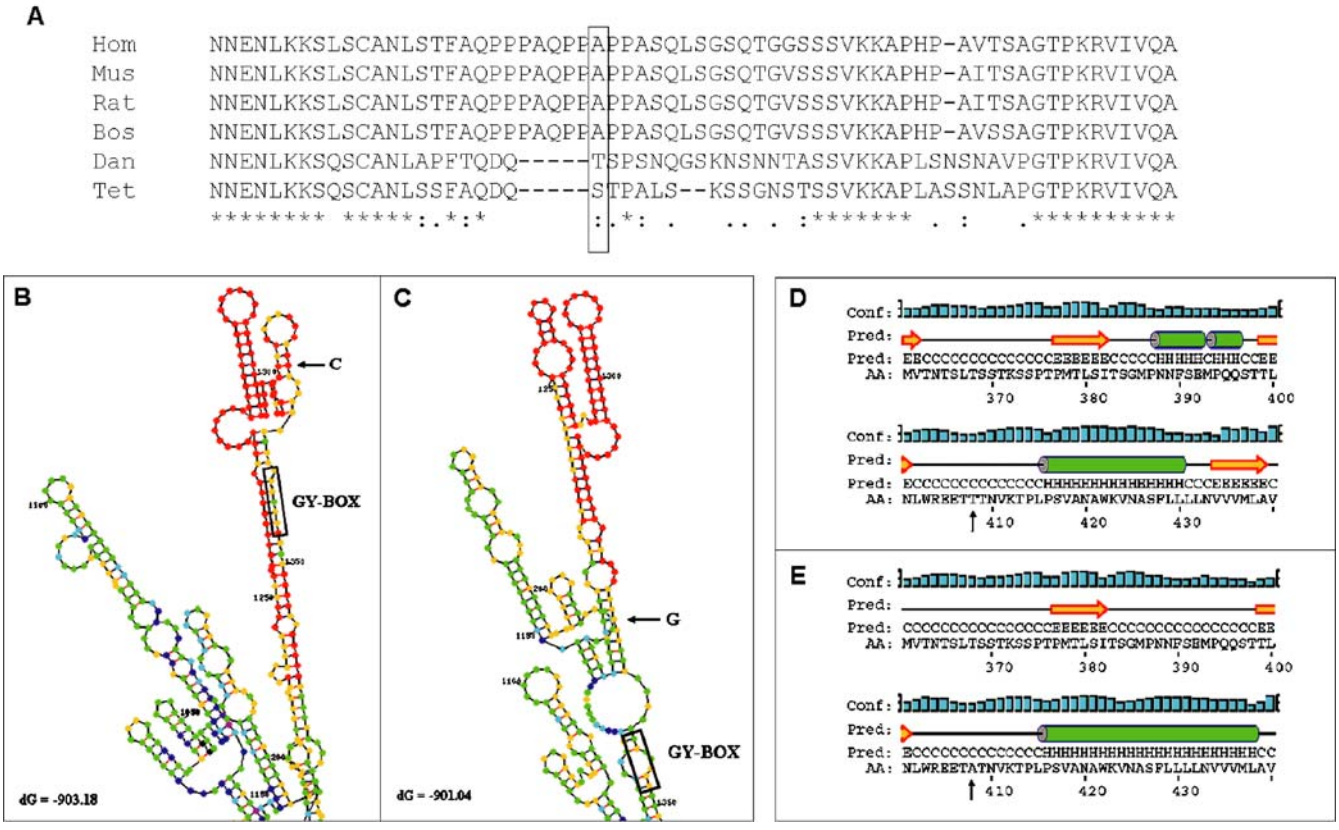

Fig. 1 **a** Multiple alignment of p35 amino acid sequence from human (*Homo sapiens*, *Hom*), mouse (*Mus musculus*, *Mus*), rat (*Rattus norvegicus*, *Rat*), cow (*Bos taurus*, *Bos*), zebra fish (*Danio rerio*, *Dan*) and green spotted pufferfish (*Tetraodon nigroviridis*, *Tet*), from amino acid 83 to amino acid 146. *Box* indicates Ala 108. *Stars* represent fully conserved residues, *single/double dots* indicate the presence of specific amino acid groups, according to the ClustalX classification. **b, c** Prediction of the *CDK5R1* 3′ untranslated region (UTR) secondary structure in the region comprising the GY-box, using the Mfold tool. **b** Wild-type structure in accordance with the secondary structure reported in UCSC database. **c** Prediction of the mutated (2254G) 3′UTR secondary structure. The free energy decrease is indicated. **d, e** Prediction of the secondary structure of OMgp (shown from amino acid 361 to amino acid 440) using the PSIPRED tool. *Arrows* indicate Thr 408 in the wild-type sequence (**d**) and Ala 408 in the mutated sequence (**e**). *Cylinders and arrows* indicate the potential α-helix or β-sheet structures, respectively. The *vertical bars* indicate the level of the prediction confidence

