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Chapter

Treatment of Facial Nerve Palsy Based on Genetic Analysis of the Facial Muscles

Hiroshi Moriyama

Abstract

Details of the molecular biological features of facial nerve palsy have not been widely reported in textbooks. I performed a genetic analysis of facial muscle specimens from Japanese patients with moderate (House-Brackmann facial nerve grading system III) and severe (House-Brackmann facial nerve grading system V) dysfunctions due to Bell's palsy and rats, after facial nerve resection (total paralysis). Microarray analysis of gene expression was performed using specimens from both the healthy and affected sides, and gene expressions were compared. Changes in gene expression were defined as a palsy/healthy side ratio >2.0 or <0.5. I observed changes of gene expression; in particular, genes for muscle, neuron, and energy function showed changes with the severity of facial nerve palsy. This study may aid the development of new treatments and diagnostic/prognostic markers based on the severity of palsy.

Keywords: facial nerve palsy, facial muscle, orbicularis oculi, microarray analysis, gene expression

1. Introduction

Bell's palsy is an acute, idiopathic, unilateral facial nerve paresis or paralysis of unknown cause. It leads to the partial or complete inability to voluntarily move facial muscles on the affected side of the face and may be occurring with equal frequency on the right and left sides of the face. Although typically self-limited, symptoms of Bell's palsy may include oropharyngeal or facial numbness, disturbed taste on the anterior part of the tongue, impaired tolerance to ordinary levels of noise, mild pain in or behind the ear, significant oral incompetence, and an inability to close the eyelid, leading to potential eye injury. Clinically important improvement without intervention occurs within 3 weeks after onset of symptoms in 85% of patients and within 3–5 months in the remaining 15% [1]. Patients failing to show signs of improvement by 3 weeks may have suffered Wallerian degeneration of the facial nerve, leading to residual paresis and abnormal branching of regenerating axons, or may have an alternative diagnosis that requires identification by specialist examination or investigations. Among patients with Bell's palsy, 71% obtained their normal facial muscle function [1]. Sequelae were slight in 12% of patients with Bell's palsy, mild in 13%, and severe in 4% [1]. Incomplete recovery of facial expression may have a long-term impact on quality of life. Some patients with Bell's palsy never recover despite treatment by pharmacotherapy and facial nerve decompression surgery. Physicians take the bold course of operation as a last resort. Contemporary

reconstructive options include neurotization procedures (a new motor nerve is used to restore innervation to a viable muscle), contiguous regional muscle transfer (most commonly temporalis muscle transfer), and microsurgical free muscle transfer [2]. Additionally, some patients do not wish to undergo plastic and reconstructive surgery, and at present, there is no effective treatment for them [3]. Research on myogenin expression in the facial muscles following resection or compression of the facial nerve [4] indicated that facial muscle gene analysis might be a promising way to predict the outcome of facial palsy even at an early stage of the disease [4]. I thus performed a genetic analysis of the facial muscles in patients with Bell's palsy and in rats after facial nerve resection and assessed its molecular biological aspects as a pathway to new treatments.

2. Materials and methods

2.1 Human specimens

The materials used to study the microarray analysis were obtained from the orbicularis oculi muscles of six Japanese patients with Bell's palsy (females in their 60s). Three patients were judged to be House-Brackmann facial nerve grading system III (moderate dysfunction), and the other three were judged to be House-Brackmann facial nerve grading system V (severe dysfunction). Both groups had previously received nonsurgical conservative treatment using steroids and so on but had not undergone facial nerve decompression surgery. All the patients were treated with plastic and reconstructive procedures at 1.5 years after the onset of facial paralysis. Both groups underwent blepharoplasty of the superior eyelid for blepharoptosis on the affected side. They also underwent blepharoplasty on the healthy side for esthetic reasons. Biopsy materials were obtained from the palpebral part of orbicularis oculi on both the healthy side and the side with palsy. The microarray analysis of gene expression was performed using Affymetrix Human Gene 1.0 ST arrays (Affymetrix Inc., Santa Clara, CA, USA), and gene expressions were compared between the two sides. Changes of gene expression were defined as a palsy/healthy side expression ratio >2.0 or <0.5.

