Current Trends in Neurology
Vol. 8, 2014

Original Communication

Segmental copy number loss of the PCSK6 gene in sporadic amyotrophic lateral sclerosis

Hidenori Sato[1], Shigeki Arawaka[1], Manabu Wada[1], Toru Kawanami[1], Fumiaki Tanaka[2], Gen Sobue[2], Mitsuru Emi[3] and Takeo Kato[1,*]

[1]Department of Neurology, Hematology, Metabolism, Endocrinology and Diabetology, Yamagata University, Faculty of Medicine, Yamagata, Japan; [2]Department of Neurology, Nagoya University School of Medicine, Nagoya, Japan. [3]Cancer Research Center, University of Hawaii, Honolulu, USA.

ABSTRACT

Sporadic amyotrophic lateral sclerosis (SALS) is regarded as a multifactorial disease; both genetic and environmental factors may contribute to the pathogenesis of SALS. To identify a possible genetic change that confers risk for SALS, we conducted whole-genome screening of a copy-number variation (CNV) with a CNV beadchip and a high-density oligonucleotide tiling microarray, followed by real-time quantitative polymerase chain reaction (qPCR). In the 1.1- to 5.5- kb region within the gene encoding the proprotein convertase subtilisin/kexin type 6 (PCSK6) on the chromosome 15q26.3 subtelomere, we found a copy-number loss in a large proportion (8 of 11; 72%) of SALS patients. Subsequent tiling microarray validated the results and revealed the fine structure of segmental loss. qPCR analysis confirmed the copy-number loss in 13 out of 23 SALS patients, as compared with 7 out of 44 controls ($p = 0.0015$, Odds Ratio: 6.63, 95%CI: 1.89–25.72). The present study suggests that a segmental copy-number loss of the PCSK6 gene may play a role in the pathogenesis of SALS.

KEYWORDS: ALS, amyotrophic lateral sclerosis, copy-number variation, deletion, motor neuron, PCSK6, polymorphism, subtelomere

*Corresponding author: tkato@med.id.yamagata-u.ac.jp

1. INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an adult-onset, fatal neurodegenerative disease which is characterized neuropathologically by the degeneration of motor neurons in the brain and spinal cord. ALS has two subgroups: familial ALS (FALS) and sporadic ALS (SALS). FALS is a rare disease which is inherited in a Mendelian fashion, and several causal genes have been identified [1, 2]. The majority (90–98%) of ALS cases are SALS. Although the etiology of SALS remains to be determined, the "British motor neuron disease twin study" demonstrated the importance of a genetic component in SALS [3]. Genome-wide association studies using single nucleotide polymorphisms (SNPs) have been performed, and some SNPs showed significant associations with SALS [4-8]; however, others did not after correcting for multiple testing [9-11]. Moreover, even the SNPs significantly associated with SALS were not necessarily replicated in the later studies [11, 12].

Recently, copy-number variations (CNVs) have been reported as inter-individual genetic variations [13]. Several CNV abnormalities also seem to be significant in the etiology of sporadic and inherited diseases [reviewed in 14]. To identify a possible genetic change that confers risk for SALS, we performed a whole-genome analysis for CNV and found a copy number gain within the region of the isopentenyl diphosphate isomerase (IDI) genes in many patients with SALS [15]. Here, we report

another gene, the proprotein convertase subtilisin/kexin type 6 (PCSK6) gene, a segmental copy-number loss of which was significantly associated with SALS.

2. MATERIALS AND METHODS

2.1. Subjects

Fifty-nine patients with SALS were included in this study. These patients fulfilled the criteria for probable or definite ALS according to the 1994 El Escorial criteria [16]. 104 community-dwelling elderly individuals with no neurological diseases were randomly selected as control [17, 18]. No significant differences in the mean age (SALS/control = 60.6/70.0 years) or the ratio of men to women (SALS/control = 0.68/0.76) were observed between the two groups ($p > 0.05$ in both). One-hundred patients with Parkinson's disease were also included as disease-control [19]. DNA was extracted and purified from peripheral blood. Written informed consent for genetic analysis was obtained from all the subjects. The Medical Ethics Committee of Yamagata University, Faculty of Medicine approved the present study.

