Uncovering Novel Prognostic Factors of Sudden Sensorineural Hearing Loss by Whole-Genome Sequencing of Cell-Free DNA

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BACKGROUND: Sudden sensorineural hearing loss is a common disease with several etiologic hypotheses, such as infection, vascular occlusion, inflammation, oxidative stress, etc. Studies have reported that the concentration of cell-free DNA in plasma will elevate in these situations. Former studies have reported that the whole-genome sequencing of cell-free DNA has high accuracy and sensitivity in inferring gene expressions. In this study, we plan to use the whole-genome sequencing of cell-free DNA to uncover novel prognostic factors of sudden sensorineural hearing loss and provide new insight into the clinical application of cell-free DNA.

METHODS: In this study, 84 sudden sensorineural hearing loss patients (47 in recovery group and 37 in no-recovery group) were enrolled. After whole-genome sequencing of the cell-free DNA, the protein–protein interaction network was constructed using the differentially expressed genes. Multinomial logistics regression analysis was used to analyze the prognostic factors of hearing improvement.

RESULTS: In this study, we found distinct patterns of expressed and unexpressed genes in cell-free DNA sequence read depth coverage in sudden sensorineural hearing loss patients. The top centrality hub genes IGF1, NOTCH1, APOE, FAM3C, RPS6KB1, and RELB were identified from the protein–protein interaction network. Multinomial logistics regression analysis demonstrated that the coverage patterns of 3 key differentially expressed genes (NOTCH1, APOE, and RELB) are significantly different in sudden sensorineural hearing loss with and without hearing recovery.

CONCLUSION: The cell-free DNA could have more applications in diverse diseases, and the coverage patterns of 3 differentially expressed genes (NOTCH1, APOE, and RELB) are independent prognostic factors of sudden sensorineural hearing loss. Their expression levels may play a critical role in the hearing improvement of sudden sensorineural hearing loss patients.

KEYWORDS: Cell-free DNA, sudden sensorineural hearing loss, prognostic factors, whole-genome sequencing

INTRODUCTION

Sudden sensorineural hearing loss (SSNHL) is a common otologic disease occurring abruptly with unilateral or bilateral hearing loss of more than 30 dB in at least 3 consecutive frequencies within 3 days. It is estimated that SSNHL affects 5-27 per 100 000 people annually. Infection, vascular occlusion, inflammation, oxidative stress, and rupture of the cochlear membrane are the most common theories of its reported etiologic mechanisms. The prognosis varies among patients with the same conventional treatment. Considering the effects on the quality of life of patients and the burden on public health, researchers focus on the prognostic factors of SSNHL. Furthermore, it is of great importance to improve the prognosis.

According to some publications, the DNA are released into the circulation in a wide range of conditions, including infection, inflammation, immune reactions, ischemic stroke, myocardial infarction, oxidative stress, pregnancy, etc. Thus, cell-free DNA (cfDNA) is used as a diagnostic material for non-invasive screening testing. In 2016, Ulz et al conducted a study on the application for inferring expressed genes by whole-genome sequencing of plasma DNA. They demonstrated that plasma DNA possesses the sensitivity and accuracy to predict whether genes are expressed and confirmed the quantitative relationship between the transcription start...
sites (TSSs) sequence read depth coverage patterns and different gene expression levels.6

As the most common etiologic theories of SSNHL cause elevated cfDNA levels, several studies have reported that the cfDNA may play a role in intercellular messenger, synchronized cell differentiation. We believe that cfDNA, which is used as a non-invasive detection method, can be a promising biomarker reflecting the body’s condition of SSNHL patients. Here, we are going to find out some novel biomarkers of the prognosis of SSNHL by using whole-genome sequencing of cfDNA. Meanwhile, we want to provide new insights into the application of cfDNA.

METHODS

Subjects and Grouping
This retrospective study was conducted in our hospital from 2015 to 2019. The study protocols were approved by the Nanfang Hospital Clinical Research Ethics Committee (No.: NFEC-2019-266). Adult patients diagnosed as SSNHL according to the American Academy of Otolaryngology Head and Neck Surgery guidelines were included in this study.1 All the patients were all-frequencies descending SSNHL. Exclusion criteria included pregnancy, bilateral hearing loss, the duration from onset to admission over 3 days, pretreated with any medicine before admission, autoimmune or malignancy diseases, conductive hearing loss, lesions in the inner ear diagnosed by imaging, a history of asthma or chronic obstructive pulmonary diseases, head trauma history, middle or external ear diseases or middle ear surgery history, a family history of hearing loss or other otologic diseases.

