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SHORT COMMUNICATION

Association study to evaluate the serotonin transporter and apolipoprotein E genes in frontotemporal lobar degeneration in Italy

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Abstract Frontotemporal lobar degeneration (FTLD) is a progressive neurodegenerative disorder characterized by behavioral and language disturbances. We performed a case-control association study in the Italian population to assess the relevance for FTLD genetic susceptibility of the serotonin (5-HT) transporter gene-linked polymorphic region [*rs4795541*, alias short (S)/long (L)] an in/del polymorphism of the promoter region of the gene coding for the 5-HT transporter (*SLC6A4*). This functional polymorphism was reported to influence the *SLC6A4* transcription rate, with the S-allele having a two-fold reduced efficiency. We collected 225 independent subjects (74 sporadic FTLD and 151 age-matched healthy controls, CT) that were genotyped for the *rs4795541,* the *SLC6A4* single nucleotide polymorphisms (SNP) *rs25531* and *rs6354*, and the apolipoprotein E (*APOE*) allelic variants. A significant correlation [$P = 0.018$, OR (95% CI): 2.1 (1.1–3.9)] between *rs4795541* S-allele presence and FTLD susceptibility was found. In summary, the *rs4795541* might be important for FTLD susceptibility in the Italian population.

Keywords Frontotemporal dementia · Serotonin · Serotonin transporter · Apolipoprotein E · Genetics

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Introduction

Frontotemporal lobar degeneration (FTLD) is a neurodegenerative disease characterized by alterations in behavior and speech (Scarpini et al. 2006). The majority of FTLD cases are sporadic, and only a minor fraction (5–10%) is monogenic due to alterations in the microtubule-associated protein tau (*MAPT*, OMIM +157140), granulin (*GRN*, OMIM *138945), valosin-containing protein (*VCP,* OMIM *601023) or the chromatin-modifying protein 2B (*CHMP2B*, OMIM +609512) (Cruts et al. 2006; Scarpini et al. 2006).

The current consensus criteria identify three clinical FTLD subtypes: progressive nonfluent aphasia (PA), semantic dementia (SD) and frontotemporal dementia (FTD) (Neary et al. 1998). Few genes have been considered as genetic susceptibility factors for sporadic FTLD, including *MAPT* itself (Fenoglio et al. 2007). The association between FTLD and *APOE-ε4* (OMIM +107741) in the Italian population is controversial (Bernardi et al. 2006; Verpillat et al. 2002).

An impairment of serotonin (5-HT) transmission in FTLD has been reported (Yang and Schmitt 2001). The 5-HT transporter is encoded by the single gene *SLC6A4* (OMIM *182138) (17q11.1-q12), and it modulates 5-HT reuptake (Lesch et al. 1994). A functional polymorphism in the promoter region of *SLC6A4*, called the 5-HT

transporter gene-linked polymorphic region (*rs4795541*), was reported by Heils et al. (1996). The *rs4795541* consists of a 43-bp deletion (short variant *S*) or insertion (long variant *L*), the S-allele reducing *SLC6A4* transcription two times (Collier et al. 1996). The *SLC6A4* promoter region contains other functional polymorphic sites, such as *rs25531* (A→G) (Wendland et al. 2008).

We performed a case-control association study in the Italian population to evaluate *rs4795541, rs25531* and *APOE-ε4* as genetic risk factors for FTLD. Moreover, we performed a dHPLC screening of our population, searching for rare coding variants.

Materials and methods

Patient recruitment

Two hundred and twenty-five independent subjects were recruited from the following clinical centers: "Fondazione Ospedale Maggiore Policlinico" (Milan, Italy) and "Luigi Sacco" Hospital (Milan, Italy). All patients underwent screening laboratory tests, neurocognitive evaluation, brain magnetic resonance imaging (MRI) or computed tomography (CT scan). Cognitive dysfunctions were assessed by the Clinical Dementia Rating (CDR), the Mini Mental State Examination (MMSE), the Frontal Assessment Battery (FAB), the Wisconsin Card Sorting Test (WCST) and the Tower of London.

Seventy-four subjects were diagnosed as FTLD, according to Neary et al. and subsequent revision by McKhann et al. (Neary et al. 1998; McKhann et al. 2001). An accurate follow-up was done to confirm the diagnosis. Age-matched healthy controls (CT) had no memory and psychobehavioral complaints. All subjects (or their relatives) gave their informed consent to participation in the study, whose protocol was approved by the human investigation scientific committee of the Italian Ministry of Health.

Blood samples collection, *rs4795541, rs25531* and *APOE* genotyping

Genomic DNA (gDNA) was isolated from whole blood using a commercial Flexigene kit (Qiagen, Hildren, Gemany).