2.2. Screening with a whole-genome CNV beadchip

Whole-genome screening was conducted by using the deCODE-Illumina CNV beadchip (57K, i-select format, Illumina Infinium system, deCODE Genetics, Inc.). The CNV probes were designed to target CNV-rich regions of the whole genome, such as megasatellites, duplicons, unSNPable regions and CNVs registered in the Database of Genomic Variants. The regions contained 15,559 CNV segments, which cover 190 Mb or 6% of the human genome. CNV data were analyzed by using the DosageMiner program as described previously [20].

2.3. Analysis with high-density oligonucleotide tiling microarray

Agilent 400 K high-density oligonucleotide tiling microarray assay was conducted on a CNV-enriched designed microarray (product #ID 02185, Agilent Technology, Inc.), which targets CNV-rich regions, median probe spacing 5 kb and average probe spacing 7 kb, in the whole-genome region. HapMap genome DNA [NA19000, JPN] was used as reference genome DNA. The intensity data were calculated by the ratio of the target genome intensities to the reference genome intensities. Abnormal copy number (loss or gain) was detected by deviation of probe $\log_2$ ratios exceeding a given threshold of 1 SD from the mean probe ratio [21, 22].

2.4. Real-time quantitative PCR (qPCR)

Copy numbers of the region of the PCSK6 gene on 15q26.3 were assayed by using TaqMan Gene Copy Number Assays of Life Technologies in basic qPCR according to the instructions of the manufacturer. The primers and probes were designed from the genomic sequence (hg18/Build 36) using proprietary web-based assay search tools (https://bioinfo.appliedbiosystems.com/genome-database/copy-number-variation.html). Each assay was run as a triplex TaqMan real-time qPCR reaction by using an FAM dye–based assay targeted to the region of the PCSK6 gene (Hs03912091_cn) and its flanking region (Hs0393814_cn) and a VIC dye–based assay for the reference gene, RNase P (PN 4316844 from Life Technologies). Each qPCR assay was performed in triplicate. Data were analyzed as described previously [23].

3. RESULTS

In search of a SALS-associated CNV, we performed whole-genome screening with the CNV beadchip for 11 SALS patients and 104 controls. We found

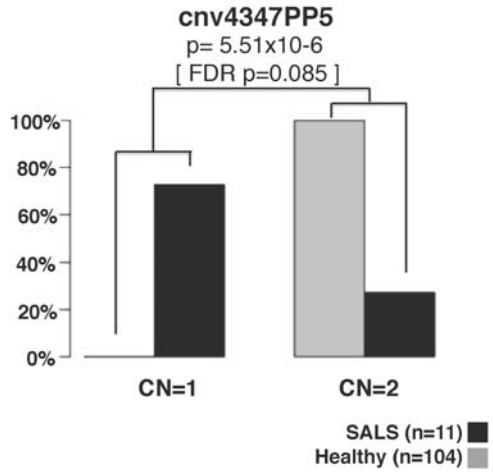

Figure 1. Frequency of copy number (CN) of the marker cnv4347PP5 in patients with sporadic amyotrophic lateral sclerosis (SALS) and healthy controls. The cnv4347PP5 is located in 3' exonic region of the PCSK6 gene. CNs were estimated by the deCODE-Illumina CNV beadchip (deCODE Genetics). CN = 1: one copy, CN = 2: two copies.

several CNV markers that were significantly associated with SALS. Among them, the marker cnv4347PP5 was located within the region of the PCSK6 gene on the 15q26.3 subtelomere. Eight of the 11 SALS patients showed a copy-number loss at the CNV marker on the PCSK6 gene region, as compared with 0 out of 104 control samples ($p = 5.51 \times 10^{-6}$ by logistic regression, $p < 0.1$ after false discovery rate correction) (Figure 1).

We then analyzed the structure of the copy-number change along the 15q26.3 subtelomeric region by using the high-density oligonucleotide tiling microarray in 50 SALS patients and 20 healthy controls. A segmental copy-number loss of 1.1–5.5 kb (Figure 2A, Figure 2B) in the 15q26.3 subtelomeric region was found in 15 of the SALS patients examined but in none of the control subjects ($p = 0.00629$, OR = 17.9, 95%CI: 1.02–315.15, Table 1).

