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Molecular classification of amyotrophic lateral sclerosis by unsupervised clustering of gene expression in motor cortex

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive and ultimately fatal neurodegenerative disease, caused by the loss of motor neurons in the brain and spinal cord. Although 10% of ALS cases are familial (FALS), the majority are sporadic (SALS) and probably associated to a multifactorial etiology. Currently there is no cure or prevention for ALS. A prerequisite to formulating therapeutic strategies is gaining understanding of its etio-pathogenic mechanisms. In this study we analyzed whole-genome expression profiles of 41 motor cortex samples of control (10) and sporadic ALS (31) patients. Unsupervised hierarchical clustering was able to separate control from SALS patients. In addition, SALS patients were subdivided in two different groups that were associated to different deregulated pathways and genes, some of which were previously associated to familiar ALS. These experiments are the first to highlight the genomic heterogeneity of sporadic ALS and reveal new clues to its pathogenesis and potential therapeutic targets.

Introduction

Amyotrophic lateral sclerosis (ALS) is a fast progressive and disabling neurodegenerative disease characterized by upper and lower motor neuron loss, leading to respiratory insufficiency and death after 3–5 years (Mitchell and Borasio, 2007). The incidence of ALS ranges from 1.7 to 2.3 cases per 100,000 population per year world-wide (Beghi et al., 2006). Despite intensive research, knowledge of the pathogenetic mechanisms and precise genetic causes of ALS remains incomplete. Although most cases of ALS are isolated or sporadic (SALS), about 10% are familial (FALS) and have been linked to the mutation of several genes (Abel et al., 2012; Andersen and Al-Chalabi, 2011; Lill et al., 2011; Simpson and Al Chalabi, 2006; Valdmanis and Rouleau, 2008; Yoshida et al., 2010), such as SOD1 (Rosen, 1993), ALSIN (Hadano et al., 2001), SETX (Chance et al., 1998; Chen et al., 2004), SPG11 (Orlacchio et al., 2010), FUS (Kwiatkowski et al., 2009; Vance et al., 2009), VAPB (Nishimura et al., 2004), ANG (Chen et al., 2010; Greenway et al., 2006), TARDBP (Gitcho et al., 2008; Kabashi et al., 2008; Sreedharan et al., 2008), Fig. 4 (Chow et al., 2009), OPTN (van Es et al., 2009), ATXN2 (Elden et al., 2010), and C9ORF72 (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Although the etiology of SALS remains largely unknown, a number of observations suggest a role for genetic factors in SALS (Andersen and Al-Chalabi, 2011). While FALS genes may account for some cases of SALS, this is currently viewed as a multi-factorial complex disease, in which multiple genetic variants, each of small effect, combine with environmental triggers and risk factors (Andersen and Al-Chalabi, 2011; Armon, 2001; Majoor-Krakauer et al., 2003; Simpson and Al Chalabi, 2006).

The pathogenic processes underlying ALS are not fully determined. In the last few years, a number of transcriptome studies in peripheral cells or postmortem nervous tissue of ALS patients have started to decipher genes and pathways involved in disease pathogenesis (Cox et al., 2010; Dangond et al., 2004; Jiang et al., 2005; Lederer et al., 2007; Malaspina et al., 2001; Offen et al., 2009; Rabin et al., 2010; Wang et al., 2006). Although comparison of results is often difficult, because of different tissues and/or microarray platforms, common alterations implicated by these transcriptome studies were related to the cytoskeleton, inflammation, protein turnover and RNA splicing (Saris et al., 2013). Due to the inherent complexity of nervous tissue and the need for postmortem material, however, the existing genomics studies of ALS were restricted to a limited number of postmortem ALS samples (≤11 motor cortex, and 14 spinal cord) (Cox et al., 2010; Dangond et al., 2004; Jiang et al., 2005; Lederer et al., 2007; Malaspina et al., 2001; Offen et al., 2009; Rabin et al., 2010; Wang et al., 2006). To uncover the entire spectrum of genes and pathways involved in ALS pathology

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we analyzed whole-genome expression profiles of motor cortex samples from control (10) and SALS (31) patients (Table 1). By unsupervised hierarchical clustering we separate control from ALS patients, and subdivide the latter in two different groups that are associated to differentially expressed genes and pathways.