All patients received systematic glucocorticoids (methylprednisolone sodium succinate, for a total of 10-day course with 80 mg/day for 4 days, then 40 mg/day for 3 days, and 20 mg/day for another 3 days; Pfizer Manufacturing Belgium NV, Puurs, Belgium). Pure tone audiometry (PTA) was evaluated pre- and 30 days post-treatment. Clinical outcomes were assessed according to modified Siegel’s criteria,9 and patients were categorized as no recovery in this study including (1) hearing gain < 15 dB and (2) final hearing threshold > 75 dB irrespective of hearing improvement.

Patients Sampling
Peripheral blood was drawn on the day of admission pre- and post-treatment for laboratory testing, and the excess unused blood was collected for the subsequent analysis. Venous blood was centrifuged to separate 600 μL of plasma.

Cell-Free DNA Extraction, Library Construction, and Sequencing
Cell-free DNA was extracted from plasma using nucleic acid isolation or purification reagent Guangzhou Darui Biotechnology Co., Ltd, (Guangdong Guangzhou,China). cDNA concentration was measured with a Qubit 2.0 Fluorometer. Ion Plus Fragment kit Life Technologies (CA, USA) was applied to construct the fragment DNA library. Whole-genome sequencing was performed on Ion Torrent Thermo Fisher Scientific (MA, USA) after library molecules were amplified.

Differential Gene Identification
The methods are the same as our previous study.10 First, the sequence reads were mapped onto the human reference genome (hg19) using BWA software. Then, the repeat and low-quality sequences were deleted. The known human protein-encoding gene information was obtained from the RefSeq database. Sequencing coverage at a 2K-TSS was extracted by SAMTools. The mapped reads were normalized by Reads Per Kilobase per Million mapped reads (RPKM). The fold change (FC) is based on the significant differential coverage at the 2K-TSSs region between patients with and without recovery and the statistical analysis was performed using the Mann–Whitney U-test. Then the Benjamini–Hochberg method was used to adjust the P-value. Only genes with $|\log_2(FC)| \geq 0.6$ and adjust-$P<0.05$ were identified as differential genes (DGs) between patients with and without recovery. The GraphPad Prism 8.0 was used to visualize the DGs.

Protein–Protein Interaction Network Analysis and Hub Gene Identification
The DGs identified were subjected to protein–protein interaction (PPI) analysis using the search functionality of STRING11 to explore the association between the DGs, and a PPI network was built simultaneously. The minimum score of >0.4 was selected as the confidence. The Cytoscape ver 3.7.212 was applied to further analyze and visualize the PPI network using the downloaded data matrix. CytoHubba was applied to discover hub genes as it has been proven that it is a valuable tool to identify hub objects and subnetworks from a complex interactome.13

Statistical Analysis
Statistical analysis methods were applied to compare the characteristics of patients with and without recovery. Continuous values with and without normal distribution were compared by unpaired Student’s t-test and the Mann–Whitney U-test, respectively. Categorical values were compared using the chi-square test or Fisher’s exact test. Multinomial logistics regression analysis was used to analyze the independent prognostic factors of SSNHL. This statistical analysis is performed by using Statistical Package for the Social Sciences v.25 (IBM SPSS Corp.; Armonk, NY, USA) and R language (R 3.6.1).

RESULTS

Clinical Characteristics of Patients
Totally 84 patients (47 in recovery group, 37 in no-recovery group) were included in this study. The age of patients with and without recovery has statistical significance and there is also a significant difference in patients with or without vertigo in the 2 groups. The other

|                          | Recovery | No Recovery | P     |
|--------------------------|----------|-------------|-------|
| Age (mean ± standard deviation) | 40.89 ± 13.90 | 47.24 ± 14.07 | .042  |
| Sex (M/F)                | 25/22    | 17/20       | .510  |
| Affected side (L/R)      | 28/19    | 18/19       | .318  |
| Duration from onset to admission | 2.11 ± 0.843 | 2.17 ± 0.816 | .734  |

Comorbidity

|                        | Recovery | No Recovery | P     |
|------------------------|----------|-------------|-------|
| Hypertension           | 7 (14.89%) | 11 (29.72%) | .100  |
| Diabetes               | 4 (8.51%) | 3 (8.11%)   | .690  |

Symptoms

|                        | Recovery | No Recovery | P     |
|------------------------|----------|-------------|-------|
| Vertigo                | 8 (17.02%) | 14 (37.84%) | .031  |
| Tinnitus               | 44 (93.62%) | 32 (86.49%) | .292  |
clinical characteristics have no significant difference (Table 1). Age and vertigo were included in the further multinomial logistics regression analysis.