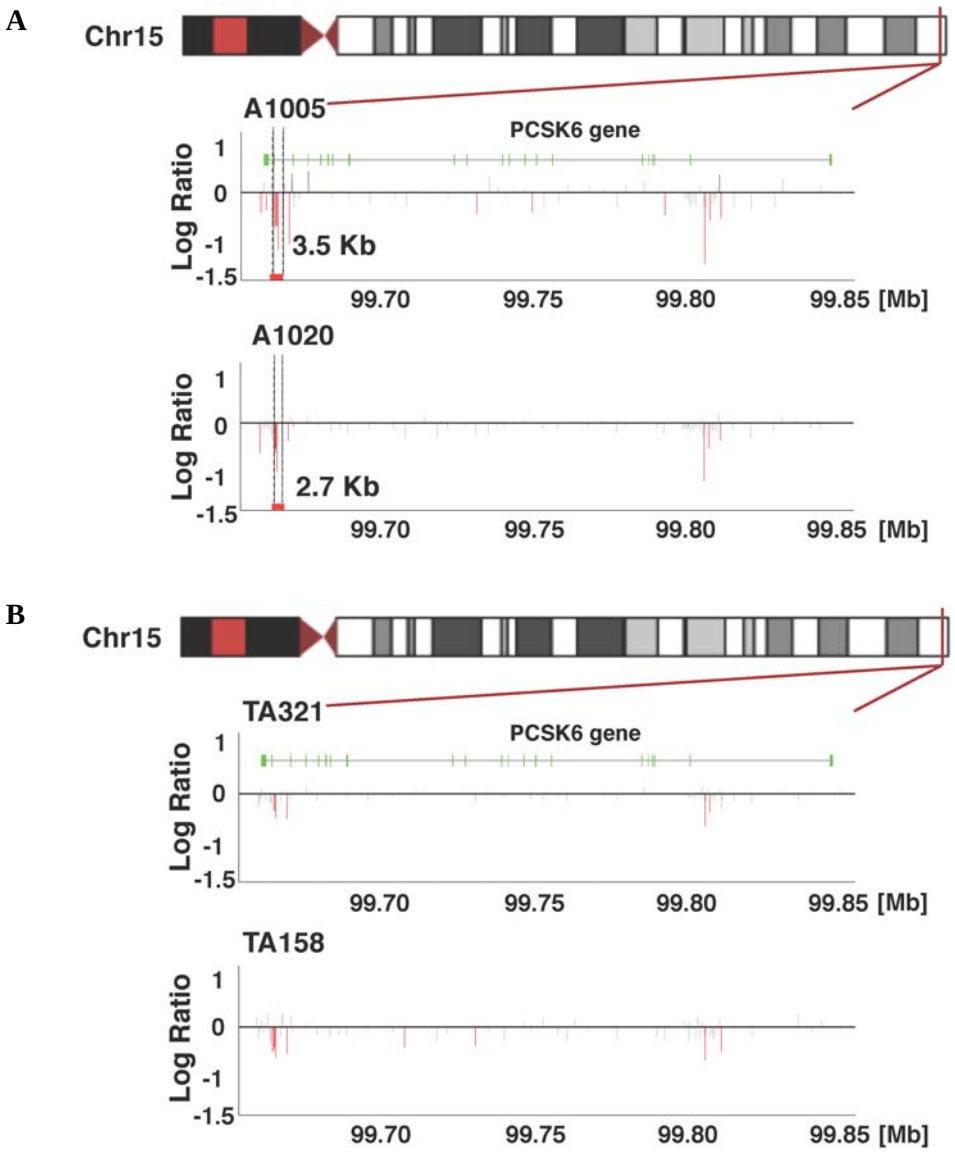

Figure 2. Fine structure of segmental copy-number loss in the PCSK6 gene region in two representative cases of SALS (A1005 and A1020) (A). The results of two healthy controls (TA321 and TA158) are shown in (B). The vertical red lines show average Log_2 Ratio. The vertical dot lines indicate the range of copy-number loss.

Table 1. Segmental copy number loss of PSCK6 gene in SALS and healthy subjects.

