Diagnostic/Genetic Screening – Approach for Genetic Diagnoses and Prevention of Cleft Lip and/or Palate

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The treatment, research and volunteer work for cleft lip and/or palate (CL/P) has been led for over 30 years by our team. Within this period, more than 4,000 cases of CL/P were treated and at the same time, and approximately 400 papers were published as the first or partner researcher in Nature Genetics, New England Journal of Medicine and others. In addition, with $20 million that was donated from companies and laypeople, and the grant from the Japanese government, CL/P centres in many countries and in Japan, the oral and craniofacial congenital anomaly gene bank in our CL/P centre was established by our leadership. In the bank there are genes from approximately more than 8,000 cases. The genes were mapped with Professor Jeffery Murray of Iowa University in the United States, the findings about genetic syndromes such as Van der Woude Syndrome and basal cell nevus syndrome were applied in clinical settings. The genetic counselling section that specialises in the oral and maxillofacial field was established by our effort for the first time in Japan. In this review, our clinical experience and approach for genetic diagnoses and prevention of cleft lip and/or palate will be discussed.

Key words: cleft lip and palate, epidemiology, genetic study, animal experimental study, prevention

About 30 years ago, the psychological issue of mothers with children born with a cleft lip and palate was first studied in Japan¹. The results showed that the congenital anomaly of cleft lip and palate was not a life-threatening disease, but more than 30% of mothers of children with cleft lips/palates thought about committing suicide. It can be said the deformity of this congenital anomaly gives mothers lots of stress. In some cases, mothers killed their children because of this disease.

One in 500 to 600 children are born with a cleft lip and palate. It was said that one in 1,500 African children was born with this disease, but recently it is thought that the incidence among African people might be much higher², and we have started operating the study in Africa.

The world’s population has reached 7 billion and approximately 14 million people suffer from this congenital anomaly³.

This review discusses the epidemiological approach of gene and genetic diagnosis and prevention.

Epidemiological approach

The Cleft Lip and Palate Centre at Aichi Gakuin University is located in the middle of Japan and the area is in the third biggest prefecture Aichi in Japan. The gross national product (GNP) of Aichi prefectures accounts for around 1% of world GNP, and produces 40% of Japanese exports. The congenital anomaly monitoring and epidemiological study targeting 11.4 million local

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residents for 30 years was conducted and the study results are used as Japanese data at the Clearing House of the World Health Organization. In the last 22 years, the study on 1,250,144 local residents was conducted, and it was found that 1,812 babies were born with cleft lip/palate, and the incidence ratio was 0.145%\(^4\). Besides epidemiological study at birthing facilities, detailed data from questionnaires with 200 questions including maternal environment were analysed using the cleft pattern modelling method\(^{10,11}\). The questionnaire is targeted at outpatients.

**From epidemiological approach to animal experimental studies**

**Spontaneous models of beagle dog, mouse and rat**

Beagle dogs with cleft lip and palate (Fig 1), with cleft palate only (Fig 2), with progenia (Fig 3) and with Robin sequence (Fig 4), mice with cleft lip and palate from spontaneous generation (Fig 5) were used for animal experiments\(^{12}\).

After the success of the experiment of A/J mouse infused hair colour gene by the congenic strain method, other experiments could be implemented. The mouse inherited 99.8% of spontaneous generation genes of cleft lip\(^{13}\) and only white hair colour was disinherited, with the colour being changed to cinnamon brown. Figure 6 is the picture of a rat with a mandibular cleft. Animals were examined\(^{14}\) for studying about the very rare congenital anomalies that occur in humans.

From epidemiological data, it was found that mothers with anaemia indicated a higher probability of delivering babies with a cleft lip and palate. So an experiment was conducted to see if pregnant mice with blood removed would deliver more babies with cleft palates\(^{15}\). The result was positive. But the result included two possibilities of:

- Anaemia causing a higher incident of cleft palate.
- The stress from the blood being withdrawn caused a higher incidence of cleft palates.

So we infused an anaemia gene to mice through the congenic method and tried to see if the factor of anaemia affected the incidence rate of cleft palates. In the experiment, the W/W\(v\) gene was infused to spontaneous generation mice with cleft lip and palates by using the congenic method and for establishing a new system, interbreeding was repeated over generations.

What should be done is that the hypothesis generated from the epidemiological study and the result from the animal test need to be verified and should be made applicable to clinical settings to bring benefit to patients.

Figure 7 shows the white A/J mice and the black mouse with a white spot on its back. The white A/J mice that were infused with the W/W\(v\) gene, which was extracted from black mice by back-cross interbreeding had black hair, apart from a white spot of hair on their backs. The result proved that the W/W\(v\) gene was interbred to the A/J mouse successfully. In Fig 8, the cleft palate is confirmed from stained osteocartilaginous tissues. The result of A/J mice and A/J-w\(+/-\) mice showed 8.8% of cleft palate babies from usual spontaneous generation mice and much higher incidence of 22.85 from A/J mice infused with the W/W\(v\) gene (Table 1).
From these results, it can be said that the anaemia encouraged the incidence of cleft lips and palates. To prevent cleft lip, it is advised to treat anaemia before pregnancy and prevent anaemia at the early stages of pregnancy.

