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Original Article

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Dynamic Transcriptional Events in Distal Sural Nerve Revealed by Transcriptome Analysis

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Compared with biochemical information available about the diseases in the central nervous system, that for peripheral neuropathy is quite limited primarily due to the difficulties in obtaining samples. Characterization of the core pathology is a prerequisite to the development of personalized medicine for genetically heterogeneous diseases, such as hereditary motor and sensory neuropathy (HMSN). Here, we first documented the transcriptome profile of distal sural nerve obtained from HMSN patients. RNA-seq analysis revealed that over 12,000 genes are expressed in distal sural nerve. Among them 4,000 transcripts are novel and 10 fusion genes per sample were observed. Comparing dataset from whole exome sequencing revealed that over 1,500 transcriptional base modifications occur during transcription. These data implicate that dynamic alterations are generated when genetic information are transitioned in distal sural nerve. Although, we could not find significant alterations associated with HMSN, these data might provide crucial information about the pathophysiology of HMSN. Therefore, next step in the development of therapeutic strategy for HMSN might be unveiling biochemical and biophysical abnormalities derived from those potent variation.

Key words: hereditary motor and sensory neuropathy (HMSN), peripheral nervous system (PNS), distal sural nerve, transcriptome

INTRODUCTION

Recent explosive advances in multi-omics have provided us with powerful tools to investigate the pathophysiological complexity of disease processes. In Mendelian diseases, the application of a single-omic approach, such as whole exome sequencing (WES), has dramatically reduced the time and effort required to identify causative mutations [1]. Thus, the next step, in which multi-omics are effectively applied to Mendelian diseases, might reveal biochemical and biophysical abnormalities that present therapeutic targets for personalized medicine.

Compared with the biochemical information available about the central nervous system (CNS), that available for the peripheral nervous system (PNS) is quite limited, thereby therapeutic approaches to the PNS diseases more difficult. Characterization of the core pathology, in conjunction with the identifications of genetic causes, is a prerequisite to the development of personalized medicine for genetically and clinically heterogeneous disorder, such as hereditary motor and sensory neuropathy (HMSN) [2].

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HMSN is frequently classified as type 1, demyelinating form (HMSN1), or type 2, axonal form (HMSN2). HMSN1 exhibits markedly reduced nerve conduction velocities (NCV), whereas HMSN2 shows slightly reduced or even normal NCVs. HMSN2 is occasionally difficult to distinguish phenotypically from distal hereditary motor neuropathy (dHMN) which is closely resembles HMSN but without sensory abnormality, because sensory signs are often lacking in HMSN2 patients [3].

Recent monumental attempts to decipher the transcriptome in the human brain have undoubtedly contributed to our anatomical understanding of the transcriptional network and disease-related changes in inaccessible tissues [4, 5]. Subsequent proteomic or metabolomic analyses will provide further crucial information in the near future. Therefore, documentation of the transcriptome in the human distal sural nerve might provide primary knowledge as a basis for understanding the pathophysiology of PNS diseases. Here, we performed the transcriptome analysis using distal sural nerve of HMSN patients.

PATIENTS AND METHODS

Patients

This study included a total of 14 individuals of Korean origin with HMSN or normal phenotype. Written informed consent was obtained from all the participants and from the parents of participants younger than 18 years, according to the protocol approved by the Institutional Review Board of Ewha Womans University, Mokdong Hospital (ECT 11-58-37).

Clinical assessments

The patients were examined for motor and sensory impairment, deep tendon reflexes, and muscle atrophy by two independent neurologists. The muscle strengths of the flexor and extensor were assessed manually using the Medical Research Council scale. Physical disabilities were expressed on two different scales: the Functional Disability Scale (FDS) [6] and Charcot-Marie-Tooth disease neuropathy score (CMTNS) [7] (see Table 1). Disease severity was determined using a nine-point FDS, which was based on the following criteria: 0, normal; 1, normal but with cramps and fatigability; 2, an inability to run; 3, walking difficulty but still possible unaided; 4, walking with a cane; 5, walking with crutches; 6, walking with a walker; 7, wheelchair bound; and 8, bedridden. In addition, we determined the CMTNS, and the patient was divided into mild (CMTNS≤10), moderate (CMTNS, 11-20), and severe (CMTNS≥21) categories.

Sural nerve biopsy

Sural nerves from 14 individuals were obtained by incision under sterile conditions and local anesthesia [8]. After obtaining about 10 mm length of nerve, sharp dissection followed to obtain pure sural nerve.

