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Supplemental Data

A Missense Mutation in *CASK*

Causes FG Syndrome in an Italian Family

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Table S1 - *Primer pairs designed for CASK mutation screening*

Exon	*Primer*		*Amplicon (bp)*	*Annealing temperature (°C)*	*Start gradient solution B (%)*	*Column temperature (°C)*
1	F	gctgggcactgagctcttgg	199	60	48	65
	R	aagaccggggcatcactgaaat				
2	F	gggaaataggaacttaagagaatcaa	217	60	50	56.5
	R	atggaataaagccagacatcaaat				
3	F	tgttcctacctaatgaagtatgctgt	197	60	50	56.5
	R	accaacattgcttcaagttttacc				
4	F	cttcctggaatttcctcttttctt	168	60	48	57
	R	tttcaacttgacctctggtgatta				
5	F	ttataaactaggagcactgtatatt	200	60	52	53
	R	tcagtatttcttcacaaacatta				
6	F	atgaatccttatcttcgggtttgt	210	60	51	58
	R	gcaagtgaccaggttttgaaga				
7	F	aacatttttccttaatgttgtt	257	60	50	58.5
	R	caggataaattaatctcaatgg				
8	F	aatgttaatgtctcatttaaggtaactatg	225	60	51	60
	R	gaagtgttcagacaaagtgtagaagg				
9	F	gttttagattaagcttctgcac	236	60	51	56
	R	tacatatttatgggcaccaa				
10	F	agccatcatctcctatatatttgact	259	60	53	53
	R	atgacccacacaggtgaataagt				
11	F	ggcttgataacctgcttgttcta	161	60	51	58
	R	aacaactacacacaaacagccaag				
12	F	tgacattgactttctatactggctgtt	223	60	50	58.5
	R	ggccaatattcaattcaccaaaaa				
13	F	caaagaattgtgagtgtttactgga	179	60	50	56
	R	caataaaggtggcaaatatgatga				
14	F	ttgtaaatcttgtgtcatgtttgta	176	60	51	53
	R	gaaagtgaggtttcagttctatgg				
15	F	tacacagaaggctgcaaaggaa	470	60	55	58.5
	R	ccccaactcctagtagcataggtt				
16	F	ttatctaccgcaaaatcacaaata	202	60	52	54
	R	taaggaaggcaaagaaagaaat				
17	F	tgatgcctctggattttgttacta	382	60	55	55
	R	gttaaagcccacactgctaaagac				
18	F	aaacttttctttctttcccaccac	171	60	49	60
	R	tacagccatcagcagacagttagt				
19	F	ctggtgttctgtgttaatgaacct	157	60	50	58
	R	agcaccaacaaaattgtcacac				
20	F	ctttcttcagcttgctgctaaat	293	60	55	55
	R	gattggctattagctgctcagttt				
21	F	tctctttcacatcactatggcatc	320	60	56	56.5

	R	ataaaattggaaaatggatggtca				
22	F	tggatgactcttctattttatcaacc	227	60	51	59
	R	cttaacccagcctcagtaacagt				
23	F	aagccatatatcataagttggattc	270	60	52	54.5
	R	aatgttttgaaagtgagaataagca				
24	F	ttgcttattctcactttcaaaacatt	314	60	53	54.5
	R	aactcattttcctccttgttcttt				
25	F	actgggtttgagtaaatctgttgg	353	60	57 and 50	58 and 62
	R	gatttcagaatctgtgcttattgg				
26	F	tttcttctttaagtcgggatttt	174	60	50	56
	R	gcttgatccttacagcttatttgg				
27	F	acgaatccaactgatttctctctt	321	60	55	59
	R	taacaaagaggcttttccacaaat				
Promoter A	F	ctccctgcgattacaaaagaaggaaa	541	65	N/A	N/A
	R	CAGAGACGCTCCCTCCTCTTTCT				
Promoter B	F	GAGTGGGCGGAGCCTGTGGTTCT	594	65	N/A	N/A
	R	AGAGACTGCGGCGCCTTCCTCTG				
Promoter C	F	CGATCCTCGCTCCATGGTCCTG	574	65	N/A	N/A
	R	gaagaccggggcatcactgaaatg				

N/A = not analyzed by DHPLC.