**Plasma DNA Read Depth Patterns**

Sequencing coverage data at 2K-TSSs of 104 healthy volunteers in the previous study were compared with plasma read depth coverage from SSNHL patients. The patterns for differential expressed genes of cfDNA in SSNHL patients correspond to those in 104 healthy volunteers and those established by micrococcal nuclease assays. Gene expression levels negatively correlated with the read depths across the 2K-TSSs regions (Figure 1). In volunteers (Figure 1A) and SSNHL patients’ cfDNA (Figure 1B), the mean relative coverage of all expressed genes and unexpressed genes are shown in red and blue, respectively (Details in the Supplementary Table 1).

**Identification of Differentially Expressed Genes**

Based on the statistical analysis of the sequencing coverage at 2K-TSSs between recovery and no-recovery group, 143 DGs were obtained with the cut-off criteria (adj. \( P \)-value < .05 and \( |\log_2(FC)| \geq 0.6 \)). Using \( \log_2(FC) \) scores and \(-\log_{10} P\) as the calculated criteria, the analysis results are presented in a volcano plot generated by GraphPad Prism 8.0 (Figure 2A). Compared with no-recovery group, the read depth of 57 DGs and 86 DGs was increased and decreased in recovery group, respectively. The top 10 DGs with significantly increased and decreased coverage were in Figure 2B. In these DGs, NOTCH1, RELB, and FAM3C are reported to correlate with the inner ear and hearing.\(^{14-16}\)

**Protein–Protein Interaction Network and Hub Gene analysis**

A PPI network was constructed by using the STRING online database, with parameters including a minimum required interaction score \( \geq 0.4 \) (medium confidence) and only query proteins being displayed (Figure 3A). Among all the DGs, 62 DGs were included in the PPI network visualized by Cytoscape software. The size of each node has a positive correlation with statistically significant differences. The red and blue nodes represent the DGs with increased and decreased coverage in recovery group, respectively. Nodes with lighter colors have a lower \( |FC| \) value than the dark ones. The top 10 ranked genes located in the center of the PPI network evaluated by 5 calculation methods (Closeness, Stress, EPC, EcCentricity, Radiality) in the CytoHubba application are shown in Supplementary Table 2. An online website (http://www.ehbio.com/ImageGP/index.php/Home/Index/VennDiagram.html)\(^{17}\) was utilized to find intersections of these 5 algorithms and generated a Venn plot (Figure 3B). The 5 most significant hub genes are NOTCH1, insulin-like growth factor 1 (IGF1), apolipoprotein E(ApoE), ribosomal protein S6 kinase beta-1(RPS6KB1), and RELB.

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**Figure 1.** Plasma DNA read depth maps at transcription start sites. (A) Plasma DNA read depth maps for the promoter regions of the unexpressed and expressed genes in 104 healthy volunteers of whom the data are acquired from the Supplementary Note of references (Ulz et al.\(^8\) 2016). The mean relative coverage of expressed genes is shown in red, and the unexpressed genes are in blue. (B) Plasma DNA read depth maps for the promoter regions of the unexpressed and expressed genes in SSNHL patients in our study. The mean relative coverage of expressed genes is shown in red, and the unexpressed genes are in blue.

**Figure 2.** (A) The volcano plot of all genes. All the dots above the green horizontal dotted line have a \( P \)-value \(<.05 \). While the dots outside the 2 vertical dotted lines have a \( |\log_2(FC)| \geq 0.6 \). Dots with the color grey have no statistical significance. The red and blue dots indicate the genes with increased and decreased coverage in recovery group, respectively. (B) Top 10 genes with increased and decreased coverage in recovery group compared with no-recovery patients, and the \( |\log_2(FC)| \geq 0.6 \) and adj. \( P \)-value \(<.05 \) set as the selection criteria. Red and blue columns indicate the genes with increased and decreased coverage, respectively.
These 5 hub genes and FAM3C were regarded as 6 key DGs for further analysis.

### Multinomial Logistic Regression Analysis
According to the sample size, 8 variables (age, vertigo, and the average coverage of 6 key DGs) were included in multinomial logistic regression analysis. The results are shown in Table 2. NOTCH1, APOE, RELB, and age have significant differences in the 2 groups.

### DISCUSSION
Previous studies have reported that genes with different expression levels have distinct coverage patterns of the cfDNA in the 2K-TSSs. In this study, we have verified that the coverage pattern of cfDNA in the 2K-TSSs of SSNHL patients is consistent with those in the former studies.8,18 It has been demonstrated that the read depth coverage has a negative correlation with the gene expression level in these studies.8

This result manifests that the gene expression level is negatively correlated with read depth coverage in SSNHL patients.