This study was approved by the local ethics review board (No. 149) at the Showa University Hospital. I conducted and had supervisory control of this study according to the Ethical Principles for Medical Research Involving Human Subjects in the Declaration of Helsinki. I provided all patients with detailed information about this study and obtained written informed consent prior to inclusion from both patients and doctors for participation in this study.

2.2 Rat specimens

Three male Slc: Wistar/ST rats weighing 300 g (12 weeks old) were used. The management of environmental variables of rats was as follows: (1) the room temperature range for rat housing between 21 and 23°C, (2) the humidity at the level of rat cages of 40–70%, (3) the room ventilation rates of about 15–20 air changes per hour, and (4) the light cycles of 12/12 hours light/dark. The animals were allowed to have food and water provided ad libitum until the end of the experiment.

Each animal was anesthetized with an intraperitoneal injection of 0.15 mg/kg of medetomidine hydrochloride, 2.0 mg/kg of midazolam, and 2.5 mg/kg of butorphanol tartrate and fixed on an experimental table in the prone position. The face was shaved and cleaned, and a skin incision was made to expose

the extratemporal portion of the facial nerve bilaterally. On the nerve resection side (right side), the main trunk of the facial nerve was cut just distal to the stylomastoid foramen, forming a 7 mm gap in the trunk to avoid regeneration. In sham surgery group, the facial nerve on the opposite side (left side) was exposed without further manipulation. The rats were returned to the animal room after completion of these procedures.

The rats were sacrificed under deep anesthesia by intraperitoneal administration of pentobarbital (150 mg/kg) on the third postoperative week. A piece of orbicularis oculi muscle was obtained from each side and immediately immersed in RNA*later* solution (QIAGEN N. V.; Venlo, Netherlands), for gene expression analysis. The microarray analysis of gene expression was performed using Affymetrix Human Gene 1.0 ST arrays (Affymetrix Inc., Santa Clara, CA, USA), and gene expression was compared between the two sides. Changes of gene expression were defined as a palsy/healthy side expression ratio >2.0 or <0.5.

Under the approval of the Ethics Committee of Nagoya City University Graduate School of Medical Sciences (No. H27M-74), the present study was conducted following the Guidelines for Animal Experimentation at the above institute. All efforts were made to minimize suffering and the number of animals used, as described in the experimental protocol.

3. Results

Table 1 summarizes the data from the three severities of facial nerve palsy (House-Brackmann facial nerve grading system III, V, and VI). Only 13 genes showed similar changes between patients with moderate dysfunction and those with severe dysfunction, and only one gene showed similar changes between patients with moderate dysfunction and rats with total paralysis. In contrast, 333 genes showed similar changes between patients with severe dysfunction and rats with total paralysis. The overall changes indicated that patients with moderate dysfunction differed from those with severe dysfunction and rats with total paralysis.

Species	Human	Human	Rat
Severity of facial nerve palsy	Moderate dysfunction	Severe dysfunction	Total paralysis
House-Brackmann facial nerve grading system	III	V	VI
Gene category			
Muscle	↑20 ↓4	↓↓	↓↓
Neuron	↓↓	↑39 ↓20	↑29 ↓18
Energy	↑	↓↓	↓↓
Immunity	→	↑	↑
Inflammation	→	↑	↑↑
Stress	→	↑	↑
Apoptosis	→	→	→
Autophagy	→	→	↑
Death	→	→	→

Table 1.
Comparison of changes in gene expression between the three severities of facial nerve palsy. Changes of gene expression were defined as a palsy/healthy side ratio > 2.0 or < 0.5. ↓↓ *indicates that most genes in the category were downregulated.* ↓ *indicates that some genes in the category were downregulated.* ↑↑ *indicates that most genes in the category were upregulated.* ↑ *indicates that some genes in the category were upregulated.* → *indicates that expression of genes in the category did not change. Numbers (mean values) on the right side of the arrows indicate the number of genes showing changes.*

3.1 Patients with moderate dysfunction

A total of 174 genes showed changes, which was a relatively low number. Genes in the neuron category tended to show downregulation, while most genes showing changes in the muscle category were upregulated. Genes related to muscle components and genes involved in muscle movement were also upregulated. Only two energy production genes were upregulated, and both were important genes related to the glycolysis pathway. I did not find any large functional clusters except for these functional categories. Some genes involved in cell proliferation and cell division showed downregulation. However, genes linked to stress markers and apoptosis did not show upregulation (**Table 1**).