To assess the *rs4795541* genotype, 50 ng of gDNA was amplified by polymerase chain reaction (PCR) using the following primers: for-5′ggcgttgccgctctgaatgc-3′ and rev-5′ gagggactgagctggacaacca-3′ (Nakamura et al. 2000).

The *rs25531* genotype was assessed by allele-specific PCR using the following primers: common rev: 5′tggagtc cgcgcgggattctggtgccacct-3′; for-allele A specific: 5′-acccc tcgcggcatccccctgcacccaca-3′; for-allele G specific: 5′-acc cctcgcggcatccccctgcacccacg-3′.

APOE genotype was determined by restriction fragment length polymorphism (RFLP) using the following primers: for-5′ tcggccgcagggcgctgatgg-3′; rev-5′ctcgcgggcccccggc cccggcctggta-3′ and CfoI as restriction enzyme (Roche, Basel, Switzerland).

dHPLC screening

SLC6A4 (**NM_001045**) exons 1–15 and the relative 5′- and 3′-intronic flanking regions (50 bp) were screened by dHPLC (Wave 3500, Transgenomic, Omaha, NE) using the experimental conditions set up by the instrument's software (Navigator, Transgenomic, Omaha, NE). The presence of an heterozygous nucleotidic substitution was confirmed by double-stand DNA sequencing.

Statistical analysis

Frequency distributions were compared using χ^2 test or Fisher's exact test. The odds ratios (OR) were calculated by a 2×2 contingency table at 95% confidence interval (CI). The statistical significance limit was set at $P = 0.05$. Analyses were performed using StatView program version 5.0 or RxC program (http://www.marksgeneticsoftware.net). The program HaploView 4.1 was used to evaluate linkage disequilibrium (LD).

Results

Case-control study to assess *rs4795541* genotype distribution

Table 1 summarizes the population demographics. Seventy patients were classified as FTD (95%), while four patients (5%) were diagnosed as PA. For subsequent analysis, FTD and PA genotypes were considered aggregated. Sex

Table 1 Demographics of the population sample recruited for the case-control studies

Diagnosis	No. of subjects (males:females)	Age at onset (years ± SD)	Age at sampling (years ± SD)
CT	151 (61:90)	–	67.0 ± 12.0
FTLD	74 (34:40)	69.4 ± 9.7	–
FTD	70 (31:39)	69.2 ± 9.7	–
PA	4 (3:1)	69.0 ± 10.5	–

CT age-matched healthy controls, *FTLD* sporadic frontotemporal lobar degeneration, *FTD* frontotemporal dementia, *PA* progressive nonfluent aphasia, *SD* standard deviation

distribution was balanced (male-to-female ratio 0.85). In controls, females were slightly over-represented (male-to-female ratio 0.7).

The genotypic frequencies of *rs4795541* for CT and FTLD respected Hardy-Weinberg equilibrium (data not shown), and we found a significant difference between CT and FTLD ($P = 0.046$, χ^2-test) (Table 2). The S-allele in the FTLD group was increased (47.3 vs. 35.8% in controls; $P = 0.019$, χ^2-test). The calculated OR with 95% CI interval for the *rs4795541* (S/L + S/S) genotypes was 2.1, with an associated *P*-value of 0.018. Sample stratification by gender gave no difference.

Case-control study to assess *rs25531* and apolipoprotein E (*APOE*) genotypes

We genotyped our sample for *rs25531* (Table 2). The results did not evidence a difference between CT and FTLD, either at the genotypic or allelic level. A similar picture was found for the *APOE-ε4* allele (Table 2). An FTD subject was excluded from *APOE* analyses due to an ambiguous genotyping result. *APOE* distribution did not differ between CT and FTLD, both for genotypic and allelic frequencies.

Once the genotypic distribution of *rs4795541, rs25531* and *APOE* had been assessed separately, we verified a possible interaction among these polymorphisms. The *APOE-ε4*(−) group had $n = 187$ subjects, and by comparing the *rs4795541* allelic frequency between CT and FTLD, an almost significant increase of the S-allele in FTLD was detectable ($P = 0.068$, χ^2-test). The same was true for the *APOE-ε4*(+) group (sample size $n = 37$; FTLD S-allele frequency 46.4 vs. 26.0% in CT; $P = 0.073$, χ^2-test). No significant or almost-significant difference was detected between *APOE-ε4*(−) and *APOE-ε4*(+) subjects for *rs25531* genotypic or allelic distribution.