Transcriptome analysis

RNA-Seq data were generated from all 14 samples. The cDNA library was prepared using the TruSeq RNA Sample Preparation Kit (Illumina, San Diego, CA). The sequencing protocol consisted of total RNA extraction from a biopsy of the distal sural nerve and polyA RNA extraction, RNA fragmentation, random-hexamer-primed reverse transcription, and 100 nt paired-end sequencing with HiSeq 2000 (Illumina). To estimate the expression levels and to identify alternatively spliced transcripts, the RNA-Seq reads were mapped to the human genome using TopHat (version 1.3.3, http://tophat.cbcb.umd.edu/index.html). Then all variants were annotated by the ANNOVAR (version 2011Feb20) program. To determine whether the variants were previously reported, dbSNP135 (http://www.ncbi.nlm.nih.gov) was used. The transcript levels were calculated and the relative transcript abundances were measured in fragments per kilobase of exon per million fragments mapped (FPKM) using Cufflinks (http://cufflinks.cbcb.umd.edu/index.html). The deFuse program (version 0.4.3, http://sourceforge.net/apps/mediawiki/defuse) was used to identify gene fusions from the RNA-Seq data because this program considered all alignments and all possible locations for fusion boundaries [9].

Whole-exome sequencing

Total DNA was purified from peripheral blood with a QIAamp Blood DNA Purification Kit (Qiagen, Hilden, Germany). The whole exome was captured using SeqCap EZ V3.0 (Roche-NimbleGen, Madison, WI), and sequencing was performed using the HiSeq 2000 Genome Analyzer (Illumina). The UCSC assembly hg19 (NCBI build 37.1) was used as the reference sequence. Variant calling was achieved for those with ≥20 SNP quality. The variants were annotated with the ANNOVAR program.

RESULTS AND DISCUSSION

To investigate the transcriptional profile in the human distal sural nerve which is the most common tissue for observation of pathophysiology of PNS, we enrolled 14 Korean individuals. RNA-Seq and subsequent analyses were used to generate mRNA expression profiles in which fragments per kilobase of transcript per million fragments mapped (FPKM) was used to quantify each transcript. Using a threshold of FPKM>1, which corresponds to approximately one transcript per cell [10], we found that over 12,000 transcripts are expressed in the human distal sural nerve

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Table 1. Patients enrolled in this study and transcriptome analysis results

ID	Age[a]	CMT type	Onset age	FDS[b]	CMTNS[c]	Causative gene	Transcriptome					Whole Exome		TBM[i]		Reference
							Transcripts[d]	Novel Transcripts[e]	Gene Fusion[f]	Variants[g]	Unreported Variants[h]	Variants[g]	Unreported Variants[h]	Variants[j]	Unreported Variants[k]	
FC285-2	33	Normal	-	0	-	-	10,985	4,871	10	5,136	2,370	-	-	-	-	-
FC257-2	35	Normal	-	0	-	-	12,378	3,094	15	5,069	1,716	-	-	-	-	13
FC183-3	51	HMSN1	18	2	9	ND[l]	11,909	3,332	17	5,224	2,214	9,366	444	1,501	1,199	-
FC285-1	8	HMSN1	0	6	27	PMP22	12,365	3,086	13	4,880	1,545	9,543	726	928	704	-
FC414-1	17	HMSN1	15	2	12	ND	12,009	5,297	7	6,269	3,245	9,521	471	2,340	2,058	-
FC176-1	13	HMSN2	8	3	17	ND	12,511	2,916	11	4,801	1,829	9,491	501	1,202	939	-
FC257-1	9	HMSN2	4	6	23	MFN2	12,777	2,587	16	4,997	1,444	-	-	-	-	13
FC283-1	64	HMSN2	52	6	26	ND	11,396	5,660	2	5,418	2,887	9,276	571	2,167	1,889	-
FC407-1	13	HMSN2	15	3	17	GDAP1	12,075	3,971	9	5,071	2,348	9,588	818	1,825	695	-
FC417-1	18	HMSN2	3	6	25	C10orf2	12,230	3,470	11	4,823	1,954	9,733	838	1,224	985	-
FC457-3	48	HMSN2	46	3	19	TFG	11,599	6,218	5	4,996	2,537	9,295	484	1,694	1,406	14
FC529-1	17	HMSN2	8	5	25	ND	11,467	6,218	15	4,499	1,827	9,487	516	1,210	977	-
FC305-1	49	dHMN	15	3	14	BSCL2	11,905	3,147	9	4,385	1,649	-	-	-	-	15
FC305-4	50	dHMN	26	3	12	BSCL2	12,332	3,094	12	4,907	1,654	-	-	-	-	15