3.2 Patients with severe dysfunction

A total of 763 genes showed changes of expression. Among 59 genes with changes in the neuron category, 39 genes showed upregulation and 20 genes showed downregulation. Most genes showing changes in the muscle and energy categories showed downregulation, but some genes related to immunity, inflammation, and stress, in contrast, showed upregulation (**Table 1**).

3.3 Rats with total paralysis

A total of 1102 genes showed changes of expression. Among 47 genes with changes in the neuron category, 29 genes were upregulated and 18 were downregulated. Some genes linked to immunity, stress, and autophagy were also upregulated. Moreover, most genes showing changes in the inflammation category were upregulated. In contrast, most genes showing changes in the muscle and energy categories showed downregulation (**Table 1**).

4. Discussion

The total number of genes showing changes of expression (mean values for the patients) in the patients with moderate dysfunction was 174, compared to 763 in the patients with severe dysfunction. The total number of genes that changed in both groups was 25, but only 13 genes showed changes of expression in the same direction. Next, the total number of genes showing changes of expression (mean values for the patients and rats) in the patients with moderate dysfunction was 174 but 1102 in the rats with total paralysis. The total number of genes that changed in both groups was 19, but only one gene showed changes of expression in the same direction. These findings indicated that the condition of moderate dysfunction differed greatly from that of severe dysfunction in patients with facial nerve palsy and rats with total paralysis.

Considering these findings, I speculate that, if facial nerve conduction is reduced in moderate dysfunction, the orbicularis oculi muscle cannot function well and the patient presents with paresis. To improve this situation, regeneration of muscle tissue is promoted. Researchers reported that innervation is required for normal energy production in muscles [5]. Therefore, due to denervation, there was the decrease in energy production in muscles with paresis, but patients with moderate dysfunction had some energy production related to regeneration of muscle tissue. There was very little energy production or regeneration of the affected muscles because of the largely abolished facial nerve conduction in patients with severe dysfunction. Accordingly, the muscles innervated by the facial nerve did not show much regeneration, but the neurons themselves showed accelerated regeneration.

On the basis of these genetic findings, I suggest that neurorrhaphy and nerve grafting of the facial nerve are appropriate for patients with moderate dysfunction. These procedures will improve facial nerve conduction, leading to the increase in energy production for the regeneration of muscle tissue. However, there was little muscle regeneration and very low energy production in patients with severe dysfunction, although they showed acceleration of neuronal regeneration. These findings may explain why neurorrhaphy and nerve grafting of the facial nerve are unsuccessful if performed more than 1 year after the onset of facial palsy. Many authors have reported that recovery is better if the interval between injury to the facial nerve and repair surgery on the facial nerve is shorter [6–8]. Because improvement of facial nerve conduction is incomplete in patients with severe dysfunction, I consider that promoting the regeneration of muscle tissue and energy production is necessary. It is possible that some form of regenerative medicine, such as the use of induced pluripotent stem (iPS) cells, could be employed to improve muscle regeneration [9]. However, before such treatments become available for clinical use, I propose administration of pyruvate or mitochonic acid 5 (MA-5) as a practical way to promote muscle regeneration and energy production. Pyruvate plays a pivotal role in central carbon metabolism. Pyruvate is generated from several sources, including the end product of glycolysis, the oxidation of lactate, or as the transamination of alanine. Pyruvate is crucial for ATP generation in mitochondria and for driving several major biosynthetic pathways intersecting the citric acid cycle. In addition, it is well known that pyruvate can eliminate hydrogen peroxide by a nonenzymatic reaction to form acetate, carbon dioxide, and water [10]. This antioxidant activity of pyruvate may be also beneficial for patients with mitochondrial disorders, because the respiratory defects increase the leakage of reactive oxygen species from the mitochondria. Thus, administration of pyruvate restores the production of adenosine 5′-triposphate (ATP) via the glycolytic pathway. MA-5 targets the mitochondrial protein mitofilin at the crista junction of the inner membrane and could be a novel treatment for diseases associated with mitochondrial dysfunction [11, 12]. In Bell's palsy patients with severe dysfunction, it seems that mitochondrial cytopathy develops with impairment of ATP synthesis. As this state is similar to that in various mitochondrial diseases, I consider that pyruvate therapy [13, 14] and the administration of MA-5 could be effective. I also propose neurovascular free muscle transfer as effective surgery for patients with severe dysfunction of facial palsy. Neurovascular free muscle transfer is one of the main reconstructive surgeries for established or long-standing facial palsy [15], but it also seems to be suitable for severe facial palsy based on genetic findings in this study, and I suggest that neurovascular free muscle transfer could be more effective than facial nerve neurorrhaphy with nerve grafting in patients with severe dysfunction.

