The Spectrum of Mutations in Progranulin

A Collaborative Study Screening 545 Cases of Neurodegeneration

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eTable 1. *GRN* Intronic Variants and Polymorphisms Observed in This Study

Coding DNA NM_002087.2	Predicted Protein NP_002078.1	MAF (Count)			Case Information				
		Cases[a] (n=1090)	Controls 1[b] (n=760)	Controls 2[c]	ID	Dx	AAO, y	FH	Source[d]
Intronic Variations (Pathogenic Potential Unknown)									
c.264+7G>A	NC	0.002 (2)	0.007 (5)	0.005 (6/1124)	ANA01	FTD	62	Yes	30-32
					ARC04	FTD	46	Possible	
					UPA118	FTLD-U	Unk	Unk	
					UPC1189	ALS	73	Yes	
c.265–27C>A	NC	0.001 (1)	0.001 (1)	NA	CBG01	CBD	81	No	Novel
c.349+62G>C	NC	0.001 (1)	NA	0.003 (1/380)	UP78	FTD	67	Possible	30
c.598+39G>A	NC	0.001 (1)	NA	NA	GB03	DLDH	40	Yes	Novel
c.708+6_+9delTGAG	?[e]	0.001 (1)	0.000 (0)	NA	UPA241	FTLD-U	69	Possible	Novel
c.709–17C>T	NC	0.001 (1)	0.000 (0)	NA	CCW01	FTD	44	Yes	Novel
c.934–19C>A	NC	0.001 (1)	NA	NA	UP230	PMA	58	Possible	Novel
c.1413+20C>T	NC	0.001 (1)	NA	NA	HOG01	FTD (MND)	61	No	Novel
c.1645–40C>T	NC	0.001 (1)	NA	NA	UP414	FTD	50	No	Novel
Polymorphism (MAF ≥0.01)									
c.55C>T (rs63750723)	p.R19W	0.001 (1)	NA	0.028 (13/450)					11, 31
c.264+21G>A (rs9897526)	NC	0.110 (116)	NA	0.103 (106/1026)					30, 32
c.350–50_–47dupGTCA (rs34424835)	NC	0.197 (206)	NA	0.236 (207/877)					31, 32
c.384T>C (rs25646)	p.D128	0.024 (25)	0.045 (34)	0.025 (26/1046)					11, 32
c.462+24G>A (rs850713)	NC	0.193 (203)	NA	0.228 (147/646)					32
c.835+7G>A	NC	0.069 (72)	NA	0.074 (77/1046)					30-32
c.*78C>T (rs5848)[f]	NC	0.261 (274)	NA	0.272 (176/646)					32

Abbreviations: AAO, age at onset; ALS, amyotrophic lateral sclerosis; CBD, corticobasal degeneration; DLDH, dementia lacking distinctive histopathology; Dx, diagnosis; FH, family history; FTD, frontotemporal dementia; FTLD-U, frontotemporal lobar degeneration with ubiquitin-positive inclusions; ID, identifier; MAF, minor allele frequency; MND, motor neuron disease; NA, not available; NC, no change; PMA, progressive muscular atrophy; Unk, unknown.

[a] Frequency in 1090 chromosomes (all cases); numbers in parentheses indicate counts.

[b] Frequency in our 760 control chromosomes; numbers in parentheses indicate counts.

[c] Combined control frequency from the references; numbers in parentheses indicate counts over chromosomes screened.

[d] Numbers represent reference numbers.

[e] Position or prediction uncertain.

[f] *Stop codon.

eTable 2. *In Silico* and *Ex Vivo* Splicing Analysis of Splice Site Variations

Variation	Allele	Sequence[a]	NNSPLICE Score		Location in Gene	Effect of Variant Allele in Minigene Splicing[b]	Predicted Protein NP_002078.1
			Donor Site	Acceptor Site			
c.264+2T>C	T	CAGAG**g**t**g**ag	0.98	NC	Intron 2	(1) Inclusion of entire intron 2	p.V90SfsX67
	C	CAGAG**c**c**c**ag	<0.005	NC		(2) Activate cryptic site in intron 2 with inclusion of 34 bp 5′ of intron 2	p.V90SfsX40
c.348A>C	A	ATC**A**Ggtgca	0.26	NC	Exon 3	(1) Exclusion of entire exon 3	p.A89VfsX139
	C	ATC**C**Ggtgca	0.04	NC		(2) Inclusion of entire intron 3	p.N118AfsX82
c.709–2A>G	A	ctc**a**gGCCAC	NC	0.95	Intron 6	Activate cryptic site in exon 7 with inclusion of 87 bp 3′ of exon 7	p.A237TfsX6
	G	ctc**g**gGCCAC	NC	<0.005			
c.1179+2T>C	T	CAGAG**g**tata	0.73	NC	Intron 9	Activate cryptic site in intron 9 with inclusion of 35 bp 5′ of intron 9	p.V395YfsX29
	C	CAGAG**c**cata	<0.005	NC			
c.1414–2A>G	A	gcc**a**gGCTGT	NC	0.97	Intron 10	Inclusion of entire intron 10	p.A472VfsX10
	G	gcc**g**gGCTGT	NC	<0.005			