We found that NOTCH1, APOE, RELB, FAM3C, IGF1, and RPS6KB1 are the potential critical DGs in SSNHL patients with different prognoses using plasma DNA whole-genome sequencing and bioinformatics analysis. Multinomial logistic regression analysis showed that NOTCH1, APOE, RELB, and age are the independent prognostic factors of SSNHL. Our results are consistent with previous research that the younger SSNHL patients have better hearing recovery19 (P = .011, odds ratio = 0.910).

According to some publications, 4 hub genes (APOE, IGF1, NOTCH1, and RELB) have been demonstrated to be associated with various kinds of hearing loss.20-23 Among them, the multinomial logistic regression analysis showed that NOTCH1, APOE, and RELB are independent

![Figure 3A](image1.png) **Figure 3.** (A) PPI network of DGs. Genes with increased coverage are in red, while the blue nodes indicate the genes with decreased coverage. The darker the color of the nodes, the greater the change folds of the genes. The size of the nodes has a positive correlation with statistical significance. (B) Venn plot. We used 5 intersecting algorithms to generate a Venn plot to identify significant hub genes by employing an online analysis tool. Different algorithms are presented in different colors. The cross areas indicate the commonly accumulated DGs. Five hub genes are shown in concurrent areas (IGF1, NOTCH1, ApoE, FAM3C, RELB, and RPS6KB1).

![Figure 3B](image2.png)

### Table 2. Multinomial Logistic Regression Analysis

| Variables | B    | Standard Error | Wald   | P     | Odds Ratio | 95% OR, CI* |
|-----------|------|----------------|--------|-------|------------|-------------|
|           |      |                |        |       | Lower Bound | Upper Bound |
| FAM3C     | 0.025| 0.044          | 0.327  | .568  | 1.026      | 0.940       | 1.119       |
| IGF1      | 0.101| 0.057          | 3.115  | .078  | 1.107      | 0.989       | 1.239       |
| RPS6KB1   | 0.035| 0.040          | 0.764  | .382  | 1.036      | 0.957       | 1.120       |
| APOE      | 0.194| 0.077          | 6.299  | .012  | 1.214      | 1.043       | 1.413       |
| RELB      | -0.127| 0.043        | 8.604  | .003  | 0.880      | 0.809       | 0.959       |
| NOTCH1    | -0.228| 0.087        | 6.926  | .008  | 0.796      | 0.672       | 0.943       |
| Vertigo   | 1.519| 0.919          | 2.729  | .099  | 4.567      | 0.753       | 27.691      |
| Age       | -0.094| 0.037         | 6.418  | .011  | 0.910      | 0.846       | 0.979       |
prognostic factors of SSNHL. NOTCH1 is a member of Notch signaling pathway. This signaling pathway mediates Hair cells (HCs) and supporting cells (SCs) differentiation during the inner ear development when NOTCH1 and some other factors in this signaling pathway are expressed in SCs.24 The study on the neonatal mouse cochlea suggested that weakened Notch signaling allows SCs to convert to HCs and plays a role in spontaneous HC regeneration.25 However, the conclusion of whether SCs can convert to HCs in response to Notch signaling inhibition in the adult cochlea is still controversial.26-29 Recent studies have reported that the expression level of microRNA-183 has a significant difference between SSNHL patients with and without hearing recovery.30 What’s more, microRNA-183 can mediate the Notch signaling pathway in the prohibition of differentiation and regeneration of hair cells from mouse cochlea and NOTCH1 is also a target gene of microRNA-183. In the current study, we observed significantly different coverage of NOTCH1 among patients with different prognoses. The multinomial logistics regression analysis showed that it is the independent prognostic factor of SSNHL suggests. Thus, NOTCH1 is likely to contribute to hearing recovery in SSNHL patients. It can be a target in the further research of SSNHL.

Previously, some publications have reported that APOE and RELB have some connection with the prognosis of SSNHL.31-34 A case-control study on 177 individuals including SSNHL patients and healthy objectives has reported that the genotype distribution of APOE was significantly different between patients and healthy control in the Iranian population. They suggest the APOE gene variant may be associated with SSNHL.31 The coverage patterns of APOE have been demonstrated to be significantly correlated with hearing recovery of SSNHL patients in this study and suggests that the expression level of APOE may be one of the prognostic factors of SSNHL. According to research, RELB is a member of the Nuclear factor-κB(NF-κB)/Rel family that plays a critical role in inflammation, immunity, cell proliferation, differentiation, and survival.22 It has also been reported that the NF-κB family is related to the prognosis of various hearing loss types.33,34 What’s more, one study has demonstrated that hyperbaric oxygen treatment can alleviate hearing loss in SSNHL patients by suppressing the inflammatory response induced by NF-κB signaling,23 which means the NF-κB signaling plays an essential role in the hearing recovery of SSNHL patients. Since we observed significantly different coverage of RELB in our study, the expression level of RELB may affect the prognosis of SSNHL in some ways.