	CN = 1	CN = 2
SALS (n = 50)	15	35
Healthy (n = 20)	0	20

p = 0.00629, OR = 17.9 (1.02-315.15)

CN: estimated copy-number.
SALS: sporadic amyotrophic lateral sclerosis.

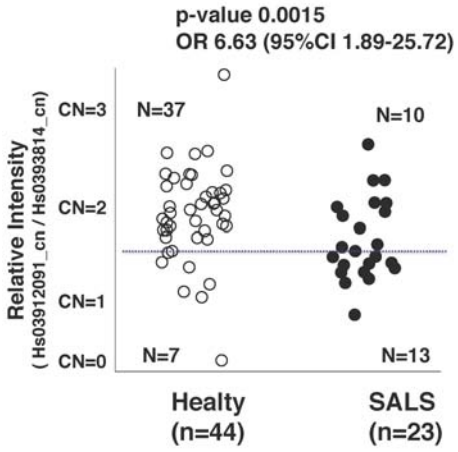

Figure 3. Quantitative PCR analysis for estimating the copy number in the region (Hs03912091_cn) of the PCSK6 gene. Its flanking region (Hs0393814_cn) is used as reference.

This region with copy-number loss contained the 3' exonic region of the PSCK6 gene (NM_138319 and NM_002570). A segmental copy-number loss in the PSCK6 gene was confirmed in 13 out of 23 (56%) SALS patients by basic real-time qPCR, as compared with 7 out of 44 controls (p = 0.0015, OR = 6.63, 95%CI: 1.89–25.72) (Figure 3). To test whether the copy-number change of the PSCK6 gene region was specific to SALS, we analyzed DNA from a total of 100 patients with Parkinson's disease. The results showed that no such change was observed in any cases of Parkinson's disease.

We have previously reported a copy-number gain in the region of the IDI genes on the 10p15.3 subtelomere in many patients with SALS [15]. We combined the present results (PCSK6 gene) with the previous results (IDI genes) designed using the same SALS-patients dataset. As shown

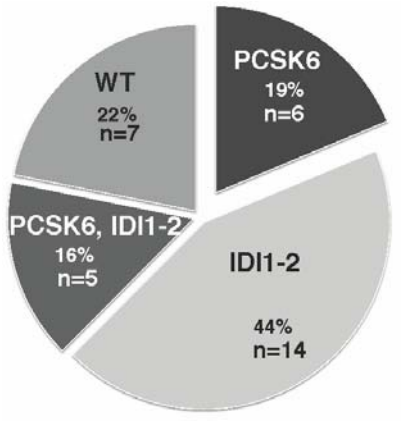

Figure 4. Frequency of copy-number loss of the PCSK6 gene region and/or copy-number gain of the IDI1-2 gene region in patients with SALS.
IDI1-2: isopentenyl diphosphate isomerase-1 and -2.

in Figure 4, among the 32 patients with SALS, 14 (44%) had a copy-number gain in the region of the IDI genes, and 6 (19%) showed a copy-number loss in the region of the PCSK6 gene. Twenty-five of 32 (78%) patients with SALS had a copy-number aberration of either gene or both.

4. DISCUSSION

The previously-reported genome-wide association studies on CNVs in SALS did not show a common CNV locus associated with SALS [24-26]. Those studies employed conventional platforms for genome-wide SNP genotyping, such as HumanHap300 and HumanHap550, which covered only 25% and 40% of the CNVs, respectively, resulting in the incapacity from capturing a large fraction of CNV [27]. CNVs not covered on the conventional SNP platforms were needed to do the examination [25]. We used a "CNV-targeted platform" that contains many CNV markers in the CNV-rich regions of the human genome. In the previous study, we reported a segmental copy-number gain in the IDI gene region on the 10p15.3 subtelomere in many SALS patients [15]. In the present study, we found another gene encoding PCSK6, the 3' region of which showed a segmental copy-number loss in 6 of 32 (19%) patients with SALS. 78% of the SALS patients had an aberration of either gene or both.

