

PRELIMINARY REPORT ON THE SHORT STATURE OF SOUTHEAST ASIAN FOREST DWELLERS, THE MANNI, IN SOUTHERN THAILAND: LACK OF AN ADOLESCENT SPURT IN PLASMA IGF-I CONCENTRATION

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Abstract. Plasma concentration of insulin-like growth factor type-I (IGF-I) was studied among the Mannis in Thailand to find a possible cause of their short stature. The Mannis are hunting and gathering indigenous tribal peoples living in Asian tropical rain forests. A total of 50 plasma specimens from three different Manni groups in southern Thailand were used in this study. The concentrations of acid-ethanol extract of plasma IGF-I were measured by radio-immunoassay. We found that (1) plasma concentration of IGF-I in the Mannis was low, (2) there was no adolescent spurt in IGF-I levels, and (3) the post adolescent plasma IGF-I level of the Manni was significantly lower than that of age-matched Japanese. Low IGF-I levels among the Mannis may account for their short stature.

INTRODUCTION

Stature depends on skeletal growth and development. The growth of the bone is regulated by various hormonal factors (Raisz, 1978). Insulin-like growth factor I (IGF-I), which used to be called somatomedin C, has been shown to play an important role in growth and development, since IGF-I takes part in the final step of transaction pathway of skeletal growth (Herington, 1991). Thus, patients showing short stature with normal growth hormone concentrations accompanied by low serum concentrations of IGF-I have been identified (Daughaday *et al.*, 1969).

Among normal human populations, the Pygmy, well known tropical forest dwellers, have been considered as a natural model for dwarfism (Merimee and Rimoin, 1986). Low levels of IGF-I and growth hormone binding protein (GHBP) have been documented among African Pygmies (Merimee *et al.*, 1981, 1987; Baumann, 1989). In Asia, the presence of hunter-gatherers with short

stature are known (Martin, 1905). They have inhabited Southeastern Asian countries such as the Philippines, Malaysia and the Andaman Islands. They have been classified into the Aeta, the Semang, and the Andamanese. They share common physical characters such as short stature, dark skin color, and frizzy hair. Somatometric descriptions of the Austro-Asiatic language speaking Semang of Southeast Asia have been traced as far back as the 19th Century and their short stature was also mentioned in Martin's masterpiece (Martin, 1905).

People of an indigenous population known as "Sakai" or "Ngo" inhabit southern Thailand. They call themselves "Manni". They have kept a nomadic mode of life and their presence was recorded in several provinces in southern Thailand until recently. However, the population size of the Mannis has decreased to ~ 100 and the range of their living area is limited to only three provinces in Thai territory. Their genealogical status is still controversial and their place among the Southeast Asian populations is unclear. Since 1989 we have carried out investigations on their biological characteristics in terms of genetic diversity and adaptation (Shimizu *et al.*, 1997). To shed light on the short stature of Asian tropical forest dwellers, we measured the blood concentration of IGF-I among the Manni.

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MATERIALS AND METHODS

Samples

A total of 50 Mannis (31 males and 19 females) from three different localities were involved in this study (Fig 1). The age of the subjects ranged between 6 and 70 years. After the oral informed consent, blood specimens were collected by venipuncture with heparin as anticoagulant between 1989 and 1993. Plasmas were separated and stored -80°C prior to use. For the comparative study of IGF-I concentration in the post adolescent phase, plasmas from Japanese aged 22~24 years were collected and stored in the same manner.



Fig 1—Locality of the Manni groups in southern Thailand. Blood specimens were collected in Palian, Satul and Tarto.

Assay for plasma IGF-I concentration

To 200 μl of mixture of 87.5% ethanol and 12.5% 2N hydrochloric acid (vol/vol) in polystyrene tube, 50 μl of plasma was added through mixing (by vortex) for 10 seconds and rotated for 30 minutes. After standing for 30 minutes at room temperature, the tubes were centrifuged at 3,000 rpm for 30 minutes at 4°C . Supernatant (100 μl) was transferred into a fresh polystyrene tube and 4 μl of 0.855M tris base was added and mixed to neutralize the acid-ethanol extract of plasma.

Two types of RIA kit, Somatomedin-C 'Eiken' (Kit I) and Somatomedin-C 'Eiken' II (Kit II) were used to measure the concentration of IGF-I. Both kits were purchased from Eiken Chemical Co Ltd, Tokyo, Japan. Prior to RIA, the extract was diluted

at a concentration of 1:20 with buffer supplied in the kits. Assays were performed in polystyrene tubes according to the methods indicated in the kits. The sensitivity of the kits was 7 mU/ml in Kit I and 0.3ng/ml in Kit II, respectively. Both inter- and intra-assay variability was less than 10% in Kit I and 15% in Kit II, respectively. The correlation between Kit I and Kit II was 0.986. The results obtained by the assay using Kit I were converted from mU/ml to ng/ml using the data that 1U corresponds to 36.1ng (Eiken Chemical Co Ltd).

