Mutations analysis of the *GRN* gene in an Emilia-Romagna cohort of dementia patients

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AIM

- ✓ Identification of the *GRN* gene variants that characterized Emilia-Romagna patients with suspected neurodegenerative dementia
- ✓ Establish the pathogenicity of these variants applying specific flow-chart

MATERIALS AND METHODS

- ✓ **Patients** (DNA from 781 patients with diagnosis of cognitive impairment, parkinsonism, and motor neuron disease)
- ✓ ***GRN* variants screening:** Sanger and Next Generation Sequencing
- ✓ **Interpretation of novel genetic variants:**
- Bioinformatics: (I) population-based exome sequencing data (ExAC) and (II) *in silico* tools for pathogenicity prediction (VarSome, Human Splicing Finder)

….where possible:

- Plasma pGRN dosage
- RNA analysis
- Neuropathological Examination

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RESULTS

✓ **Known pathogenic variants (AD&FTD; HGMD; Pubmed)**

GRN mutation	N° Patients	Clinical Phenotype
p.Thr272SerfsTer10	6	Behavioral Variant Frontotemporal Dementia
p.Gly79AspfsTer39	1	Behavioral Variant Frontotemporal Dementia
p.Met1?	1	Behavioral Variant Frontotemporal Dementia
c.709-2A>T	1	Behavioral Variant Frontotemporal Dementia

✓ **Previously reported "unclear" variants (AD&FTD; HGMD; Pubmed)**

GRN variant	N° Patients	Clinical Phenotype
p.Arg19Trp	1	Amyotrophic Lateral Sclerosis
p.Arg298His	1	Amyotrophic Lateral Sclerosis
c.-2C>T	1	Frontotemporal Dementia / Amyotrophic Lateral Sclerosis

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✓ Novel variants

GRN variant	N° Patients	Clinical Phenotype
c.1179+3A>G	4	Behavioral Variant Frontotemporal Dementia, Corticobasal Syndrome
p.Val279Val	1	Amyotrophic Lateral Sclerosis
p.Ala505Gly	1	Amyotrophic Lateral Sclerosis

✓ Analysis of novel genetic variants:

GRN variant	ExAC	Varsome	Human Splicing Finder	Plasmatic pGRN concentration*
c.1179+3A>G	-	VUS: Benign supporting	Alteration of the WT donor site, most probably affecting splicing.	43.92 ± 7.67 ng/ml (n=3)
p.Val279Val	-	VUS	Activation of an exonic cryptic acceptor site. Potential alteration of splicing.	106 ng/ml
p.Ala505Gly	0.00009914	VUS: Benign suppoting	Alteration of an exonic ESE site. Potential alteration of splicing.	76.2 ng/ml

* Normal range normal value >100 ng/mL, the mean value in FTD patients 61.2 ng/mL) (Ghidoni et al., 2012)

