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Brief communication

Investigation of next-generation sequencing technologies as a diagnostic tool for amyotrophic lateral sclerosis

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ABSTRACT

The future of genetic diagnostics will see a move toward massively parallel next-generation sequencing of a patient's DNA. Amyotrophic lateral sclerosis (ALS) is one of the diseases that would benefit from this prospect. Exploring this idea, we designed a screening panel to sequence 25 ALS-linked genes and examined samples from 95 patients with both familial and sporadic ALS. Forty-three rare polymorphisms were detected in this cohort. A third of these have already been reported with respect to ALS, leaving 28 novel variants all open for further investigation. This study highlights the potential benefits of next-generation sequencing as a reliable, cost and time efficient, diagnostic, and research tool for ALS.

1. Introduction

Rapid diagnosis of amyotrophic lateral sclerosis (ALS) and other neurologic disorders is vital if future treatments are to be applied at an early disease stage. For genetic causes of these diseases, the current technology lies with sequential Sanger sequencing. However, with an array of multiple genes causing each disease and, additionally, numerous alterations within each gene being potentially harmful, it can be time consuming and costly to diagnose a patient suspected of harboring a detrimental genetic variation. Furthermore, the range of genetic tests at each institution can be limited. It is now plausible that next-generation sequencing (NGS) technologies will eliminate many of these issues. To test this possibility, we have developed a single comprehensive assay containing 25 genes which have, to varying degrees, been implicated in ALS.

2. Methods

2.1. Probe design

The ALS gene panel was designed using Illumina TruSeq Custom Amplicon and implemented on an Illumina MiSeq platform. This utilizes polymerase chain reaction amplicon-based target enrichment and screens for variants in 25 ALS disease genes. These were split into 2 groups depending on the desired coverage. The first

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Table 1
A list of the variants detected in this study

Gene	Variant	AA change	No. of patients	PhyloP prediction	SIFT prediction	PolyPhen prediction	Mutation taster prediction	dbSNP	ExAC frequency in European (non-Finnish)	Quality score	Genomic position	Disease-causing (number of patients)	Patient status (number of patients)	Reference if previously reported
ALS2	T1081C	S361P	1	N	T	B	N	—	0	2283	202625636	No	SALS	
ALS2	A280G	I94V	3	N	T	B	N	rs3219154	0.02	391–897	202626437	Yes (1); no (2)	SALS (3)	Hand et al. (2003), NS; Herzfeld et al. (2009), spastic paraplegia
ALS2	G3905A	R1302H	1	N	—	B	N	—	0.00003	256	202580494	No	FALS	
BSCL2	T1280C	L427P	2	N	T	—	N	rs145649423	0.006	1066–1263	62457948	Unknown	FALS (1); SALS (1)	
BSCL2	C629T	S210L	1	C	T	—	D	—	0.00001	3992	62462041	Unknown	SALS	
CEP112	C1759T	R587C	1	C	D	D	D	rs77905043	0.0007	2778	63957694	Unknown	SALS	
CEP112	A2080G	I694V	1	N	T	B	D	rs74004953	0.00006	398	63898353	Unknown	FALS	
CEP112	C1091G	A364G	1	C	T	D	D	rs146009782	0.008	1387	64049981	Unknown	SALS	
CEP112	T1985C	V662A	1	C	T	P	N	rs144307988	0	1004	63898448	Unknown	SALS	
FUS	C1561T	R521C	1	N	D	D	D	rs121909670	0	2690	31202739	Yes	FALS	Vance et al. (2009); Drepper et al. (2009); Suzuki et al. (2010)
MATR3	C460T	P154S	1	C	T	B	N	—	0	2372	138643564	Yes	SALS	Johnson et al (2014).
OPTN	T1340G	M447R	1	C	D	D	D	—	0	2363	13169842	Likely	FALS	
OPTN	1401+2T>G	N/A	1	—	—	—	—	—	0	3818	13169905	Likely	SALS	Del Bo et al. (2011), similar mutation
PON1	A55G	N19D	1	N	T	B	N	rs141948033	0.002	362	94953733	No	SALS	
PON2	286delA	R96fs	1	—		—	—	—	0	2390	95041705	No	SALS	
PRPH	G26A	R9Q	3	C	D	P	D	rs57451017	0.01	2524–5762	49689009	Likely	FALS (1), SALS (2)	Gros-Louis et al. (2004).
SOD1	T341C	I114T	2	C	—	D	D	rs121912441	0	727–1100	33039672	Yes	FALS (2)	Gellera et al. (2001); Stewart et al. (2006)
SPG11	G6319A	V2107I	2	C	T	D	D	rs115970214	0.00007	2611–8675	44864905	Unknown	SALS (2)	
SPG11	G4923C	K1641N	2	N	T	D	D	rs150218102	0.00004	4223–5060	44878032	Unknown	SALS (2)	
SPG11	A6224G	N2075S	1	N	T	B	N	rs140824939	0.004	2630	44865000	Unknown	SALS	
SPG11	G808A	V270I	2	C	T	P	N	rs80338868	0.007	6261–6704	44949354	Unknown	SALS (2)	Stevanin et al. (2008), NS
SPG11	G1108A	E370K	3	C	T	P	N	rs77697105	0.02	2095–5475	44944037	Unknown	SALS (3)	
SPG11	A6944C	N2315T	1	C	T	D	D	—	0.0001	757	44858107	Unknown	SALS	
SPG11	A6943C	N2315H	1	C	T	D	D	—	0	227	44858108	Unknown	SALS	
SPG11	A5204G	H1735R	1	C	T	D	D	—	0	3765	44876674	Unknown	SALS	
SPG11	C7168T	P2390S	1	C	T	D	D	—	0	673	44855483	Unknown	SALS	
SPG11	A3037G	K1013E	2	C	T	B	N	rs111347025	0.01	827–1740	44907562	Unknown	SALS (2)	
SPG11	G2083A	A695T	1	C	D	P	N	rs78183930	0.02	248	44918690	Unknown	SALS	
TARDBP	T14C	L5P	2	—	—	—	—	rs61730366	0.006	193–259	11073982	Likely	FALS (1), SALS (1)	Guerreiro et al. (2008); Gijselinck et al. (2009); Kirby et al. (2010).
VEGFA	G334C	G112R	1	N	—	D	N	—	0	777	43738777	No	SALS	

ExAC frequencies from Exome Aggregation Consortium, Cambridge, MA (URL: http://exac.broadinstitute.org) [accessed 10/12/14].
Key: FALS, familial amyotrophic lateral sclerosis; NS, not significant; SALS, sporadic amyotrophic lateral sclerosis; SIFT, sorting intolerant from tolerant.