[a]Age at sural nerve biopsy; [b]Functional disability scale [6]; [c]Charcot-Marie-Tooth disease neuropathy score [7]; [d]Expressed transcripts were determined as FPKM>1. Original data are available in Supplementary Material; [e]Novel transcripts determined with the Cufflinks program and FPKM>1; [f]Gene fusion was analyzed with the deFuse software; [g]Nonsynonymous splicing site, stop gain/loss, and coding indels; [h]Variants unreported in dbSNP135; [i]Transcriptional base modifications; [j]Number of transcriptome variants that were not identified in the whole-exome analysis; [k]Nonsynonymous splicing site, stop gain/loss, and coding indels that are not reported in dbSNP135; [l]causative gene was not defined.

(Table 1 and Supplementary Material) is almost equivalent to the number expressed in human lymphoblastoid cells (11,000 active transcripts), which was calculated by a similar analytical parameter [11].

RNA-Seq also identified over 4,000 unreported transcripts (FPKM > 1) per sample. As genome tiling arrays revealed over 10,000 novel transcripts in the human liver [12], the transcriptional events in the human distal sural nerve are also dynamic. We also found that around 10 transcripts per sample were fused in both the controls and patients (Table 1). However, we have no clue to the function or biochemical significance of these unreported and fused transcripts in this tissue.

Our transcriptome analysis also revealed over 5,000 functionally significant variants (nonsynonymous splicing site, stop gain/loss, and coding indels) in each sample. Because these variants might primarily derive from changes in the genomic DNA, we analyzed the genomic variants in nine samples from peripheral blood DNA using WES. WES revealed that over 9,000 functionally significant variants are present per sample. Therefore, the variants of transcriptome are fewer than those of genome. Next, we calculated transcriptional base modifications (TBMs) by comparing the two data sets. TBM analysis revealed that there are over 1,500 functionally significant variants. Among them 77% (over 1,200 variants) were unreported to dbSNP135. Although these might include many false positives, arising from methodological or analytical limitations and the discordance of data sources, these data imply that dramatic alterations occur when genetic information is actively transitioned in specific tissues. The significance of TBM is still unclear, until its correlation with disease onset, progression, susceptibility, or penetrance is reported.

Based on this expression profile, we analyzed the changes in the expression patterns of HMSN-causative genes and tried to identify the genes that were differentially expressed in the patients and the controls. Several changes in putative mechanistic/metabolic pathways were identified in the HMSN patient groups (data not shown), but the heterogeneity and size of the samples limited the statistical validity of the results. Nonetheless, future well-designed *in vitro* experimental approaches might convert parts of these data into valid information, which will allow us to understand the pathophysiology of the disease and may suggest therapeutic strategies.

In summary, we first generated a transcriptional profile of the human distal sural nerve, providing basic information about the gene expression in this tissue based on pathophysiological studies of HMSN. Our data also suggest that considerable numbers of events occur beyond the information encoded in the DNA, which has been the main source of biomedical information in the pre-omics era. The contribution of multi-omics might accelerate the biomedical approaches in the PNS by presenting systematic information. Subsequent studies that characterize these novel transcripts and TBMs and validate the HMSN-related changes in the expression profile will provide crucial information about

the pathological mechanism of HMSN and provide therapeutic options for each genetic causative.

