Importance of Genetic Factors in Stroke: An Evidence Obtained by Selective Breeding of Stroke-prone and -resistant SHR

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SPONTANEOUSLY hypertensive rats (SHR)¹⁻³ are now regarded as the best animal model so far for human essential hypertension⁴⁻⁵. However, so far as cerebrovascular lesions were concerned, the incidence was relatively lower around 10 per cent before F₂₀ generation⁶⁻⁷ in comparison with men in whom nearly a quarter of the population as in Japan, die of cerebrovascular diseases, so called stroke. Experimental alteration of environmental factors such as stress⁸ or salt-loading⁹⁻¹⁵ augmented hypertension and aggravated hypertensive cardiovascular diseases in SHR. Although these environmental factors increased the incidence of cerebrovascular lesions, these effects, which were different in various families or among substrains of SHR, indicated the importance of genetic disposition as well as the environmental factors for the development of stroke. Consequently, we tried to separate the strain of SHR which would develop stroke spontaneously in high incidence from among the substrains of SHR with a greater susceptibility to cerebrovascular lesions.

Successive Selection of the Stroke-prone SHR

Since symptoms of stroke were used to be observed only in the advanced stage up to F₂₃ generation before selection, it was impossible to breed the offspring from SHR which had a definite sign of stroke. Therefore, SHR in A₃ and A₁ sb substrains, which were supposed to have a greater genetic disposition to stroke, were mated as many as possible to get the offspring in advance (at least two generations ahead) and only the offspring of the SHR which developed stroke spontaneously were maintained to proceed the further selection. All SHR were macroscopically and microscopically examined after natural death to confirm the cerebrovascular lesions. We have obtained by such a selective brother-sister breeding the offspring of SHR, one or both parents of which had a stroke for 6 or 7 generations successively. The incidence of stroke in adult males over 100 days of age in these selectively-bred SHR was greatly increased up to 77 per cent in average (92 out of 119 rats) from F₂₇ to F₂₉ generations, while the incidence of stroke under the process of the selection was 39 per cent in average (45 out of 115) from F₂₄ to F₂₆ generations. On the other hand, the incidence of stroke in adult females over 150 days of age was somewhat lower than that in males, 58 per cent in average (23 out of 40) in F₂₇ and F₂₈ generations, but the selection effect was obvious also in females, because the incidence under the process of the selection had been 15 per cent (15 out of 97) from F₂₄ to F₂₆ generations. The incidences of stroke after F₂₇ both in males and

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in females were nearly stable and seemed to have reached almost the maximum plateau level. It is partly because of the difficulty in decreasing further the death rate due to other causes such as pneumonia during a long-term observation up to 2 years under conventional state, but this fact also indicates that the selection of SHR which develop stroke spontaneously may be almost completed. Therefore, these selectively-bred SHR were named as the stroke-prone SHR and the establishment of the stroke-prone SHR as well as their main characteristics in blood pressure and cerebrovascular lesions were first reported by Okamoto, Yamori and Nagaoka \(^6\) Fig. 1 shows two typical examples of macroscopical cerebral hemorrhage and softening observed in the stroke-prone SHR. (For the details of the macroscopical or microscopical findings of these cerebral lesions and the symptomatology of these stroke-prone SHR, see the reference\(^5\)).

On the contrary to such a high incidence of stroke in the stroke-prone SHR, the incidences were very low in the selectively-bred and maintained offspring from B\(_1\), B\(_2\) and C substrains: 5 per cent in average (6 out of 127) in adult males from F\(_{24}\) to F\(_{28}\) generations and 1 per cent in average (2 out of 142) in adult females from F\(_{24}\) to F\(_{28}\) generations. These offspring were called stroke-resistant SHR. The successful separation of stroke-prone and -resistant SHR by selective inbreeding itself obviously confirmed the importance of genetic factors for the development of stroke.

**Genealogy of the Stroke-prone SHR**

Gnenalogical analysis provided with the further evidence for the importance of genetic factors.
Fig. 2. Pedigree of one family of the selectively-bred stroke-prone SHR (as of Nov. 1973). The brain of a rat with asterisks was not examined. See the notes in the figure for other legends.

Factors in stroke. As shown in the pedigree of one family of the stroke-prone SHR (Fig. 2), for example, the incidence of stroke was increased in F$_{27}$ and thereafter, i.e., in the offspring obtained by the successive selection of SHR with stroke for 3 generations. Since the younger rats developed stroke in the later generations of selective breeding, genetic accumulation seemed to affect the development of stroke and to accelerate it as shown in the gradual shortening of the life span of SHR which died with cerebrovascular lesion. Furthermore, in the family of F$_{29}$ generation from the parents, both of which died with cerebrovascular lesions, most members died with stroke or showed clear symptoms of stroke during the observation up to 290 days after birth. However, the other family of F$_{29}$ generation, partly shown in the pedigree of Fig. 2, was derived from the parents, only one of which had cerebrovascular lesions at autopsy and the other was still alive without any signs of stroke during the observation up to 420 days after birth. In none of the family obvious signs of stroke were observed during the observation for 290 days, but the members were supposed to have the traits of stroke because one of their offspring developed stroke in so young age as 139 days after birth. Such a comparative study on the incidence of stroke in relation to their family history substantiated the view of the importance
of heredity in stroke.