Even though the multinomial logistics regression analysis shows that the IGF1 is not the independent prognostic factor of SSNHL in our study, the Japanese researchers have demonstrated that topical IGF1 therapy has a positive effect on SSNHL patients who failed systemic corticosteroid treatment.19 Moreover, as a secreted hormone, it can distribute to the inner ear through general blood circulation and is found to be locally expressed in the cochlear and vestibular ganglia of mice.26 Therefore, the different expression levels of IGF1 might impact the hearing improvement of SSNHL patients. And we believe that patients might be benefited from the local glucocorticoid therapy at the beginning of the onset. However, it needs more studies to validate.

Since there are only 84 patients here, the small sample size is one of the limitations of this study. What’s more, only adult patients were included in our study. More extensive studies including patients of all ages are required in the further studies. Another limitation is that the result is only based on the cfDNA sequencing and bioinformatics analysis. More studies are essential to validate our results and confirm the associations between our results and clinical outcomes.

CONCLUSION
This is the first time to infer the prognostic factors of SSNHL by whole-genome sequencing of cfDNA. We validated that the plasma coverage patterns in SSNHL patients are similar to the former study, which has confirmed the cfDNA has high accuracy and sensitivity in inferring gene expression levels. These suggest that cfDNA sequencing may have a broader application in various diseases. Some key genes (NOTCH1, APOE, RELB, and IGF1) may play a critical role in the prognosis of SSNHL. Since our findings are predicted by cfDNA sequencing, more solid studies are planned to validate in further research.

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Informed Consent: N/A.

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Supplementary Table 1a. Plasma DNA Read Depth Maps for the Promoter Regions of the Unexpressed and Expressed Genes in Healthy Volunteers in the Reference (Ulz et al, 2016)8

| pos  | Expressed genes | Unexpressed genes |
|------|-----------------|-------------------|
| -1000| 0.895038443     | 0.94413901        |
| -990 | 0.893420237     | 0.946795372       |
| -980 | 0.88966116      | 0.940403695       |
| -970 | 0.885040628     | 0.938994231       |
| -960 | 0.882981772     | 0.93257963        |
| -950 | 0.88233489      | 0.937162079       |
| -940 | 0.880311011     | 0.93674593        |
| -930 | 0.88163784      | 0.9407257         |
| -920 | 0.88411029      | 0.942384741       |
| -910 | 0.890202496     | 0.947780036       |
| -900 | 0.893179427     | 0.949804657       |
| -890 | 0.896065918     | 0.95352255        |
| -880 | 0.896651479     | 0.9541782         |
| -870 | 0.897806472     | 0.95605266        |
| -860 | 0.897026532     | 0.953151954       |
| -850 | 0.892010072     | 0.946079195       |
| -840 | 0.884413399     | 0.941744153       |
| -830 | 0.874892451     | 0.939197621       |
| -820 | 0.868759604     | 0.95003118        |
| -810 | 0.860468542     | 0.931042932       |
| -800 | 0.85159708      | 0.930863448       |
| -790 | 0.84339117      | 0.932377746       |
| -780 | 0.837721296     | 0.931556557       |
| -770 | 0.838572397     | 0.929013895       |
| -760 | 0.839900082     | 0.930797301       |
| -750 | 0.841753889     | 0.931351147       |
| -740 | 0.845493418     | 0.930814278       |
| -730 | 0.849669914     | 0.934952072       |
| -720 | 0.85435473      | 0.939276872       |
| -710 | 0.858079772     | 0.943210025       |
| -700 | 0.859315853     | 0.945955409       |
| -690 | 0.85946436      | 0.956019998       |
| -680 | 0.852708042     | 0.955417401       |
| -670 | 0.845872643     | 0.95357285        |
| -660 | 0.835850099     | 0.952080184       |
| -650 | 0.824259425     | 0.946612758       |
| -640 | 0.813810673     | 0.941540454       |
| -630 | 0.801804771     | 0.926023308       |
| -620 | 0.795227301     | 0.926175956       |
| -610 | 0.790836444     | 0.922376801       |
| -600 | 0.787428154     | 0.917509768       |
| -590 | 0.788773147     | 0.91562045        |
| -580 | 0.793075542     | 0.923941961       |
| -570 | 0.797602084     | 0.924574451       |
| -560 | 0.802647112     | 0.926911753       |
| -550 | 0.807130176     | 0.929920676       |
| -540 | 0.812207823     | 0.934177738       |
| -530 | 0.814878312     | 0.933591234       |
| -520 | 0.813364466     | 0.932399966       |
| -510 | 0.811753029     | 0.934074366       |