VUS: Variant of uncertain significance

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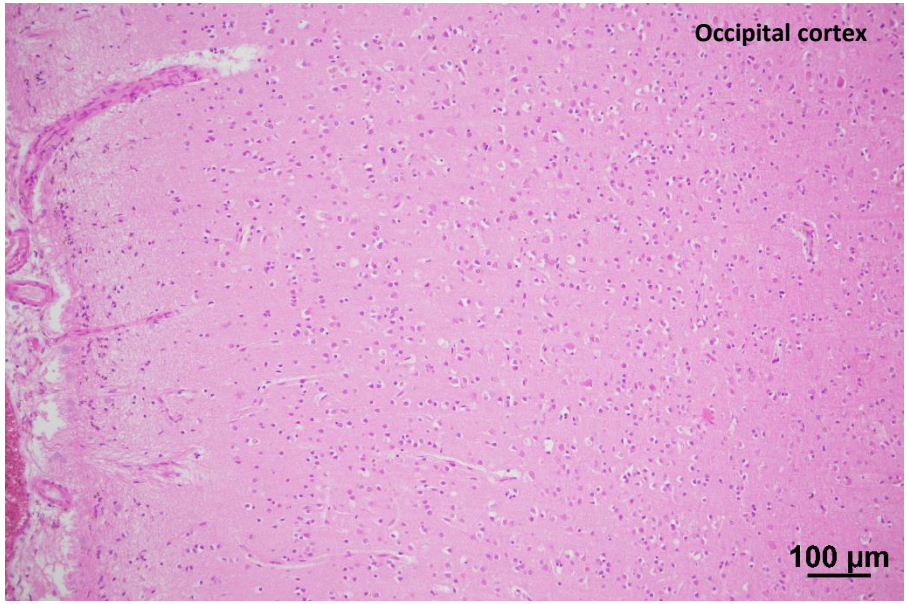
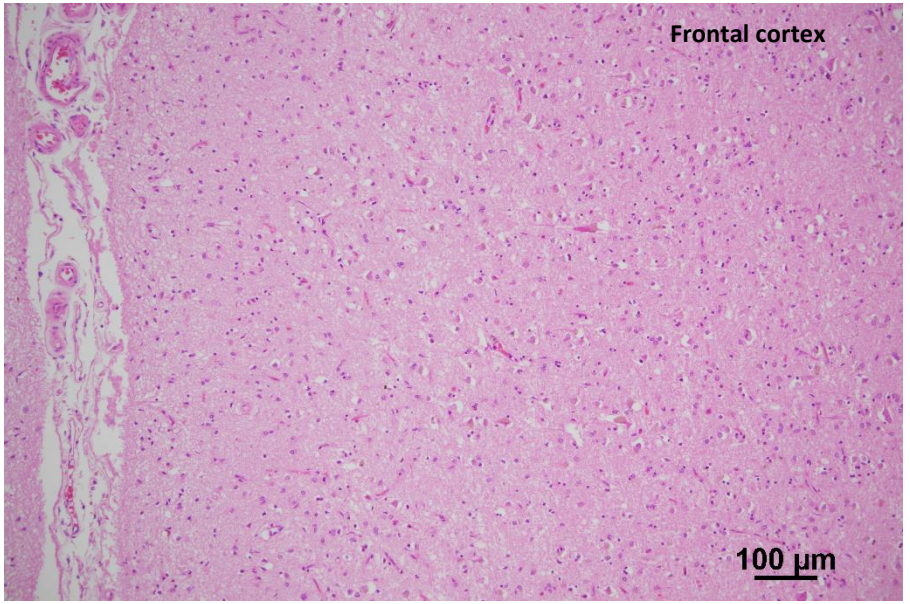
- ✓ **Neuropathological findings for the c.1179+3A>G variant**





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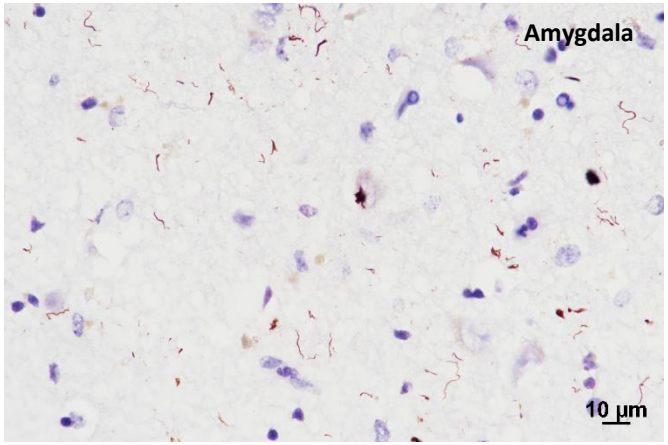
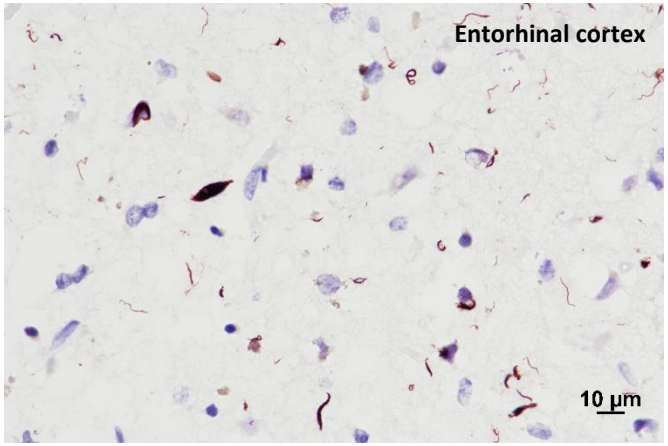
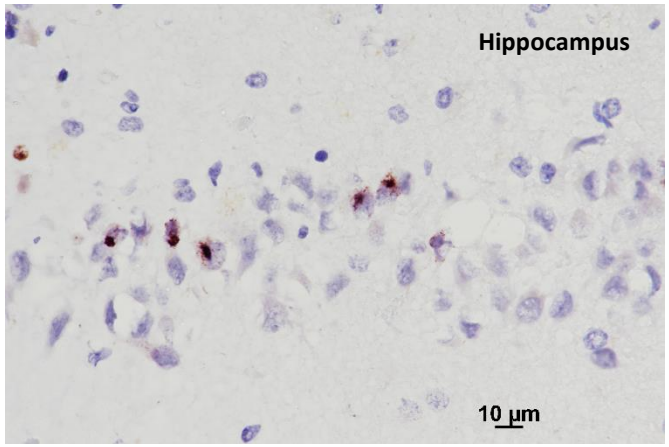
✓ **Neuropathological findings for the c.1179+3A>G variant: TDP-43 staining**