Many bioactive peptides are first synthesized as precursors that require proteolytic processing by the proprotein convertases [28]. The PCSK6

(also known as PACE4) gene, which is located on the chromosome 15q26.3 subtelomere, encodes a proprotein convertase that belongs to the subtilisin-like proprotein convertase family [29]. The PCSK6 protein has been reported to process several proteins, including neurotrophins (NTs), such as nerve growth factor (NGF) [30], neurotrophin 3 (NT3) [31], and brain-derived neurotrophic factor (BDNF) [31], from their latent precursors into biologically active products. The PCSK6 protein is expressed in various organs, including the brain and spinal cord [32, 33]. These NTs have been shown to regulate neuronal survival and death [34]. For example, NGF binds to its specific receptor (TrkA) and promotes neurite elongation and survival. On the other hand, proNGF, a precursor of NGF, binds to the p75 neurotrophin receptor (p75NTR) and induces apoptosis [34]. In the present study, a segmental copy-number loss was observed in the PCSK6 gene, suggesting that the function of PCSK6 as a proprotein convertase may be impaired. This may result in a decrease in the amount of biologically active, mature NTs and in an increase in the precursors of NTs. NTs and their receptors have been implicated as being involved in the pathological process of a mouse model of ALS and human SALS [35-39]. Indeed, a knockdown of p75NTR significantly delayed locomotor impairment and mortality in a mouse model of ALS [40, 41]. Taken together, it seems that neuronal death may be induced by the dysfunction of the PCSK6 gene in SALS.

CONCLUSION

In conclusion, we found a copy-number loss in the PCSK6 gene in a considerable number of patients with SALS. Further studies on the function of PCSK6 in motor neurons may contribute to the elucidation of the pathogenesis of SALS, leading to the identification of a novel therapeutic target of the disease.

ACKNOWLEDGEMENTS

This study was supported in part by Research on Measures for Intractable Diseases (ALS) from the Ministry of Health, Labour and Welfare of Japan.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

REFERENCES

1. Lagier-Tourenne, C., Polymenidou, M. and Cleveland, D. W. 2010, Hum. Mol. Genet., 19, R46.
2. Maruyama, H., Morino, H., Ito, H., Watanabe, Y., Kinoshita, Y., Kamada, M., Nodera, H., Suzuki, H., Komure, O., Matsuura, S., Kobatake, K., Morimoto, N., Abe, K., Suzuki, N., Aoki, M., Kawata, A., Hirai, T., Kato, T., Ogasawara, K., Hirano, A., Takumi, T., Kusaka, H., Hagiwara, K., Kaji, R. and Kawakami, H. 2010, Nature, 465, 223.
3. Graham, A. J., MacDonald, A. M. and Hawkes, C. H. 1997, J. Neurol. Neurosurg. Psychiatry, 62, 562.
4. van Es, M. A., van Vught, P. W., Blauw, H. M., Franke, L., Saris, C. G., Andersen, P. M., Van Den Bosch, L., de Jong, S. W., van't Slot, R., Birve, A., Lemmens, R., de Jong, V., Baas, F., Shelhass, H. J., Sleegers, K., Van Broeckhoven, C., Wokke, J. H., Wijmenga, C., Robberecht, W., Veldink, J. H., Ophoff, R. A. and van den Berg, L. H. 2007, Lancet Neurol., 6, 869.
5. Dunckley, T., Huentelman, M. J., Craig, D. W., Pearson, J. V., Szelinger, S., Joshipura, K., Halperin, R. F., Samper, C., Jensen, K. R., Letizia, D., Hesteriee, S. E., Pestronk, A., Levine, T., Bertorini, T., Graves, M. C., Mozaffar, T., Jackson, C. E., Bosch, P., McVey, A., Dick, A., Barohn, R., Lomen-Hoerth, C., Rosenfeld, J., O'connor, D. T., Zhang, K., Crook, R., Ryberg, H., Hutton, M., Katz, J., Simpson, E. P., Mitsumoto, H., Bowser, R., Miller, R. G., Appel, S. H. and Stephan D. A. 2007, N. Engl. J. Med., 357, 775.
6. van Es, M.A., van Vught, P. W., Blauw, H. M., Franke, L., Saris, C. G., Van den Bosch, L., de Jong, S. W., de Jong, V., Baas, F., van't Slot, R., Lemmens, R., Schelhaas, H. J., Birve, A., Sleegers, K., Van Broeckhoven, C., Schymick, J. C., Traynor, B. J., Wokke, J. H., Wijmenga, C., Robberecht, W., Andersen, P. M., Veldink, J. H., Ophoff, R. A. and van den Berg, L. H. 2008, Nat. Genet., 40, 29.
7. Cronin, S., Berger, S., Ding, J., Schymick, J. C., Washecka, N., Hernandez, D. G., Greenway, M. J., Mradley, D. G., Traynor, B. J. and Hardiman, O. 2008, Hum. Mol. Genet., 17, 768.