GY-box motif has first been described in many 3′UTRs of genes involved in Notch signalling in *Drosophila* where it mediates negative post-transcriptional regulation [32]. GY-box motif is complementary to the 5′ end of some *Drosophila* microRNA (miRNAs) and has been validated as a miR-7 target [33]. Transcripts bearing the GY-box are negatively regulated by complementarity to the corresponding miRNAs in vivo [34]. The 3′UTR of *CDK5R1* mRNA also includes potential sequence elements such as the AUUUA pentamer and other AU-rich regions, known to play a role in mRNA stability and degradation through the binding to specific factors [31].

We thus sequenced the two portions of 3′UTR of *CDK5R1* gene in which putative regulatory elements (GY-box and AU-rich elements) were predicted. Two novel mutations were found in these regions: c.2254C>G and c.3452G>A. Notably, the nucleotide change c.2254C>G is only 10 bp upstream of the GY-box element. The secondary structure of *CDK5R1* 3′UTR was predicted with Mfold and compared with the normal structure available from the UCSC Genome Browser. This analysis allowed us to see a significantly different folding of the RNA in the portion surrounding residue 2254G compared with the wild-type residue 2254C (Fig. 1b,c). This change in the RNA secondary structure causes a different positioning of the GY-box element which may affect its correct functioning. The mutation was found in patient MR-44 and in her mother, who also displays MR. Apart from the proband and her mother, three other mentally retarded sibs, one of whom generated a MR child, were in this pedigree, but unfortunately, no further family members were available to verify segregation of the mutation with the MR. Mutation c.3452G>A was found in patient MR-74, who displays MR, language retardation, isolation propensity and attention deficit. The variation is located in the AU-rich portion but outside the predicted AU-rich elements of the 3′UTR of *CDK5R1*. Indeed, no differences in 3′UTR folding were predicted using Mfold, compared with the wild-type structure. Further studies are required to determine whether this mutation falls inside an as yet unknown UTR regulatory element and whether the nucleotide change might affect the regulation of transcript degradation.

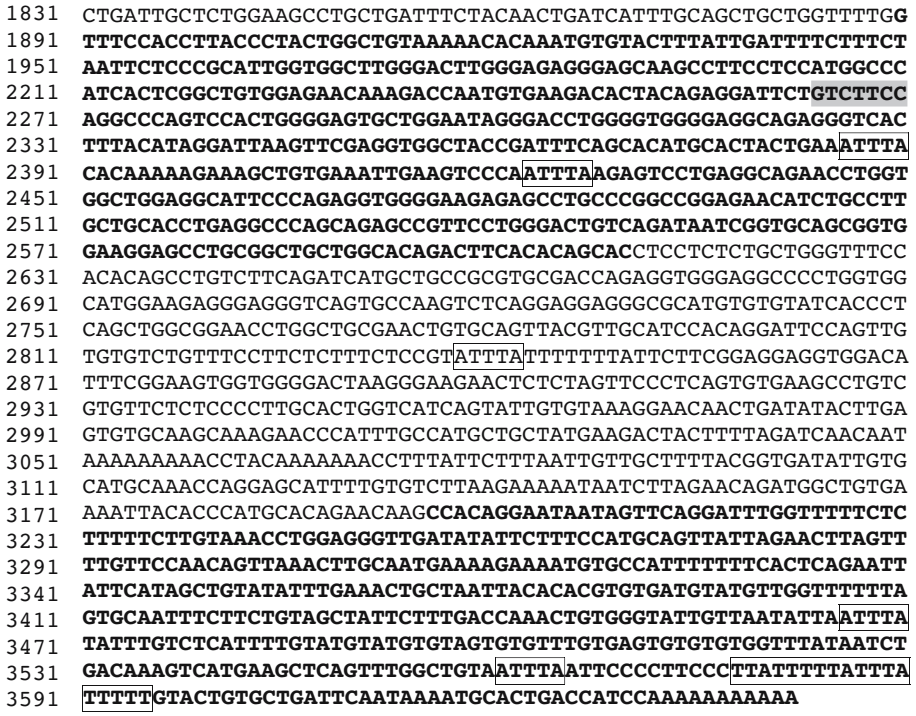

Fig. 2 3′UTR sequence of the *CDK5R1* cDNA, from nucleotide 1831 to poly-A tail. The GY-box element is *shaded*, while AU-rich elements are *boxed*. The amplicons used for mutation analyses are indicated in *bold*