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REFERENCES

1. Ng SB, Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, Huff CD, Shannon PT, Jabs EW, Nickerson DA, Shendure J, Bamshad MJ (2010) Exome sequencing identifies the cause of a mendelian disorder. Nat Genet 42:30-35.
2. Park J, Hyun YS, Kim YJ, Nam SH, Kim SH, Hong YB, Park JM, Chung KW, Choi BO (2013) Exome sequencing reveals a novel PRPS1 mutation in a family with CMTX5 without optic atrophy. J Clin Neurol 9:283-288.
3. Nakhro K, Park JM, Kim YJ, Yoon BR, Yoo JH, Koo H, Choi BO, Chung KW (2013) A novel Lys141Thr mutation in small heat shock protein 22 (HSPB8) gene in Charcot-Marie-Tooth disease type 2L. Neuromuscul Disord 23:656-663.
4. Hawrylycz MJ, Lein ES, Guillozet-Bongaarts AL, Shen EH, Ng L, Miller JA, van de Lagemaat LN, Smith KA, Ebbert A, Riley ZL, Abajian C, Beckmann CF, Bernard A, Bertagnolli D, Boe AF, Cartagena PM, Chakravarty MM, Chapin M, Chong J, Dalley RA, Daly BD, Dang C, Datta S, Dee N, Dolbeare TA, Faber V, Feng D, Fowler DR, Goldy J, Gregor BW, Haradon Z, Haynor DR, Hohmann JG, Horvath S, Howard RE, Jeromin A, Jochim JM, Kinnunen M, Lau C, Lazarz ET, Lee C, Lemon TA, Li L, Li Y, Morris JA, Overly CC, Parker PD, Parry SE, Reding M, Royall JJ, Schulkin J, Sequeira PA, Slaughterbeck CR, Smith SC, Sodt AJ, Sunkin SM, Swanson BE, Vawter MP, Williams D, Wohnoutka P, Zielke HR, Geschwind DH, Hof PR, Smith SM, Koch C, Grant SG, Jones AR (2012) An anatomically comprehensive atlas of the adult human brain transcriptome. Nature 489:391-399.
5. Konopka G, Friedrich T, Davis-Turak J, Winden K, Oldham MC, Gao F, Chen L, Wang GZ, Luo R, Preuss TM, Geschwind DH (2012) Human-specific transcriptional networks in the brain. Neuron 75:601-617.
6. Birouk N, LeGuern E, Maisonobe T, Rouger H, Gouider R, Tardieu S, Gugenheim M, Routon MC, Léger JM, Agid Y, Brice A, Bouche P (1998) X-linked Charcot-Marie-Tooth disease with connexin 32 mutations: clinical and electrophysiologic study. Neurology 50:1074-1082.
7. Shy ME, Blake J, Krajewski K, Fuerst DR, Laura M, Hahn AF, Li J, Lewis RA, Reilly M (2005) Reliability and validity of the CMT neuropathy score as a measure of disability. Neurology 64:1209-1214.
8. McPherson A, Hormozdiari F, Zayed A, Giuliany R, Ha G, Sun MG, Griffith M, Heravi Moussavi A, Senz J, Melnyk N, Pacheco M, Marra MA, Hirst M, Nielsen TO, Sahinalp SC, Huntsman D, Shah SP (2011) deFuse: an algorithm for gene fusion discovery in tumor RNA-Seq data. PLoS Comput Biol 7:e1001138.
9. Sommer CL, Brandner S, Dyck PJ, Harati Y, LaCroix C, Lammens M, Magy L, Mellgren SI, Morbin M, Navarro C, Powell HC, Schenone AE, Tan E, Urtizberea A, Weis J; Peripheral Nerve Society (2010) Peripheral Nerve Society Guideline on processing and evaluation of nerve biopsies. J Peripher Nerv Syst 15:164-175.
10. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B (2008) Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods 5:621-628.
11. Ju YS, Kim JI, Kim S, Hong D, Park H, Shin JY, Lee S, Lee WC, Kim S, Yu SB, Park SS, Seo SH, Yun JY, Kim HJ, Lee DS, Yavartanoo M, Kang HP, Gokcumen O, Govindaraju DR, Jung JH, Chong H, Yang KS, Kim H, Lee C, Seo JS (2011) Extensive genomic and transcriptional diversity identified through massively parallel DNA and RNA sequencing of eighteen Korean individuals. Nat Genet 43:745-752.
12. Bertone P, Stolc V, Royce TE, Rozowsky JS, Urban AE, Zhu X, Rinn JL, Tongprasit W, Samanta M, Weissman S, Gerstein M, Snyder M (2004) Global identification of human transcribed sequences with genome tiling arrays. Science 306:2242-2246.
13. Chung KW, Suh BC, Cho SY, Choi SK, Kang SH, Yoo JH, Hwang JY, Choi BO (2010) Early-onset Charcot-Marie-Tooth patients with mitofusin 2 mutations and brain involvement. J Neurol Neurosurg Psychiatry 81:1203-1206.
14. Lee SS, Lee HJ, Park JM, Hong YB, Park KD, Yoo JH, Koo H, Jung SC, Park HS, Lee JH, Lee MG, Hyun YS, Nakhro K, Chung KW, Choi BO (2013) Proximal dominant hereditary motor and sensory neuropathy with proximal dominance association with mutation in the TRK-fused gene. JAMA Neurol 70:607-615.
15. Choi BO, Park MH, Chung KW, Woo HM, Koo H, Chung HK, Choi KG, Park KD, Lee HJ, Hyun YS, Koo SK (2013) Clinical and histopathological study of Charcot-Marie-Tooth neuropathy with a novel S90W mutation in BSCL2. Neurogenetics 14:35-42.

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