8. van Es, M. A., Veldink, J. H., Saris, C. G., Blauw, H. M., van Vught, P. W., Birve, A., Lemmens, R., Shelhaas, H. J., Groen, E. J., Huisman, M. H., van der Kooi, A. J., de Visser, M., Dahlberg, C., Estrada, K., Rivadeneira, F., Hofman, A., Zwarts, M. J., van Doormaal, P. T., Rujescu, D., Strengman, E., Giegling, I., Muglia, P., Tomik, B., Slowik, A., Uitterlinden, A. G., Hendrick, C., Waibel, S., Meyer, T., Ludolph, A. C., Glass, J. D., Purcell, S., Cichon, S., Nöthen, M. M., Wichmann, H. E., Schreiber, S., Vermeulen, S. H., Klemeney, L. A., Wakko, J. H., Cronin, S., McLaughlin, R. L., Hardman, O., Fumoto, K., Pasterkamp, R. J., Meininger, V., Melki, J., Leigh, P. N., Shaw, C. E., Landers, J. E., Al-Chalabi, A., Brown, Jr. R. H., Robberecht, W., Andersen, P. M., Ophoff, R. A. and van den Berg, L. H. 2009, Nat. Genet., 41, 1083.
9. Schymick, J. C., Scholz, S. W., Fung, H. C., Britton, A., Arepalli, S., Gibbs, J. R., Lombardo, F., Matarin, M., Kasperaviciute, D., Hemandez, D. G., Crews, C., Brujin, L., Rothstein, J., Mora, G., Restagno, G., Chi, A., Singleton, A., Hardy, J. and Traynor, B. J. 2007, Lancet Neurol., 6, 322.
10. Cronin, S., Tomik, B., Bradley, D. G., Slowik, A. and Hardiman, O. 2009, Eur. J. Hum. Genet., 17, 213.
11. Chiò, A., Schymick, J. C., Restagno, G., Scholz, S. W., Lombardo, F., Lai, S. L., Mora, G., Fung, H. C., Britton, A., Arepalli, S., Gibbs, J. R., Nalls, M., Berger, S., Kwee, L. C., Oddone, E. Z., Ding, J., Crews, C., Rafferty, I., Washecka, N., Hernandez, D., Ferrucci, L., Bandinelli, S., Guralnik, J., Macciardi, F., Torri, F., Lupoli, S., Chanock, S. J., Thomas, G., Hunter, D. J., Gieger, C., Wichmann, H. E., Calvo, A., Mutani, R., Battisini, S., Giannini, F., Caponnetto, C., Mancardi, G. L., La Bella, V., Valentino, F., Monsurrò, M. R., Tedeschi, G., Marinou, K., Sabatelli, M., Conte, A., Mandrioli, J., Sola, P., Salvi, F., Bartolomei, I., Siciliano, G., Carlesi, C., Orrell, R. W., Talbot, K., Simmons, Z., Connor, J., Pioro, E. P., Dunkley, T., Stephan, D. A., Kasperaviciute, D., Fisher, E. M., Jabonka, S., Sendtner, M., Beck, M., Bruijn, L., Rothstein, J., Schmidt, S., Singleton, A., Hardy, J. and Traynor, B. J. 2009, Hum. Mol. Genet., 18, 1524.
12. Fernández-Santiago, R., Sharma, M., Berg, D., lllig, T., Anneser, J., Meyer, T., Ludolph, A. and Gasser, T. 2011, Neurobiol. Aging, 32, 511.e1.
13. Redon, R., Ishikawa, S., Fitch, K. R., Feuk, L., Perry, G. H., Andrews, T. D., Flegler, H., Shapero, M. H., Carson, A. R., Chen, W., Cho, E. K., Dallaire, S., Freeman, J. L., Gratacòs, J. R., Huang, J., Kalaitzopoulos, D., Komura, D., MacDonald, J. R., Marshall, C. R., Mei, R., Montgomery, L., Nishimura, K., Okumura, K., Shen, F., Somerville, M. J., Tchinda, J., Valsesia, A., Woodwark, C., Yang, F., Zhang, J., Zerjai, T., Zhang, J., Armengol, L., Conrad, D. F., Estivill, X., Tyler-Smith, C., Carter, N. P., Aburatani, H., Lee, C., Jones, K. W., Scherer, S. W. and Hurles, M. E. 2006, Nature, 444, 444.
14. Lupski, J. R. 2007, Nat. Genet., 39, S43.
15. Kato, T., Emi, M., Sato, H., Arawaka, S., Wada, M., Kawanami, T., Katagiri, T., Tsuburaya, K., Toyoshima, I., Tanaka, F., Sobue, G. and Matsubara, K. 2010, Biochem. Biophys. Res. Commun., 402, 438.
16. Brooks B. R. 1994, J. Neurol. Sci., 124 (Suppl.), 96.
17. Nagasawa, H., Wada, M., Arawaka, S., kawanami, T., Kurita, K., Daimon, M., Adachi, M., Hosoya, T., Emi, M., Muramatsu, M. and Kato, T. 2007, Eur. J. Neurol., 14, 428.
18. Iseki, C., Kawanami, T., Nagasawa, H., Wada, M., Koyama, S., Kikuchi, K., Arawaka, S., Kurita, K., Daimon, M., Mori, E. and Kato, T. 2009, J. Neurol. Sci., 277, 54.
19. Arawaka, S., Wada, M., Goto, S., Karube, H., Sakamoto, M., Ren, C-H., Koyama, S., Nagasawa, H., Kimura, H., Kawanami, T., Kurita, K., Tajima, K., Daimon, M., Baba, M., Kido, T., Saino, S., Goto, K., Asao, H., Kitanaka, C., Takashita, E., Hongo, S., Nakamura, T., Kayama, T., Suzuki, Y., Kobayashi, K., Katagiri, T., Kurokawa, K., Kurimura, M., Toyoshima, I., Tsuchiya, K., Iwatsubo, T., Muramatsu, M., Matsumine, H. and Kato T. 2006, J. Neurosci., 26, 9227.