Table S2 - *Mixes of pooled DNAs for CMS analysis*

#	***pooled DNAs***	***Notes***	***Heteroduplex***
1	CM	Healthy control male	
2	II.8	Carrier female from pedigree	*
3	III.26 + CM	Affected male (proband) from pedigree + control male	*
4	II.11 + CM	Affected male (maternal uncle) from pedigree + control male	*
5	II.17 + CM	Affected male (maternal uncle) from pedigree + control male	*
6	II.6 + II.9 + II.13 + II.18	Four unaffected males from pedigree	
7	II.4 + III.22	Two unaffected females from pedigree	
8	CF1 + CF2	Two unrelated healthy control females	
9	CF3 + CF4	Two unrelated healthy control females	
10	CF5 + CF6	Two unrelated healthy control females	
11	CF7 + CF8	Two unrelated healthy control females	
12	CF9 + CF10	Two unrelated healthy control females	

For members of the FGS family under study, DNA samples are numbered according to their position in the pedigree. An asterisk (*) indicates where the heteroduplex is expected for mutations co-segregating with the phenotype.

Table S3 - *Primer pairs designed for RT-PCR and real-time RT-PCR*

Forward		***Reverse***		***Amplicon (bp)***	***Annealing temperature (°C)***
CASK16/F	GTGCTGTTCGAGGATGTGTACGAG	CASK268/R	CCATGTAAAGCATTCCATCTGAGCTA	253 (140)*	63
CASK16/F	GTGCTGTTCGAGGATGTGTACGAG	CASK744/R	ACTTTCAGAGATATGGCTCCACTGC	729 (616)*	63
CASKsp1-2/F	GTGATCGGAAA**AG**GGTCCCTTC	CASK268/R	CCATGTAAAGCATTCCATCTGAGCTA	220	65
CASKsp1-3/F	CGAGGTGATCGGAAA**AA**TCTAAAGC	CASK398/R	TGGCAGTAGCGTAGAGCTTCCA	240	65
CASK2310/F	CAGACCTCCAAAGAAAGACGAAGAA	CASK2545/R	CAAAAGGAGCAAACTCTGCAGTTCT	236	65
GAPDH/F	GCCTGCTTCACCACCTTCTT	GAPDH/R	CGTAGGACGTGGTGGTTGAC	346	65

An asterisk (*) indicates the expected length for *CASK* exon 2-skipped products. Nucleotides at exon-exon junctions are typed in bold.

Table S4 - *Comparison of H-bonds network at the ligand-binding site*

CASK wild type		***CASK p.R28L***	
Donor	Acceptor	Donor	Acceptor
K37 (NZ-HZ3)	**E58 (OE1)**	**K37 (NZ-HZ3)**	**E58 (OE1)**
K37 (NZ-HZ3)	**E58 (OE2)**	**K37 (NZ-HZ3)**	**E58 (OE2)**
H 135 (NE2-HE2)	**G157 (O)**	**H 135 (NE2-HE2)**	**G157 (O)**
H 135 (NE2-HE2)	*D137 (O)*		
R136 (NH2-HH22)	**V161 (O)**	**R136 (NH2-HH22)**	**V161 (O)**
R136 (NH1-HH12)	**V161 (O)**	**R136 (NH1-HH12)**	**V161 (O)**
R136 (NE-H)	V161 (O)	R136 (NE-HE)	V161 (O)
R136 (NH2-HH22)	**Y192 (OH)**	**R136 (NH2-HH22)**	**Y192 (OH)**
		R136 (NE-HE)	Y192 (OH)
D137 (N-H)	**H135 (ND1)**	**D137 (N-H)**	**H135 (ND1)**
D137 (N-H)	**H135 (O)**	**D137 (N-H)**	**H135 (O)**
K139 (NZ-HZ3)	**D137 (OD1)**	**K139 (NZ-HZ3)**	**D137 (OD1)**
K139 (NZ-HZ3)	**D137 (OD2)**	**K139 (NZ-HZ3)**	**D137 (OD2)**
K139 (HZ1-HZ2)	**D137 (O)**	**K139 (NZ-HZ3)**	**D137 (O)**
		K139 (N-H)	D137 (O)
T178 (OG1-HG1)	**D137 (OD1)**	**T178 (OG1-HG1)**	**D137 (OD1)**
T178 (OG1-HG1)	**D137 (OD2)**	**T178 (OG1-HG1)**	**D137 (OD2)**
T178 (N-H)	**D137 (OD1)**	**T178 (N-H)**	**D137 (OD1)**
T178 (N-H)	**D137 (OD2)**	**T178 (N-H)**	**D137 (OD2)**
AMP (O3'-HAA)	**I14 (O)**	**AMP (03'-HAA)**	**I14 (O)**
AMP (N6-HA)	**M90 (N)**	**AMP (N6-HAC)**	**M90 (N)**
AMP (O2'-HAB)	*A93 (O)*		
AMP (O2'-HAB)	**H141 (O)**	**AMP (O2'-HAB)**	**H141 (O)**
AMP (O3'-HAA)	H141 (ND1)	AMP (O3'-HAA)	H141 (O)
AMP (O3'-HAA)	*H141 (O)*		
S20 (OG-HG)	*AMP (O2P)*		
S20 (OG-HG)	*AMP (O3P)*		
K37 (NZ-HZ3)	AMP (O5')	K37 (NZ-HZ3)	AMP (O2P)
K37 (NZ-HZ3)	AMP (O2P)	K37 (NZ-HZ3)	AMP (O3P)
M90 (N-H)	**AMP (N1)**	**M90 (N-H)**	**AMP (N1)**
		M90 (N-H)	AMP (N6)
		K139 (NZ-HZ3)	AMP (O1P)
H141 (NE2-HE2)	**AMP (O2P)**	**H141 (NE2-HE2)**	**AMP (O2P)**
H141 (NE2-HE2)	*AMP (O3P)*		
H141 (NE2-HE2)	**AMP (O1P)**	**H141 (NE2-HE2)**	**AMP (O1P)**
H141 (NE2-HE2)	*AMP(O5')*		