Abbreviations: bp, base pairs; NC, no change; NNSPLICE, Splice Site Prediction by Neural Network.[39]
[a] Exon in uppercase and intron in lowercase. Nucleotide variants are presented in boldface.
[b] Numbers in parentheses represent intensity ranking of the spliced polymerase chain reaction bands in Figure 2 of the accompanying article.

eTable 3. Functional Analysis and Prediction of Missense Variations[a]

Coding DNA NM_002087.2	Predicted Protein NP_002078.1	MAF in Ctrl (Chr)[b]	Property of AA		PGRN Domain[c]	Deleterious (Pathological) Prediction			RESCUE-ESE Analysis	Splice Assay
			Original	Substitute		PMut[d]	PolyPhen[e]	SIFT[f]		
c.26C>A	p.A9D	0.000 (0/500)	NP	Acidic	NA	**Yes** (0.62, 2)	**Yes** (1.63)	No (0.60)	NC	NC
c.55C>T	p.R19W	0.028 (13/450)	Basic	NP	P (no)	**Yes** (0.83, 6)	No (0.42)	**Yes** (0.02)	NC	ND
c.208G>A	p.G70S	0.001 (1/760)	NP	UP	G (no)	**Yes** (0.57, 1)	**Yes** (1.72)	No (0.58)	NC	ND
c.229G>A	p.V77I	0.000 (0/760)	NP	NP	G (no)	No (0.01, 9)	No (0.42)	No (0.21)	NC	ND
c.313T>C	p.C105R	0.000 (0/760)	UP	Basic	G (**yes**)	**Yes** (0.89, 7)	**Yes** (4.12)	No (0.11)	NC	NC
c.545C>T	p.T182M	0.000 (0/760)	UP	NP	NA	**Yes** (0.51, 0)	**Yes** (1.53)	No (0.12)	NC	NC
c.634C>T	p.R212W	0.001 (1/760)	Basic	NP	B (no)	**Yes** (0.88, 7)	**Yes** (1.64)	**Yes** (0.02)	**Addition**	ND
c.752C>G	p.T251S	0.000 (0/760)	UP	UP	B (no)	No (0.02, 9)	No (0.99)	No (0.75)	NC	NC
c.827C>T	p.A276V	0.000 (0/760)	NP	NP	NA	No (0.17, 6)	No (0.83)	No (0.33)	NC	**Yes**
c.893G>A	p.R298H	0.000 (0/760)	Basic	Basic	H (no)	**Yes** (0.70, 4)	No (0.49)	No (0.53)	NC	ND
c.970G>A	p.A324T	0.008 (3/367)	NP	UP	A (no)	No (0.34, 3)	No (0.02)	No (0.55)	NC	ND
c.1058G>A	p.S353N	0.001 (1/760)	UP	UP	NA	No (0.02, 9)	No (1.45)	No (0.58)	NC	ND
c.1070C>G	p.P357R	0.000 (0/760)	NP	Basic	NA	**Yes** (0.84, 6)	No (0.89)	No (0.54)	NC	ND
c.1253G>A	p.R418Q	0.002 (2/896)	Basic	UP	Q (no)	No (0.36, 2)	No (0.33)	No (0.58)	NC	NC
c.1297C>T	p.R433W	0.005 (9/1690)	Basic	NP	NA	**Yes** (0.97, 9)	**Yes** (1.91)	No (0.20)	NC	NC
c.1544G>C	p.G515A	0.004 (6/1406)	NP	NP	NA	**Yes** (0.62, 2)	No (1.12)	No (0.85)	NC	ND

Abbreviations: AA, amino acid; Chr, chromosome; Ctrl, controls; ESE, exon splicing enhancer; MAF, minor allele frequency; NA, not applicable; NC, no change; ND, not done; NP, nonpolar; PGRN, progranulin; UP, uncharged polar.
[a] Boldface type highlights positive results.
[b] Counts over number of chromosomes screened.
[c] Yes and no in parentheses indicate alteration of consensus sequence in motif.
[d] Neural network output and reliability scores are listed in parentheses.
[e] Positron-specific independent count score differences are listed in parentheses.
[f] Probabilities are listed in parentheses.

eTable 4. Functional Analysis and Prediction of Silent Variations

Coding DNA NM_002087.2	Protein NP_002078.1	MAF in Ctrl (Chr)[a]	RESCUE-ESE Analysis		Effect of Variant Allele	
			ESE Site	Sequence	Heterologous	Minigene
c.99C>T	p.D33	0.008 (11/1314)	Addition	TGGATC	No	NA
c.159G>A	p.L53	0.000 (0/760)	Addition	AAGCAG	No	NA
c.384T>C	p.D128	0.033 (60/1806)	NC	NA	NA	NA
c.903G>A	p.S301	0.003 (4/1406)	NC	NA	Yes	No

Abbreviations: Chr, chromosome; Ctrl, controls; ESE, exon splicing enhancer; MAF, minor allele frequency; NA, not available; NC, no change.
[a] Counts over number of chromosomes screened.