(Continued)
| pos | Expressed genes | Unexpressed genes |
|-----|-----------------|-------------------|
| 0   | 0.556470064     | 0.83185983       |
| 10  | 0.548666373     | 0.83312471       |
| 20  | 0.550596166     | 0.833063445      |
| 30  | 0.55595616      | 0.833193462      |
| 40  | 0.579451106     | 0.837885404      |
| 50  | 0.618565774     | 0.854305152      |
| 60  | 0.645727303     | 0.866386829      |
| 70  | 0.677900122     | 0.87774643       |
| 80  | 0.712557361     | 0.891815087      |
| 90  | 0.771771        | 0.89710493       |
| 100 | 0.82507394      | 0.91691879       |
| 110 | 0.83434053      | 0.918055707      |
| 120 | 0.838676192     | 0.91729189       |
| 130 | 0.838503092     | 0.914878068      |
| 140 | 0.828622719     | 0.917049864      |
| 150 | 0.822166278     | 0.915077015      |
| 160 | 0.814447733     | 0.913659461      |
| 170 | 0.809439371     | 0.91305305       |
| 180 | 0.804101321     | 0.91136907       |
| 190 | 0.799908543     | 0.90959344       |
| 200 | 0.80147335      | 0.907929132      |
| 210 | 0.807447613     | 0.909670725      |
| 220 | 0.815378191     | 0.91131705       |
| 230 | 0.825704031     | 0.915382612      |
| 240 | 0.834253385     | 0.91984795       |
| 250 | 0.846793366     | 0.920235294      |
| 260 | 0.857744718     | 0.922230717      |
| 270 | 0.867037811     | 0.923311087      |
| 280 | 0.874663096     | 0.92287559       |
| 290 | 0.877440496     | 0.921989688      |
| 300 | 0.879281176     | 0.922837231      |
| 310 | 0.877550553     | 0.926106932      |
| 320 | 0.871478967     | 0.926206513      |
| 330 | 0.866454444     | 0.927568495      |
| 340 | 0.86131941      | 0.929835991      |
| 350 | 0.855103467     | 0.929015007      |
| 360 | 0.851258234     | 0.931398108      |
| 370 | 0.849006724     | 0.932441819      |
| 380 | 0.851724815     | 0.927496033      |
| 390 | 0.856372009     | 0.930773174      |
| 400 | 0.862475004     | 0.933527516      |
| 410 | 0.871537971     | 0.939734248      |
| 420 | 0.881883497     | 0.942134355      |
| 430 | 0.891572646     | 0.946149797      |
| 440 | 0.906593288     | 0.95653814       |

Supplementary Table 1a. Plasma DNA Read Depth Maps for the Promoter Regions of the Unexpressed and Expressed Genes in Healthy Volunteers in the Reference (Ulz et al, 2016) (Continued)
**Supplementary Table 1b. Plasma DNA Read Depth Maps for the Promoter Regions of the Unexpressed and Expressed Genes in Ssnhl Patients in our Study**