RESULTS

Plasma IGF-I levels among the Manni are plotted in Fig 2a. One male aged 20 years showed the highest value (424.0 ng/ml), whereas, one male aged 6 years showed the lowest concentration (29.8 ng/ml). An age-dependent decrease in IGF-I concentration was observed in the post-maturation period. In juveniles, IGF-I values were distributed close to the lower limit of those in the normal young Japanese group (unpublished data, Special Reference Laboratory Ltd, Tokyo, Japan) and a spurt of

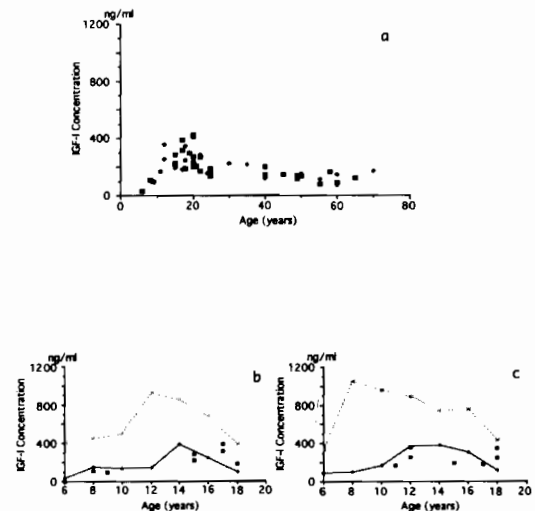


Fig 2—Plasma IGF-I concentration among the Manni in southern Thailand (\square : male; \diamond : female) (a), the young males (b) and females (c). Upper and lower lines indicate the normal range of IGF-I levels in age matched Japanese boys (b) and girls (c), respectively (Special Reference Laboratory Ltd, Japan).

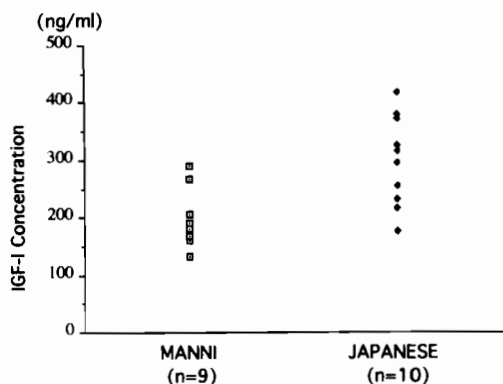


Fig 3—Distribution of plasma IGF-I concentration among age matched (21~25 years) Manni and Japanese.

IGF-I in adolescence was not observed (Fig 2b, c). Two adults showed IGF-I concentrations under 100 ng/ml. IGF-I levels in age-matched (21~25 years) subjects from the Manni and Japanese are shown in Fig 3 where the average value of IGF-I in the Manni (195.9 ± 16.9 ng/ml) was significantly lower than that in age matched Japanese (297.6 ± 24.8 ng/ml) ($p < 0.005$).

DISCUSSION

The growth and development of bones depends indirectly on GH but rather directly on IGF-I that plays important roles in the stature. One of the GH resistant diseases, Laron syndrome (Laron *et al*, 1966), which shows low concentration of blood IGF-I and GHBP in patients substantially demonstrated significant roles of IGF-I in the stature. The African Pygmy has been considered as a natural model of dwarfism and the concentration of humoral factors responsible for the stature, such as GH, IGF, testosterone and GHBP have been measured (Merimee and Rimoin, 1986; Merimee *et al*, 1987; Baumann *et al*, 1989). Low levels of plasma IGF-I and GHBP concentration have been demonstrated in the Pygmies, suggesting the low IGF-I level is responsible for their short stature.

In Asia, equivalent populations are distributed in Southeast Asian tropical forest. The Manni, one of the groups of Semang, inhabit southern Thailand. Living in an autonomous local band, they are traditional nomadic hunters, using blowguns to hunt small game, and gatherers of wild roots and

fruits. For shelter they use caves of rock overhangs or build leaf-covered windscreens. They show a rather short stature, dark skin color and frizzy hair. Recorded averaged height of the male and female Semang was 152cm and 142 cm, respectively (Sebesta and Lebzelter, 1928).

In this study, we found that (1) plasma concentration of IGF-I in the Manni was low, (2) there was no adolescent spurt in IGF-I levels, and (3) the post adolescent plasma IGF-I level of the Manni was significantly lower than that of age-matched Japanese. Low IGF-I levels and/or the lack of adolescent spurt in IGF-I concentration among the Manni may account for their short stature. These results are comparable, in general, with those found in the Pygmies in Africa (Merimee *et al*, 1987) yet we note here some different features in the plasma IGF-I profiles among the Manni. In children and adolescents, the Manni showed a similar pattern of age-dependent changes in IGF-I levels to the Pygmy, however, a different feature was observed in adults. The mean value of adult IGF-I in the Manni (185.0 ± 14.0 ng/ml) was double the mean value in that of the Pygmy (94.0 ng/ml; data from Merimee *et al*, 1987). Post adolescence decline of plasma IGF-I concentration was less in the Mannis. This may explain the slightly short stature of the Asian tropical forest dwellers and indicate levels of IGF-I in between adolescence and adult is critical for the final stature.

It is a classical argument that the short stature of tropical forest dwellers reflects genetic adaptation or epigenetic adaptation. Malnutrition is a major epigenetic cause of short stature and most if not all of such tropical rain forest dwellers are under sub-nutrition. One piece of evidence which may possibly support genetic predisposition of their short stature is that cells from Pygmies show low responsiveness to the GH stimulation *in vitro* (Merimee *et al*, 1989). Lymphocytes from Pygmies produced lower levels of IGF-I under the stimulation of GH *in vitro* than those from normal controls (Merimee *et al*, 1989). This prompted these authors to speculate that the short stature of the Pygmies was defined by genetic background. If this low responsiveness to GH is generalized from lymphocytes to epiphyseal cells of bones, it would be quite interesting to test Manni lymphocytes in an *in vitro* GH stimulation experiment to determine if tropical forest dwellers share the same cellular traits which reflect their genetic background or not. We plan to establish EBV immortalized cells of the Mannis

and will test them in the same manner to do this.

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