H-bonds important to stabilize functional protein geometry of the binding and catalytic site[29] are typed in bold, while H-bonds lost in the p.R28L mutant CaM-kinase domain of CASK are typed in italics.

Table S5 - *Comparison of the clinical signs observed in patients with CASK mutations*

	Najm at al.					*Piluso et al.*		
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient II.11	Patient II.17	Patient III.26
Ethnic origin	German (C)	German (C)	American (C)	Turkish	American (C)	Italian (C)	Italian (C)	Italian (C)
Sex	F	F	F	F	M	M	M	M
Living	yes	yes	yes	yes	Died at 2 weeks	yes	yes	yes
OFC (SD)	-2.7 (at birth)	-2 (at birth)	-2 (at birth)	-2 (at birth)	-1 (at birth)	N/D	N/D	+1.3 (at 30 months)
Length (SD)	+0.1 (at birth)	-1 (at birth)	-1.5 (at birth)	+0.6 (at birth)	-1.1 (at birth)	N/D	N/D	+0.2 (at 30 months)
Weight (SD)	-0.5 (at birth)	-1 (at birth)	-0.9 (at birth)	-0.5 (at birth)	+0.1 (at birth)	N/D	N/D	-0.6 (at 30 months)
Developmental delay	Severe	Severe	Moderate	Severe	N/A	Severe	Severe	Severe
Congenital hypotonia	-	+	-	+	+	+ (in infancy)	+ (in infancy)	+ (in infancy)
Speech	-	-	-	-	N/A	+	+	+
Walking	-	-	-	-	N/A	+	+	+
Feeding difficulties	+	+	+	+	+	-	-	-
Seizures	+	+	-	-	-	+	+	+
EEG	Hypersynchronous, low amplitude activity	Spike and wave discharges with slow background	N/A	Normal	N/A	Widely abnormal	N/D	Mildly abnormal
Sensorineural hearing loss	+	+	-	-	N/A	+	N/A N/D	+
MRI scan	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal	Normal		Normal
Facial anomalies	Hypertelorism, broad nasal bridge and tip, smooth philtrum, large ears, small jaw, all mild	Broad nasal bridge, large ears, both mild	Plagiocephaly	Broad nasal bridge and tip, large ears, small jaw, all mild	Downslanting palpebral fissures, small jaw (father with small jaw)	Prominent forehead, hypertelorism, high broad long philtrum	Prominent forehead, frontal upsweep of the hair, long nasal philtrum.	Prominent forehead and frontal upsweep of the hair, hypertelorism, saddled root of the nose with a long philtrum and half-open mouth, micrognathia
Other abnormalities	Episodic hyperpnea, mild regression of motor skills at 2 years	Scoliosis	Spasticity	-	Absent gag, no suck, hypoventilation, apnea	Severe constipation	Severe constipation, bilateral epicantus, cryptorchidism	Severe constipation, scoliosis

+ = present; – = absent; C = Caucasian; N/A = not analyzed; N/D = not documented; SD = standard deviation.

Clinical data for patients 1-5 were previously reported by Najm et al.[67]. Clinical data for the affected males of the FGS family under study (II.11, II.17 and III.26) were also previously reported by Piluso et al.[12].