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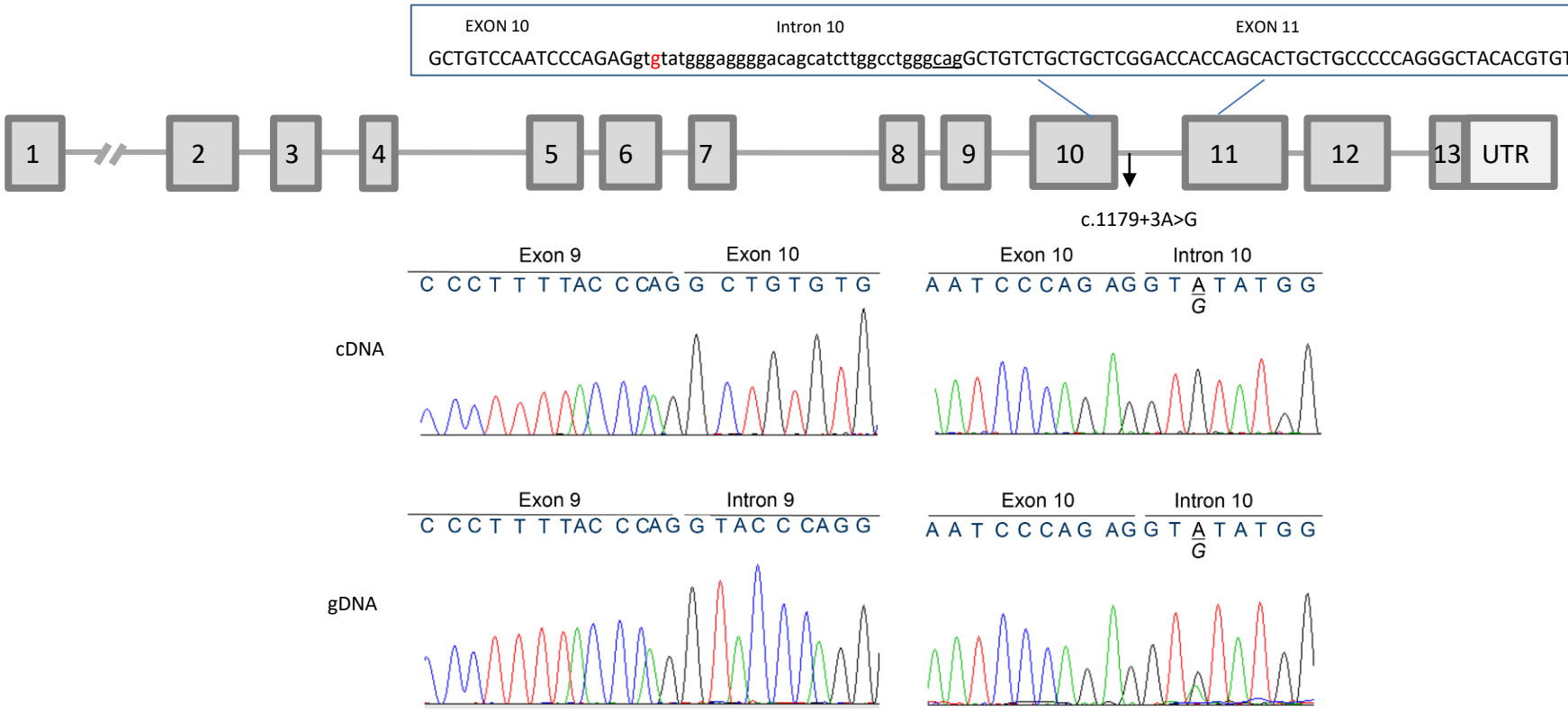
✓ **RNA analysis for the c.1179+3A>G variant**



GRN variant	Interpretation
c.1179+3A>G	**PATHOGENIC**
p.Val279Val	BENIGN VARIANT
p.Ala505Gly	UNCLEAR SIGNIFICANCE

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DISCUSSION

- ✓ The known pathogenic mutations p.Thr272SerfsTer10 is the most frequent in the screened cohort
- ✓ The second most common pathogenic mutation is a never reported variant, the c.1179+3A>G. The pathogenic mechanism involves the partial retention of the intron 10 causing a premature Stop Codon in the exon 11
- ✓ Other missense novel variants were identified in ALS patients, however it was not possible establish their pathogenicity
  - ➢ These rare variants are likely to be benign rare variants, however we cannot exclude the possibility that they are risk factors that modify other pGRN functions or the downstream effects