We linked *rs4795541*, *rs25531* and *APOE-ε4* genotypes to FTLD age at onset. No correlation came to light (data not shown).

dHPLC screening of *SLC6A4*

We performed a dHPLC screening of the *SLC6A4* gene in FTLD and CT searching for coding or splicing variants. In

Table 2 Genotypic and allelic frequencies of *SLC6A4* and *APOE* polymorphisms

rs4795541	Genotype count (%)			Allele count (%)		OR[95% CI] (*P*-value)	*P*-value
	S/S	S/L	L/L	S	L		
CT (151)	21 (14.0)	66 (43.7)	64 (42.3)	108 (35.8)	194 (64.2)		**0.046**[a]
FTLD (74)	15 (20.3)	40 (54.1)	19 (25.7)[a]	70 (47.3)	78 (52.7)[b]	S/L + S/S: 2.1[1.1–3.9] (**0.018**)	**0.019**[b]

rs25531	Genotype count (%)			Allele count(%)		*P*-value
	A/A	A/G	G/G	A	G	
CT(151)	130 (86.0)	21 (14.0)	0 (0.0)	281 (93.0)	21 (7.0)	0.47[c]
FTLD(74)	61 (82.4)	13 (17.6)	0 (0.0)[c]	135 (91.2)	13 (8.8)[b]	0.49[b]

rs6354	Genotype count (%)			Allele count(%)		*P*-value
	A/A	A/C	C/C	A	C	
CT(151)	138 (91.4)	12 (7.9)	1 (0.7)	288 (95.4)	14 (4.6)	0.70[c]
FTLD(74)	68 (91.9)	6 (8.1)	0 (0.0)[c]	142 (95.9)	6 (4.1)[b]	0.90[b]

APOE	Genotype count (%)						Allele count (%)			*P*-value
	ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	ε2	ε3	ε4	
CT(151)	1 (0.7)	14 (9.2)	1 (0.7)	113 (74.8)	21 (14.0)	1 (0.7)	17 (5.6)	261 (86.4)	24 (8.0)	0.56[c]
FTLD(73)	1 (1.4)	9 (12.3)	2 (2.7)	49 (67.2)	12 (16.4)	0 (0.0)[c]	13 (8.9)	119 (81.5)	14 (9.6)[b]	0.33[b]

Bold values indicate $P < 0.05$

OR [95% CI]: odds ratio and [confidence interval] at 95%

CT age-matched healthy controls, *FTLD* sporadic frontotemporal lobar degeneration

[a] χ^2-test *P*-value for FTLD versus CT genotype count distribution

[b] χ^2-test *P*-value for FTLD versus CT allelic count distribution

[c] *P*-value for Fisher's exact test for FTLD versus CT genotype count distribution

6 independent patients and 12 CTs, we found the nucleotidic change (A→C, nt121 of **NM_001045**) that mapped within the 5′-untranslated region (5′-UTR), had already been described in databank (*rs6354*) and was then genotyped by direct sequencing in all FTLDs and CTs. The polymorphism respected Hardy-Weinberg equilibrium (data not shown), and no significant difference was found (Table 2).

Discussion

We aimed at evaluating *rs4795541, rs25531* and *APOE-ε4* as risk factors for FTLD in Italy. For *rs4795541*, we found a significant difference between CT and FTLD, the presence of at least one S-allele associating to an increased FTLD risk of 2.1 times. Interestingly, a 5-HT deficit in FTLD was reported by autopsy, imaging and biochemical studies that evaluated the CSF content of 5-hydroxyindoleacetic acid (5-HIAA, a 5-HT metabolite) (Huey et al. 2006).

We were unable to link *rs25531* to FTLD susceptibility. The dHPLC screening found a polymorphism in *SLC6A4* 5′-UTR (*rs6354*) whose frequency did not differ between FTLD and CT. Our dHPLC analysis suggests a low frequency of *SLC6A4* coding mutations. We also evaluated LD among *rs4795541*, *rs25531* and *rs6354*, but no LD was found (Fig. 1).

Our data about *APOE-ε4* are not in agreement with a paper finding a positive association between *APOE-ε4* and FTLD in the Italian population (Bernardi et al. 2006). However, the FTLD sample was composed not only by sporadic ($n = 54$), but also by familial ($n = 46$) FTLD subjects (even if monogenic FTLD cases were excluded), thus making a rigorous comparison difficult. As for *rs4795541*, *rs25531* or *APOE-ε4* status and FTLD age at onset, our failure in detecting any relation underlines the need for a more comprehensive approach to perform this kind of analysis (Borroni et al. 2008).