In addition, within the sequenced 3′UTR fragments, two novel rare polymorphisms were found (c.1005G>A and c.2160C>T). The rare allele 1005A was observed in one patient and one normal control only, while the rare allele 2160C showed a frequency of 0.025 in the MR patients and of 0.04 in the control sample (370 chromosomes). We also calculated the allelic frequencies of the known polymorphism c.1043G>A (ref SNP ID: rs8192474) in our cohort of MR patients which were found not to significantly differ from those in 185 control chromosomes and those registered in online databases.

As regards the *OMG* gene, we found in its coding region a novel mutation c.1222A>G (p.T408A) in patient MR-27, who displays MR and language impairment. This mutation leads to the substitution to alanine of threonine 408, which is positioned in the S/T-rich domain of the protein and is conserved in several mammal species [35]. Secondary structure and function of this domain are not known. Secondary structure prediction of the OMgp protein with PSIPRED shows that p.T408A substitution might cause conformation changes in the surrounding regions, in particular in the short hydrophobic region at C terminus of the protein (Fig. 1d,e). We looked for the variation in the DNA of the parents of patient MR-27 which was found in the unaffected mother. A possible incomplete penetrance of the phenotype might explain the finding of the p.T408A substitution in a normal carrier, and this does not necessarily exclude a disease causative role for the mutation.

We also evaluated the allelic frequencies of the known polymorphism c.62G>A (p.G21D) in both patients and controls, which were found to be similar to those reported by Vourc'h et al. [36].

We extended the mutation analysis to *OMG* 5′UTR, which is characterized by a large size, including an intron, where nine uAUG and seven predicted uORFs were found. uORFs are recognized and translated by ribosomes and have been proposed to act in the control of translation by means of different mechanisms, e.g. altering the mRNA stability or causing the ribosome to stall during the elongation/termination phase of uORF translation, creating a blockade to additional ribosome scanning [25]. However, no 5′UTR regulatory elements were predicted using the UTRScan program.

We detected in this region two novel mutations (c.−1028A>C and c.−734A>G). Mutation c.−1028A>C, which abolishes a stop codon not belonging to any predicted uORF, was found in patient MR-75, who displays MR, speech and language impairment, short attention span and hyperactivity. Mutation c.−734A>G, in patient MR-71, showing isolated MR, is localized within the IVS between exon 1 and exon 2. No effect of this mutation on splice sites was predicted by analysis with Splice View and NNSPLICE0.9.

The sequence alterations identified in *CDK5R1* and *OMG* genes have been classified according to the allele frequencies as mutations and polymorphisms. We think that the observed sequence changes might concur with variation at other loci in the susceptibility to MR and/or neurodegenerative diseases.

Mutation screening of *CDK5R1* and *OMG* enhanced definition of the repertoire of their variant alleles which might help future association studies.

In addition, a possible synergic role of the two gene products in the central nervous system development may be proposed on the basis of the possible sharing of a

common signal transduction pathway: together with other ligands of the RTN4R receptor (e.g. myelin associated glycoprotein, MAG), OMgp might stimulate the phosphorylation of neurofilaments (NFs) and microtubule-associated proteins (MAPs) through the activation of the CDK5/p35 complex [37].

Moreover, findings from this study might help in addressing the functional analysis of both the regulatory transcriptional elements of UTRs and the gene products. Further investigations are necessary to establish whether or not the reported mutations cause a decreased activity of the respective gene products. A gene product dosage-dependent effect of mutated genes would simulate the effect of the pathogenetic mechanism based on gene haploinsufficiency reported for microdeletion syndromes.

In perspective, a better assessed role of *CDK5R1* and *OMG* in MR might enhance the genotype–phenotype correlation in patients with NF1 microdeletion syndrome.

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