| pos  | Expressed genes | Unexpressed genes |
|------|-----------------|-------------------|
| -1000| 0.752623        | 0.96619           |
| -990 | 0.751946        | 0.973815          |
| -980 | 0.750781        | 0.980633          |
| -970 | 0.750479        | 0.983241          |
| -960 | 0.748643        | 0.993261          |
| -950 | 0.748716        | 0.991985          |
| -940 | 0.748696        | 0.987415          |
| -930 | 0.74818         | 0.985321          |
| -920 | 0.74815         | 0.982599          |
| -910 | 0.74864         | 0.986853          |
| -900 | 0.74909         | 0.982038          |
| -890 | 0.748134        | 0.976745          |
| -880 | 0.747           | 0.974438          |
| -870 | 0.746942        | 0.978483          |
| -860 | 0.745434        | 0.977809          |
| -850 | 0.743637        | 0.972305          |
| -840 | 0.741712        | 0.965313          |
| -830 | 0.739964        | 0.959414          |
| -820 | 0.738523        | 0.951395          |
| -810 | 0.737875        | 0.947388          |
| -800 | 0.736508        | 0.951648          |
| -790 | 0.735244        | 0.948294          |
| -780 | 0.734229        | 0.94815           |
| -770 | 0.733267        | 0.947606          |
| -760 | 0.732645        | 0.943507          |
| -750 | 0.731291        | 0.939388          |
| -740 | 0.729584        | 0.929747          |
| -730 | 0.727657        | 0.927882          |
| -720 | 0.72585         | 0.920652          |
| -710 | 0.724647        | 0.926943          |
| -700 | 0.723061        | 0.927932          |
| -690 | 0.72184         | 0.928777          |
| -680 | 0.720203        | 0.9352            |
| -670 | 0.718047        | 0.934812          |
| -660 | 0.716913        | 0.936489          |
| -650 | 0.715607        | 0.937816          |
| -640 | 0.713233        | 0.935956          |
| -630 | 0.710563        | 0.925813          |
| -620 | 0.709594        | 0.92227           |
| -610 | 0.708473        | 0.920583          |
| -600 | 0.706967        | 0.915108          |
| -590 | 0.70417         | 0.916933          |
| -580 | 0.702312        | 0.91374           |
| -570 | 0.700474        | 0.910675          |
| -560 | 0.699666        | 0.905007          |
| -550 | 0.698053        | 0.898302          |
| -540 | 0.696752        | 0.894911          |
| -530 | 0.694928        | 0.887216          |
| -520 | 0.693395        | 0.881242          |
| -510 | 0.691893        | 0.873146          |
| -500 | 0.690294        | 0.870876          |
| -490 | 0.689018        | 0.87128           |
| -480 | 0.687193        | 0.870674          |
| -470 | 0.685331        | 0.874679          |
| -460 | 0.683469        | 0.869793          |
| -450 | 0.682302        | 0.866585          |
| -440 | 0.681128        | 0.866624          |
| -430 | 0.68008         | 0.867077          |
| -420 | 0.678458        | 0.866186          |
| -410 | 0.676713        | 0.86266           |
| -400 | 0.675407        | 0.863894          |
| -390 | 0.673857        | 0.864181          |
| -380 | 0.672686        | 0.861037          |
| -370 | 0.670363        | 0.857608          |
| -360 | 0.668273        | 0.855659          |
| -350 | 0.665818        | 0.85745           |
| -340 | 0.663568        | 0.859401          |
| -330 | 0.660874        | 0.861044          |
| -320 | 0.658767        | 0.861372          |
| -310 | 0.656546        | 0.863451          |
| -300 | 0.653233        | 0.866577          |
| -290 | 0.650724        | 0.86951           |
| -280 | 0.648786        | 0.873752          |
| -270 | 0.645789        | 0.876606          |
| -260 | 0.641859        | 0.879628          |
| -250 | 0.637642        | 0.88085           |
| -240 | 0.633567        | 0.882212          |
| -230 | 0.629045        | 0.885012          |
| -220 | 0.623683        | 0.885685          |
| -210 | 0.618312        | 0.888772          |
| -200 | 0.610785        | 0.886722          |
| -190 | 0.603718        | 0.886589          |
| -180 | 0.595842        | 0.888137          |
| -170 | 0.587854        | 0.884189          |
| -160 | 0.580575        | 0.881128          |
| -150 | 0.573092        | 0.879939          |
| -140 | 0.566036        | 0.878104          |
| -130 | 0.557683        | 0.878978          |
| -120 | 0.551255        | 0.878675          |
| -110 | 0.54577         | 0.878252          |
| -100 | 0.539559        | 0.890608          |
| -90  | 0.535044        | 0.900604          |
| -80  | 0.530658        | 0.908736          |
| -70  | 0.527575        | 0.912542          |
| -60  | 0.526481        | 0.916275          |
| -50  | 0.526712        | 0.915944          |
| -40  | 0.527923        | 0.918109          |
| -30  | 0.530256        | 0.92241           |
| -20  | 0.534759        | 0.929604          |
| -10  | 0.539873        | 0.928253          |