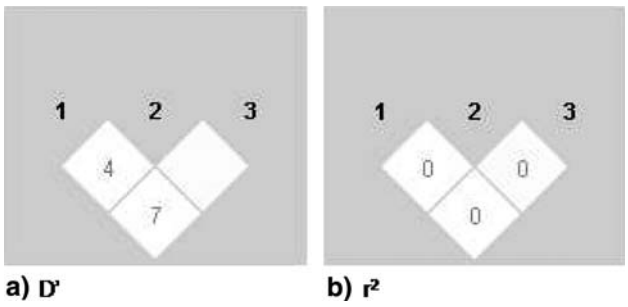

Fig. 1 Linkage disequilibrium coefficients D' and r^2 calculated by HalpoView among the *SLC6A4* polymorphisms *rs4795541*, *rs25531* and *rs6354* (1, 2 and 3, respectively, in the graphs)

In conclusion, our data highlight *rs4795541* as possible genetic modulator of FTLD susceptibility in Italy to be confirmed in larger association studies.

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Conflict of interest statement None.

References

Bernardi L, Maletta RG, Tomaino C, Smirne N, Di Natale M, Perri M, Longo T, Colao R, Curcio SA, Puccio G, Mirabelli M, Kawarai T, Rogaeva E, St George Hyslop PH, Passarino G, De Benedictis G, Bruni AC (2006) The effects of APOE and tau gene variability on risk of frontotemporal dementia. Neurobiol Aging 27:702–709

Borroni B, Grassi M, Agosti C, Premi E, Archetti S, Alberici A, Bellelli G, Caimi L, Di Luca M, Padovani A (2008) Establishing short-term prognosis in frontotemporal lobar degeneration spectrum: role of genetic background and clinical phenotype. Neurobiol Aging doi:10.1016/j.neurobiolaging.2008.04.004

Collier DA, Stober G, Li T, Heils A, Catalano M, Di Bella D, Arranz MJ, Murray RM, Vallada HP, Bengel D, Muller CR, Roberts GW, Smeraldi E, Kirov G, Sham P, Lesch KP (1996) A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in susceptibility to affective disorders. Mol Psychiatry 1:453–460

Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, Rademakers R, Vandenberghe R, Dermaut B, Martin JJ, van Duijn C, Peeters K, Sciot R, Santens P, De Pooter T, Mattheijssens M, Van den Broeck M, Cuijt I, Vennekens K, De Deyn PP, Kumar-Singh S, Van Broeckhoven C (2006) Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 442:920–924

Fenoglio C, Galimberti D, Piccio L, Scalabrini D, Panina P, Buonsanti C, Venturelli E, Lovati C, Forloni G, Mariani C, Bresolin N, Scarpini E (2007) Absence of TREM2 polymorphisms in patients with Alzheimer's disease and frontotemporal lobar degeneration. Neurosci Lett 411:133–137

Heils A, Teufel A, Petri S, Stöber G, Riederer P, Bengel D, Lesch P (1996) Allelic variation of human serotonin trasporter gene expression. J Neurochem 66:2621–2624

Huey ED, Putnam KT, Grafman J (2006) A systematic review of neurotransmitter deficits and treatments in frontotemporal dementia. Neurology 66:17–22

Lesch KP, Balling U, Gross J, Strauss K, Wolozin BL, Murphy DL, Riederer P (1994) Organization of the human serotonin transporter gene. J Neural Transm Gen Sect 95:157–162

McKhann G, Albert M, Grossman M, Miller B, Dickson D, Trojanowski J (2001) Clinical and pathological diagnosis of frontotemporal dementia. Arch Neurol 58:1803–1809

Nakamura M, Ueno S, Sano A, Tanabe H (2000) The human serotonin transporter gene linked polymorphism (rs4795541) shows ten novel allelic variants. Mol Psychiatry 5:32–38

Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, Freedman M, Kertesz A, Robert P, Albert M, Boone K, Miller B, Cummings J, Benson D (1998) Frontotemporal lobar

degeneration: a consensus on clinical diagnostic criteria. Neurology 51:1546–1554

Scarpini E, Galimberti D, Bresolin N (2006) Genetics and neurobiology of frontotemporal lobar degeneration. Neurol Sci 27(Suppl 1):S32–S34

Verpillat P, Camuzat A, Hannequin D, Thomas-Anterion C, Puel M, Belliard S, Dubois B, Didic M, Lacomblez L, Moreaud O, Golfier V, Campion D, Brice A, Clerget-Darpoux F (2002) Apolipoprotein E gene in frontotemporal dementia: an association study and meta-analysis. Eur J Hum Genet 10:399–405

Wendland JR, Moya PR, Kruse MR, Ren-Patterson RF, Jensen CL, Timpano KR, Murphy DL (2008) A novel, putative gain-of-function haplotype at SLC6A4 associates with obsessive-compulsive disorder. Hum Mol Genet 17:717–723

Yang Y, Schmitt HP (2001) Frontotemporal dementia: evidence for impairment of ascending serotoninergic but not noradrenergic innervation. Immunocytochemical and quantitative study using a graph method. Acta Neuropathol 101:256–270