Grading of facial function is necessary for evaluating and communicating the spontaneous course and the results of medical and surgical treatment of facial palsy [16, 17]. Several grading systems for the assessment of facial nerve function using gross (e.g., the House-Brackmann grading system) [16] or regional (e.g., the Yanagihara grading system) [18] scales have been proposed. In this study, patients with severe dysfunction who were judged as grade V by the House-Brackmann system also scored 8–14 points with the Yanagihara system. The clinical description in patients with severe dysfunction is barely perceptible motion, whereas that in patients with total paralysis (House-Brackmann grading system VI) is no movement. As these two clinical descriptions differ widely, I compared the changes in gene expression between patients with severe dysfunction and rats with total paralysis (**Table 1**). The total number of genes that changed in both groups was 403 and that of gene expression in the same direction accounted for around 83% (333 genes). Some genes linked to immunity, inflammation (most

genes showing changes in rats), and stress were upregulated in both groups. In the neuron category, the number of upregulated genes tended to be higher than the number of downregulated genes in both groups. Moreover, in both groups, most genes showing changes in the energy and muscle categories were downregulated. As mentioned above, the condition of moderate dysfunction differed widely from that of severe dysfunction in patients with facial nerve palsy and rats with total paralysis, but the overall changes indicated that gene expressions in patients with severe dysfunction were similar to those in rats with total paralysis. In general, the molecular biological changes after neurotmesis are as follows: (1) nerve transection produces morphological changes in the neuronal perikarya known as chromatolysis or axon reaction, and the changes include swelling of the cell body, nucleolar enlargement, displacement of the nucleus to the periphery, and dissolution of Nissl bodies [19] and (2) axotomized neurons respond by upregulation of regeneration-associated genes in association with conversion of the neuron from a transmitting to a growth state [20]. Remarkable molecular biological changes after neurotmesis, as described above, lasted for 10–20 days. Rat specimens in this study were obtained on the third postoperative week. Therefore, the rat models in this study closely resembled patients with total paralysis (House-Brackmann grading system VI). Synthesizing these results based on molecular biological analyses, I suggest that clinicians treat patients with severe dysfunction (House-Brackmann grading system V) with the same treatment as for patients with total paralysis (House-Brackmann grading system VI).

5. Conclusions

I conclude that the gene expression in facial nerve palsy changes with the degree of facial nerve palsy. This study will aid in the development of new treatments and diagnostic/prognostic markers based on the severity of facial nerve palsy.

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Conflicts of interest

The author has no conflicts of interest to declare.

Ethics approval of research

The local Ethics Review Board of Showa University Hospital approved the study protocol (No. 149) according to the principles of the Declaration of Helsinki. All the patients were provided with detailed information about the study and provided written informed consent prior to their inclusion. I abided by the Ethical Principles for Medical Research Involving Human Subjects outlined in the Declaration of Helsinki. With regard to animal experimentation, the

present study was conducted following the Guidelines for Institutional Animal Experimentation under the approval of the Ethics Committee of Nagoya City University Graduate School of Medical Sciences (No. H27M-74). I made every effort to minimize pain, discomfort for the animals and the number of animals used, as written in the experimental protocol.