20. Stefansson, H., Rujescu, D., Cichon, S., Pletilälnen, O. P., Ingason, A., Steinberg, S., Fossdai, R., Sigurdsson, E., Sigmundsson, T., Bulzer-Voskamp, J. E., Hansen, T., Jakobsen, K. D., Mugila, P., Francks, C., Matthews, P. M., Gylfason, A., Halldorsson, B. V., Gudbjartsson, D., Thorgeirsson, T. E., Sigurdsson, A., Jonasdottir, A., Jonasdottir, A., Bjornsson, A., Mattiasdottir, S., Blondal, T., Haraldsson, M., Magnusdottir, B. B., Giegling, I., Möller, H. J., Hartmann, A., Shianna, K. V., Ge, D., Need, A. C., Crombie, C., Fraser, G., Walker, N., Lonnqvist, J., Suvisaari, J., Tuulio-Henriksson, A., Paunio, T., Toulopoulou, T., Bramon, E., Di Forti, M., Murray, R., Ruggeri, M., Vassos, E., Tosato, S., Walshe, M., Li, T., Vasilescu, C., Mühleisen, T. W., Wang, A. G., Ullum, H., Djurovic, S., Melle, I., Olessen, J., Kiemeney, L. A., Franke, B., Sabatti, C., Freimer, N. B., Gulcher, J. R., Thorsteinsdottir, U., Kong, A., Andreassen, O. A., Ophoff, R. A., Georgi, A., Rietschel, M., Werge, T., Petursson, H., Goldstein, D. B., Nöthen, M. M., Peltonen, L., Collier, D. A., St. Clair, D. and Stefansson, K. 2008, Nature, 455, 232.
21. de Smith, A. J., Tsalenko, A., Sampas, N., Scheffer, A., Yamada, N. A., Tsang, P., Ben-Dor, A., Yakhini, Z., Ellis, R. J., Bruhn, L., Laderman, S., Froguel, P. and Blakemore, A. I. 2007, Hum. Mol. Genet., 16, 2783.
22. Sharp, A. J., Hansen, S., Selzer, R. R., Regan, R., Hurst, J. A., Stewart, H., Price, S. M., Blair, E., Hennekam, R. C., Fitzpatrick, C. A., Segraves, R., Richmond, T. A., Guiver, C., Albertson, D. G., Pinkel, D., Els, P. S., Schwartz, S., Knight, S. J. and Eichler, E. E. 2006, Nat. Genet., 38, 1038.
23. Livak, K. J. and Schmittgen T. D. 2001, Methods, 25, 402.
24. Blauw, H. M., Veldink, J. H., van Es, M. A., van Vught, P. W., Saris, C. G., van der Zwaag, B., Franke, L., Burbach, J. P., Wokke, J. H., Ophoff, R. A. and van den Berg, L. H. 2008, Lancet Neurol., 7, 319.
25. Cronin, S., Blauw, H. M., Veldink, J. H., van Es, M. A., Ophoff, R. A., Bradley, D. G., van den Berg, L. H. and Hardman, O. 2008, Hum. Mol. Genet., 17, 3392.