This result is applied for prevention at the clinical setting. People who are from cleft lip and palate families are instructed to treat anaemia before pregnancy.

Also from the results, taking enough daily protein may prevent cleft lips and palates, which can be seen from epidemiological data. This was verified with animal tests. In addition to the importance of treating anaemia before pregnancy for prevention, the importance of taking enough quality protein to prevent cleft lips and palates is also provided from the study\textsuperscript{16,17}.

From other epidemiological research, the results showed that mothers who took green vegetables every day delivered healthy babies. On the other hand, mothers who did not take vegetables had a higher incidence rate of delivering babies with cleft lips and palates in comparison with mothers who ate vegetables every day\textsuperscript{18}. Recently, mothers have been instructed to eat lots of vegetables and mothers who do not like vegetables have been instructed to take supplements.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
 & No of mothers & Implantation & Live foetuses & Resorption & Dead foetuses & Incidence of CLP \\
\hline
A/J mice (average) & 51 & 563 & 374 & 139 & 50 & 33 (8.8\%) 0.65 \\
 & & 11.03 & 7.33 & 2.73 & 0.98 & \\
\hline
A/J-Wv/+mice (average) & 24 & 197 & 131 & 52 & 14 & 37 (28.2\%) 1.54 \\
 & & 8.20 & 5.46 & 2.17 & 0.58 & \\
\hline
\end{tabular}
\caption{Result of A/J mice and A/J-Wv/+mice}
\end{table}
Epochal approach for prevention by gender selection

In previous epidemiological researches, cleft patterns had not been analysed in detail. The degree of cleft and incidence was analysed with the cleft pattern method. It is well known that there is a high incidence of cleft palate only females, but it did not correspond with the research – a high incidence of cleft uvula was confirmed in males. No gender difference was confirmed in cleft soft palate. Significantly higher incidences of hard and soft cleft palates were confirmed in females. In general, incidences in females were slightly higher. The result of this research provided that there is a strong relation between the degree of cleft and gender and also that gender contributes to the incidence rate of cleft palates (Table 2).

In addition, the research of family histories showed an important finding. It is well known that cleft palate is handed down to babies. The finding was that babies with cleft palate who had parents with cleft palate were only female – no male babies were confirmed. This result gave us an opportunity to develop the epochal prevention method. Patients with cleft palates tend to not marry or have babies because of the high incidence rate of inheriting cleft palates. But, there is a possibility that if the couple with the first child with cleft lip and palate take gender selection to the next child, their next son will have no cleft or a very mild cleft such as an uvula cleft (Table 3).

Genetic approach

In the experiments with mice, transplanting fertilised eggs from spontaneous generated mice with cleft lip and palate to mice without cleft lip and palate resulted in there being no foetus with cleft lip and palate. The results indicate that if the maternal body environment is controlled, cleft palates can be prevented. But the occurrence of cleft lip and palates was confirmed in the next generation. It may be said that these congenital anomalies are closely related to genes and genetic research is strongly needed. The genetic research system organised by Prof Natsume in Japan (Fig 9) is capable of storing up to one million cases and the storage of 9,000 genes has been completed.

Many patients without a diagnosis can get an appropriate diagnosis by gene analysis. Gene analysis was conducted of IRF6, TGFB3, MSX1, PAX9, RYK, which are considered to be related to cleft lips and palates. Genes of patients with cleft lips and palates are banked at World Cleft Gene Banking. Many important findings about genes related to cleft lips and palates have been found.

Genetic diagnosis

Van der Woude syndrome

The world biggest family tree of Van der Woude syndrome (VWS) was analysed and from this family, the IRF-6 gene was found, and this finding made very mild VWS confirmed diagnosis (Fig 10). The IRF-6 gene, which was extracted from fingernail DND using the genome amplification method, also had the mutation (Fig 11).
Basal cell nevus syndrome

Patients with Basal cell nevus syndrome without cleft lip could not be diagnosed until the onset of disease. Because of this, some patients died from Basal cell nevus syndrome. Owing to genetic analysis, people without the Basal cell nevus syndrome gene can feel secure and those with the gene can get diagnosed and treated at an early stage. Due to improving genetic diagnoses, informed consent of patients is very important. We opened the genetic counselling office, which specialises in cleft lip and palate counselling.

At the genetic counselling office, couples with a child who has a cleft lip and palate are given counselling regarding the risk of their second child also inheriting a cleft lip and palate. They are advised about genetic diagnosing.

Strategy for prevention

The Japanese government grants gene banking for the next 5 years and it should be focusing on genetic analysis and diagnosis, and prevention of cleft lips and palates. The genetic screening for mothers who had difficulty metabolising folic acid was conducted and Methylene-tetrahydrofolate reductase (MTHFR) was analysed for screening. The relation between plasma homocysteine and folic acid in MTHFR C677T was found. People with type T/T tend to show high values of homocysteine and low values of folic acid in comparison with those with type C/C or C/T. It may be said that screening by MTHFR will find mothers who need folic acid treatment. The prevention of cleft palate only by gender selection, besides controlling anaemia, taking daily protein products and folic acid will be conducted. Genetic diagnosis of cleft lips and palates is positively facing a new age.

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