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This work was supported by the annual budget of Showa University for scientific research. I certify that the funding agencies had no involvement in the design, data collection, analysis, or interpretation of the results. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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References

[1] Peitersen E. Bell's palsy: The spontaneous course of 2,500 peripheral facial nerve palsies of different etiologies. Acta Oto-Laryngologica. Supplementum. 2002;**549**:4-30

[2] Garcia R, Hadlock T, Klebuc M, Simpson R, Zenn M, Marcus J. Contemporary solutions for the treatment of facial nerve paralysis. Plastic and Reconstructive Surgery. 2015;**135**:1025e-1046e

[3] Holland J, Bernstein J. Bell's palsy. BMJ Clinical Evidence. 2011;**2011**:1204

[4] Teraoka M, Hato N, Takahashi H, Komobuchi H, Sawai N, Okada M, et al. Myogenin expression in facial muscle following damage to the facial nerve. Acta Oto-Laryngologica. 2012;**132**:783-787

[5] Sato D, Shinzawa G, Kusunoki M, Matsui T, Sasaki H, Feng Z, et al. Effects of electrical microstimulation of peripheral sympathetic nervous fascicle on glucose uptake in rats. Journal of Artificial Organs. 2013;**16**:352-358

[6] Conley J, Baker DC. Hypoglossal-facial nerve anastomosis for reinnervation of the paralyzed face. Plastic and Reconstructive Surgery. 1979;**63**:63-72

[7] Gavron JP, Clemis JD. Hypoglossal-facial nerve anastomosis: A review of forty cases caused by facial nerve injuries in the posterior fossa. The Laryngoscope. 1984;**94**:1447-1450

[8] Kunihiro T, Kanzaki J, Yoshihara S, Satoh Y, Satoh A. Hypoglossal-facial nerve anastomosis after acoustic neuroma resection: lnfluence of the time of anastomosis on recovery of facial movement. ORL: Journal for Otorhinolaryngology and Its Related Specialties. 1996;**58**:32-35

[9] Masumoto H, Ikuno T, Takeda M, Fukushima H, Marui A, Katayama S, et al. Human iPS cell-engineered cardiac tissue sheets with cardiomyocytes and vascular cells for cardiac regeneration. Scientific Reports. 2014;**4**:6716. DOI: 10.1038/srep06716

[10] Nath K, Ngo E, Hebbel R, Croatt A, Zhou B, Nutter L. alph-Ketoacids scavenge H2O2 in vitro and in vivo and reduce menadione-induced DNA injury and cytotoxicity. The American Journal of Physiology. 1995;**268**:C227-C236

[11] Suzuki T, Yamaguchi H, Kikusato M, Hashizume O, Nagatoishi S, Matsuo A, et al. Mitochonic acid 5 binds mitochondria and ameliorates renal tubular and cardiac myocyte damage. Journal of the American Society of Nephrology. 2016;**27**:1925-1932

[12] Suzuki T, Yamaguchi H, Kikusato M, Matsuhashi T, Matsuo A, Sato T, et al. Mitochonic acid 5 (MA-5), a derivative of the plant hormone indole-3-acetic acid, improves survival of fibroblasts from patients with mitochondrial diseases. The Tohoku Journal of Experimental Medicine. 2015;**236**:225-232

[13] Tanaka M, Nishigaki Y, Fuku N, Ibi T, Sahashi K, Koga Y. Therapeutic potential of pyruvate therapy for mitochondrial diseases. Mitochondrion. 2007;**7**:399-401

[14] Kerr DS. Review of clinical trials for mitochondrial disorders: 1997-2012. Neurotherapeutics. 2013;**10**:307-319

[15] Takushima A, Harii K, Asato H, Kurita M, Shiraishi T. Fifteen-year survey of one-stage latissimus dorsi muscle transfer for treatment of longstanding facial paralysis. Journal of Plastic, Reconstructive & Aesthetic Surgery. 2013;**66**:29-36

[16] House JW, Brackmann DE. Facial nerve grading system. Otolaryngology and Head and Neck Surgery. 1985;**93**:146-147

[17] House JW. Facial nerve grading systems. The Laryngoscope. 1983;**93**:1056-1069

[18] Yanagihara N. Grading of facial palsy. In: Fisch U, editor. Proceedings of the Third International Symposium on Facial Nerve Surgery. Zurich: Kugler Medical Publications; 1976. pp. 533-535

[19] Kreutzberg GW. Microglia, the first line of defense in brain pathologies. Arzneimittel-Forschung. 1995;**45**:357-360

[20] Boyd JG, Gordon T. Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. Molecular Neurobiology. 2003;**27**:277-324