26. Wain, L. V., Pedroso I., Landers J. E., Breen, G., Shaw, C. E., Leigh, P. N., Brown, R. H., Tobin, M. D. and Al-Chalabi, A. 2009, PLoS ONE, 4, e8175.
27. Cooper, G. M, Zerr, T., Kidd, J. M., Eichler, E. E. and Nickerson, D. A. 2008, Nat. Genet., 40, 1199.
28. Steiner, D. F. 1998, Curr. Opin. Chem. Biol., 2, 31.
29. Kiefer, M. C., Tucker, J. E., Joh, R., Landsberg, K. E., Saltman, D. and Barr, P. J. 1991, DNA Cell. Biol., 10, 57.
30. Seidah, N. G., Benjannet, S., Pareek, S., Savaria, D., Hamelin, J., Goulet, B., Laliberte, J., Lazure, C., Chrétien, M. and Murphy, R. A. 1996, Biochem. J., 314, 951.
31. Seidah, N. G., Benjannet, S., Pareek, S., Chretien, M. and Murphy, R. A. 1996, FEBS Lett., 379, 247.
32. Zheng, M., Seidah, N. G. and Pintar J. E. 1997, Dev. Biol., 181, 268.
33. Hajebrahimi, Z., Mowla, S. J., Movahedin, M. and Tavallaei M. 2008, Neurosci. Lett., 441, 261.
34. Lee, R., Kermani, P., Teng, K. K. and Hempstead, B. L. 2001, Science, 294, 1945.
35. Anand P., Parrett A., Martin J., Zeman, S., Foley, P., Swash, M., Leigh, P. N., Cedarbaum, J. M., Lindsay, R. M., Williams-Chestnut, R. E. and Sinicropi, D. V. 1995, Nat. Med., 1, 168.
36. Küst, B. M., Copray, J. C. V. M., Brouwer, N., Troost, D. and Boddeke, H. W. G. M. 2002, Exp. Neurol., 177, 419.
37. Barbeito, L. H., Pehar, M., Cassina, P., Vargas, M. R., Peluffo, H., Viera, L., Estévez, A. G. and Beckman, J. S. 2004, Brain Res. Rev., 47, 263.
38. Copray, J. C., Jaarsma, D., Küst, B. M., Bruggeman, R. W., Mantingh, I., Brouwer, N. and Boddeke, H. W. 2003, Neuroscience, 116, 685.
39. Pehar, M., Cassina, P., Vargas, M. R., Castellanos, R., Vlera, L., Beckman, J. S., Estévez, A. G. and Barbeito, L. 2004, J. Neurochem., 89, 464.
40. Küst, B. M., Brouwer, N., Mantingh, I. J., Boddeke H. W. and Copray J. C. 2003, Amyotroph. Lateral Scler. Other Motor Neuron Disord., 4, 100.
41. Turner, B. J., Cheah, I. K., Macfarlane, K. J., Lopes, E. C., Petratos, S., Langford, S. J. and Cheema, S. S. 2003, J. Neurochem., 87, 752.