**Analytical Trials on the Genetic Factors of Stroke**

In order to clarify the genetic factors related to the spontaneous development of stroke in SHR, we analyzed at first the relationship between the incidence of stroke and blood pressure. As we maintain 8 genealogically different SHR-substrains with different incidences of stroke, a comparative study among these substrains is available. Since we noted a clear significant positive correlation between the incidence of stroke and the initial increase of blood pressure among several SHR-substrains, we compared the developmental course of hypertension in the stroke-prone SHR with those in the stroke-resistant SHR and other substrains between them. As summarized in Fig. 3, the stroke-prone SHR developed hypertension over 180 mmHg significantly earlier than the stroke-resistant SHR. The blood pressure in the former also surpassed the level of 200 mmHg earlier than those in other substrains and became higher than

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the level of 230 mmHg which the stroke-resistant SHR rarely attained. The statistical analysis between the incidence of stroke and the severity or the developmental course of hypertension among these substrains clarified the following points:  

a) The incidence (%) of cerebral lesions [Y] was closely correlated to the severity of hypertension, the highest blood pressure (mmHg) observed during lifetime [X]: \( r = 0.953 \) (p < 0.01), \( Y = 1.6X - 320 \).  
b) The incidence (%) of cerebral lesions [Y] was inversely correlated to the age (weeks) when blood pressure surpassed 180 mmHg [X]: \( r = -0.806 \) (p < 0.01) \( Y = -11.8X + 158 \).  
c) The incidence (%) of cerebral lesion [Y] was positively correlated to the initial increment of blood pressure (mmHg/week) observed from 5 to 15 weeks of age [X]: \( r = 0.933 \) (p < 0.01), \( Y = 16.4X - 60 \).  

These results clearly indicated that the severe hypertension as well as rapidly developed hypertension in the younger age were closely related to the higher incidence of stroke and the genetic disposition to stroke seemed to be related to the genetic factors influencing such characteristics of hypertension.

However, such differences in the severity and the developmental course of hypertension could not explain all the difference in the incidence of stroke between stroke-prone and -resistant SHR, or among various substrains of SHR. For example, experimental salt-loading of 1 per cent salt in drinking water clearly accelerated the development of hypertension over 230 mmHg both in A\(_3\) substrain with a greater incidence of spontaneous cerebral lesions and in C substrain which rarely developed stroke.  

In spite of the same grade of severe hypertension augmented under salt-loading there were still significant differences in the incidence of stroke between A\(_3\) (84%) and C (50%) and also in the age of death between the former (123 ± 6 days) and the latter (185 ± 19 days).

Parabiosis, which was one of the useful experimental measures for the detection of humoral factors, if any, in hypertension was made between the stroke-prone and -resistant SHR by Nagaoaka et al.  

However, there seemed to be no strong humoral transmissible factors for stroke.

Although the further analyses are necessary to clarify the genetic factors involved in stroke, the mode of heredity in stroke seems to be multifactorial so far as blood pressure, which is determined by multifactorial inheritance in SHR, is closely related to the development of stroke.

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Discussion:
Chairman: Dr. J. FUJII

Dr. EBISARA (2nd Dpt. Int. Med. Univ. Tokyo): You have showed a close relationship between the cerebral bleeding and the rise in blood pressure. Is there any relationship between the cerebral softening and arteriosclerotic changes of the cerebral arteries?

Dr. YAMORI: As you know, the cerebral bleeding developed on the basis of angioneerosis of the cerebral arteries. The cerebral softening, I suppose, might be based on proliferative changes of the cerebral arteries which were considered as a repair process for the angioneerosis. A thrombotic process was sometimes superimposed on the arterial lesions.

Dr. EBISHARA: Did you find a myocardial infarction in animals with cerebral softening?

Dr. YAMORI: We often found small fibrotic scars in the myocardium but rarely found a massive myocardial infarction.

Dr. EBISHARA: We can observe multiple cerebral petechial bleedings as well as a massive cerebral bleeding in rats and rabbits with renal hypertension. Did you find such petechial bleedings in the brain?

Dr. YAMORI: Yes, we also found such lesions in SHRs in the early stage of stroke.

Dr. SEKI (Inst. Adult Dis. Asahi Life Fdn.): Was the cerebral softening responsible for the cause of death?

Dr. YAMORI: About one half of the cerebral softenings might be related to the cause of death. Dr. ODA (Ohmiya City Hosp.): According to your studies the incidence of stroke was significantly higher in male than female SHRs. How do you explain the sex difference?

Dr. YAMORI: The level of blood pressure was higher in male than in female SHRs and hypertension developed in male earlier than in female SHRs. The difference in the severity of hypertension may account for the sex difference in stroke.

Dr. ONOYAMA (2nd Dpt. Intern. Med. Kyushu Univ.): Why the cerebral bleeding developed predominantly in the subcortical areas?

Dr. YAMORI: The cortical and subcortical areas are supplied with blood by the endartery in areas are supplied with blood by recurrent branches in rats. This is one reason accountable for the predilection sites of cerebral bleeding.

Dr. IKEDA (3rd Dpt. Intern. Med. Univ. Tokyo): Have you found the acute vascular lesions in other organs?

Dr. YAMORI: The vascular lesions developed also in other organs in the stroke prone SHR. But organ specificity of the vascular disease is an interesting problem. In the future a coronary artery disease prone or a renal artery disease prone strain of SHR may be separated.

CHAIRMAN: Human pathology has indicated that the vascular disease responsible for cerebral bleeding is quite different from that responsible for cerebral softening. The former results from angioneerosis of the cerebral arterioles and the latter results from the atherosclerotic thrombosis of the larger cerebral arteries. Did you find thrombotic changes in larger cerebral arteries?

Dr. YAMORI: Thrombotic changes developed on the basis of angioneerosis in the stroke prone SHR. Accordingly the size of the affected artery responsible for cerebral softening was the same as that responsible for cerebral bleeding.

CHAIRMAN: Dr. Yamori presented an interesting paper which was concerned chiefly with separation of a stroke prone SHR. We would like to hear the nature of the vascular disease in the future.