(Continued)
### Supplementary Table 1b. Plasma DNA Read Depth Maps for the Promoter Regions of the Unexpressed and Expressed Genes in SSNHL Patients in our Study. (Continued)

| pos | Expressed genes | Unexpressed genes |
|-----|-----------------|-------------------|
| 430 | 0.658068 | 0.862425 |
| 440 | 0.66268 | 0.864916 |
| 450 | 0.669638 | 0.86526 |
| 460 | 0.673011 | 0.864543 |
| 470 | 0.676847 | 0.864205 |
| 480 | 0.680449 | 0.861473 |
| 490 | 0.684452 | 0.864916 |
| 500 | 0.687338 | 0.864066 |
| 510 | 0.68957 | 0.864312 |
| 520 | 0.692614 | 0.865027 |
| 530 | 0.69467 | 0.865368 |
| 540 | 0.69669 | 0.862427 |
| 550 | 0.699293 | 0.861547 |
| 560 | 0.701084 | 0.86162 |
| 570 | 0.703637 | 0.860257 |
| 580 | 0.703226 | 0.856891 |
| 590 | 0.706977 | 0.862946 |
| 600 | 0.710001 | 0.865912 |
| 610 | 0.713479 | 0.864113 |
| 620 | 0.716952 | 0.863233 |
| 630 | 0.720387 | 0.859703 |
| 640 | 0.722731 | 0.861787 |
| 650 | 0.724989 | 0.862676 |
| 660 | 0.727449 | 0.861665 |
| 670 | 0.729418 | 0.861235 |
| 680 | 0.731096 | 0.861033 |
| 690 | 0.732197 | 0.861818 |
| 700 | 0.73388 | 0.865619 |
| 710 | 0.735127 | 0.867461 |
| 720 | 0.735695 | 0.871256 |
| 730 | 0.736843 | 0.873118 |
| 740 | 0.73713 | 0.875661 |
| 750 | 0.738858 | 0.875789 |
| 760 | 0.740932 | 0.87561 |
| 770 | 0.742514 | 0.876952 |
| 780 | 0.744967 | 0.878444 |
| 790 | 0.746615 | 0.878974 |
| 800 | 0.747928 | 0.879495 |
| 810 | 0.749484 | 0.880887 |
| 820 | 0.751136 | 0.878811 |
| 830 | 0.753087 | 0.87984 |
| 840 | 0.75346 | 0.880724 |
| 850 | 0.75458 | 0.87803 |
| 860 | 0.755545 | 0.874857 |
| 870 | 0.756953 | 0.86453 |
| 880 | 0.757209 | 0.858935 |
| 890 | 0.757709 | 0.856962 |
| 900 | 0.758501 | 0.849493 |
| 910 | 0.759698 | 0.845942 |
| 920 | 0.76047 | 0.845035 |
| 930 | 0.761932 | 0.844318 |
| 940 | 0.763363 | 0.851328 |
| 950 | 0.765028 | 0.846968 |
| 960 | 0.766469 | 0.846435 |
| 970 | 0.767507 | 0.845973 |
| 980 | 0.769208 | 0.850575 |
| 990 | 0.770621 | 0.851756 |
| 1000 | 0.77195 | 0.851302 |
## Supplementary Table 2. CytoHubba Calculation

| name     | Closeness | name     | Eccentricity | name     | EPC | name     | Radiality | name     | Stress |
|----------|-----------|----------|--------------|----------|-----|----------|-----------|----------|--------|
| NOTCH1   | 17.65     | NOTCH1   | 0.09836      | NOTCH1   | 9.469 | NOTCH1   | 6.30632   | NOTCH1   | 1070   |
| RPS6KB1  | 15.38571  | SEMA3G   | 0.08431      | RPS6KB1  | 8.702 | RPS6KB1  | 6.07026   | SCRIB    | 432    |
| IGF1     | 14.99286  | SCRIB    | 0.08431      | IGF1     | 8.595 | IGF1     | 6.0534    | RPS6KB1  | 418    |
| APOE     | 14.10952  | RELB     | 0.08431      | APOE     | 7.8  | APOE     | 6.00281   | APOE     | 402    |
| RELB     | 13.58571  | APOE     | 0.08431      | PPP2R5C  | 7.392 | SCRIB    | 5.93536   | LPP      | 370    |
| PPP2R5C  | 13.41905  | MAP3K1   | 0.08431      | PSMB3    | 7.08  | RELB     | 5.9185    | PPAP2A   | 310    |
| PSMB3    | 13.03571  | TCF3     | 0.08431      | PSME4    | 6.956 | MAP3K1   | 5.83419   | FAM20C   | 296    |
| SCRIB    | 12.77619  | RPS6KB1  | 0.08431      | RELB     | 6.931 | TCF3     | 5.83419   | IGF1     | 252    |
| KLF6     | 12.71071  | IGF1     | 0.08431      | KLF6     | 6.879 | SEMA3G   | 5.73302   | RELB     | 240    |
| MAP3K1   | 12.61905  | PON2     | 0.07377      | PON2     | 6.306 | KLF6     | 5.73302   | PPP2R5C  | 238    |