Materials and methods

Characteristics of subjects

Two groups of patient samples were used in this study: 31 motor cortex from SALS patients, and 10 motor cortex from control individuals. Fresh-frozen samples were obtained from the department of Pathology of the Academic Medical Center (University of Amsterdam) and selected for post-mortem intervals (PMI) prior to freezing not exceeding 24 h (mean PMI: 7.07 h for controls and 6.62 h for SALS). All SALS patients (mean patient age of 57) fulfilled the El Escorial diagnostic criteria (Brooks et al., 2000) and underwent genetic screening for genes associated to ALS. Diagnosis was independently confirmed by two neuropathologists according to standard histopathological criteria (Ince et al., 1998; Piao et al., 2003). The control samples (mean patient age of 55 years) were obtained from patients who had died from a non-neurological disease (cause of death: myocardial infarction, renal failure, pulmonary embolism). Both ALS and control patients included in the study displayed no signs of infection before death. Informed consent was obtained for the use of brain tissue and for access to medical records for research purposes and approval was obtained from the relevant local ethical committees for medical research.

Detailed information related to origin, source code, age, gender, race, disease state, survival time from diagnosis date and PMI of patient samples is given in Table 1.

Sample preparation

Individual slices of 10 μm were produced from tissue samples at −20 °C by a Leica CM1510S cryostat (Leica Microsystems) and stored at −80 °C until further processing. Two slices per sample were stained by hematoxylin/eosin staining (Bio Optica) and for Nissl substance (with a microfiltered solution of cresyl violet, Sigma-Aldrich), respectively, to assess integrity of cellular and tissue morphology. Ten adjacent slices per sample were pooled and used for RNA extraction with Trizol (Life Technologies) RNA integrity was confirmed by using a RNA chip and a 2100 Bioanalyzer (Agilent Technologies, Italy) with the protocol outlined by the manufacturer.

Table 1
Characteristics of subjects.

Patient code	Race	Gender	Age	PMI (hours)	Disease state	Survival time from diagnosis date (months)	Unsupervised cluster
1	Caucasian	Male	31	8	Control	n/a	Control
2	Caucasian	Male	59	7	Control	n/a	Control
3	Caucasian	Male	68	8	Control	n/a	Control
4	Caucasian	Female	71	9	Control	n/a	Control
5	Caucasian	Male	48	4	Control	n/a	Control
6	Caucasian	Male	58	7	Control	n/a	Control
7	Caucasian	Male	60	6.5	Control	n/a	Control
8	Caucasian	Male	44	9	Control	n/a	SALS 1
9	Caucasian	Male	73	10	Control	n/a	SALS 2
10	Caucasian	Male	39	8	Control	n/a	SALS 2
11	Caucasian	Male	67	8	SALS	90	SALS 1
12	Caucasian	Male	41	10	SALS	96	SALS 1
13	Caucasian	Male	65	6.5	SALS	38	SALS 1
14	Caucasian	Male	68	6	SALS	30	SALS 1
15	Caucasian	Female	67	8	SALS	27	SALS 1
16	Caucasian	Male	43	6	SALS	38	SALS 1
17	Caucasian	Male	54	3	SALS	31	SALS 1
18	Caucasian	Male	38	7	SALS	42	SALS 1
19	Caucasian	Male	45	6.5	SALS	38	SALS 1
20	Caucasian	Female	46	7	SALS	31	SALS 1
21	Caucasian	Female	65	7	SALS	52	SALS 1
22	Caucasian	Male	54	8	SALS	49	SALS 1
23	Caucasian	Male	51	4	SALS	60	SALS 1
24	Caucasian	Male	69	10	SALS	20	SALS 1
25	Caucasian	Male	68	7	SALS	18	SALS 1
26	Caucasian	Female	68	8	SALS	22	SALS 1
27	Caucasian	Male	61	3	SALS	11	SALS 1
28	Caucasian	Female	57	4	SALS	7	SALS 1
29	Caucasian	Female	40	5	SALS	130	SALS 2
30	Caucasian	Male	41	3	SALS	72	SALS 2
31	Caucasian	Female	61	6	SALS	43	SALS 2
32	Caucasian	Female	61	10	SALS	29	SALS 2
33	Caucasian	Female	51	7	SALS	29	SALS 2
34	Caucasian	Male	63	7.5	SALS	27	SALS 2
35	Caucasian	Female	70	4	SALS	30	SALS 2
36	Caucasian	Female	69	9	SALS	52	SALS 2
37	Caucasian	Female	64	5.3	SALS	71	SALS 2
38	Caucasian	Male	46	7.5	SALS	48	SALS 2
39	Caucasian	Male	55	6	SALS	18	SALS 2
40	Caucasian	Male	51	8	SALS	23	SALS 2
41	Caucasian	Male	59	8	SALS	13	SALS 2

Fresh-frozen motor cortex samples were obtained from the Department of Neuropathology of the Academic Medical Center, University of Amsterdam, The Netherlands. Control patients died from a non-neurological disease (myocardial infarction, renal failure, or pulmonary embolism). All patients included in the study displayed no signs of infection before death. Informed consent was obtained for the use of brain tissue and for access to medical records for research purposes, approval was obtained from the local ethical committees for medical research.