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Short Communication

HIGH INCIDENCE OF A POLYMORPHIC VARIANT OF ERYTHROCYTE MEMBRANE PROTEIN, BAND 3-MEMPHIS, ON A WESTERN JAPANESE ISLAND

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Summary Band 3 is the major membrane protein of erythrocytes, which binds membrane skeletal proteins, glycolytic enzymes, and hemoglobin and transports various kinds of anions. Band 3-Memphis is a variant of Band 3, the amino terminal fragment of which depicts a slow electrophoretic mobility on sodium dodecyl sulfate-polyacrylamide gel. The frequency of Band 3-Memphis varies among populations, with a higher frequency among the Japanese. We investigated the frequency of Band 3-Memphis in a western Japanese island which is relatively isolated from the main islands, finding that the frequency of Band 3-Memphis of the inhabitants of this island is significantly higher than the frequency of the Japanese based on the survey in Kyushu Island. This indicates that there may be differences of population in the frequency of Band 3-Memphis in Japan and that Band 3-Memphis may be a good marker to genetically differentiate each population.

Key Words Band 3-Memphis, Band 3

Band 3 is the major membrane protein of erythrocytes, with a molecular weight of 90,000–100,000 (Jay and Cantley, 1986). Band 3 consists of two domains, the amino terminal domain and the carboxy terminal domain. The amino terminal domain binds membrane skeletal proteins, glycolytic enzymes, and hemoglobin, while the carboxy terminal domain acts as a transporter of various anions such

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as chloride, bicarbonate, phosphate, and phosphoenolpyruvate (Bennett, 1990; Hamasaki and Kawano, 1987; Jay and Cantley, 1986).

Band 3-Memphis was originally reported as a variant of Band 3, which gives rise to two fragments of 60 kDa and 63 kDa molecular weight by pronase or chymotrypsin digestion, while normal Band 3 produces only 60 kDa molecular weight fragments (Mueller and Morrison, 1977). Later, the variant protein that produces a 63 kDa-fragment was named Band 3-Memphis (Ranney *et al.*, 1990). Sequence analysis of the amino terminal portion of Band 3-Memphis revealed that the lysine at position 56 was replaced by a glutamic acid, which is thought to lead to abnormal electrophoretic mobility on sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) (Yannoukakos *et al.*, 1991). Carriers of Band 3-Memphis are asymptomatic and show no morphologic abnormalities of their erythrocytes (Ranney *et al.*, 1990). Functional analyses of erythrocytes containing Band 3-Memphis in homozygotes demonstrated that the transport rate of phosphoenolpyruvate was slightly decreased (Ideguchi *et al.*, 1992).

Some studies to examine the frequencies of Band 3-Memphis in each population have been performed, proving that Band 3-Memphis is not a rare polymorphism and that the gene frequency of Band 3-Memphis varies among different populations (0.04–0.10), with a higher frequency among the Japanese (0.156) (Ideguchi *et al.*, 1992; Mueller and Morrison, 1977; Palatnik *et al.*, 1990; Ranney *et al.*, 1990). However, so far no survey has been done in a small population.

In this study, in order to explore the possibility that the frequency of Band 3-Memphis varies among populations in Japan, we analyzed the frequency of Band 3-Memphis of the inhabitants in a western Japanese island which is relatively isolated from Kyushu Island.

Materials and Methods

Red blood cells were collected into EDTA-containing tubes from the inhabitants in Himeshima Island, which is located about 4 km west of Kyushu Island, 33 km northwest of Fukuoka city in western Japan (Fig. 1).

Digestion of intact red blood cells with chymotrypsin was carried out as described previously (Ideguchi *et al.*, 1992). Briefly, washed red blood cells with phosphate buffered saline (PBS: pH 7.4) were treated with chymotrypsin (final 0.5 mg/ml) at 37°C for 1 hr at 50% hematocrit in PBS containing 5 mM glucose. The reaction was stopped by addition of PBS containing 0.2 mM phenylmethylsulfonyl fluoride, and ghosts were obtained by hypotonic lysis (Fairbanks *et al.*, 1971). Electrophoresis was conducted on a 9% polyacrylamide gel at the constant voltage of 120 V and gels were stained with Coomassie Brilliant Blue (Laemmli, 1970).

Results and Discussion

For this survey, the red blood cells of 75 inhabitants (male 28, female 47) of this island were examined from the total population of 142 (52.8%). There were

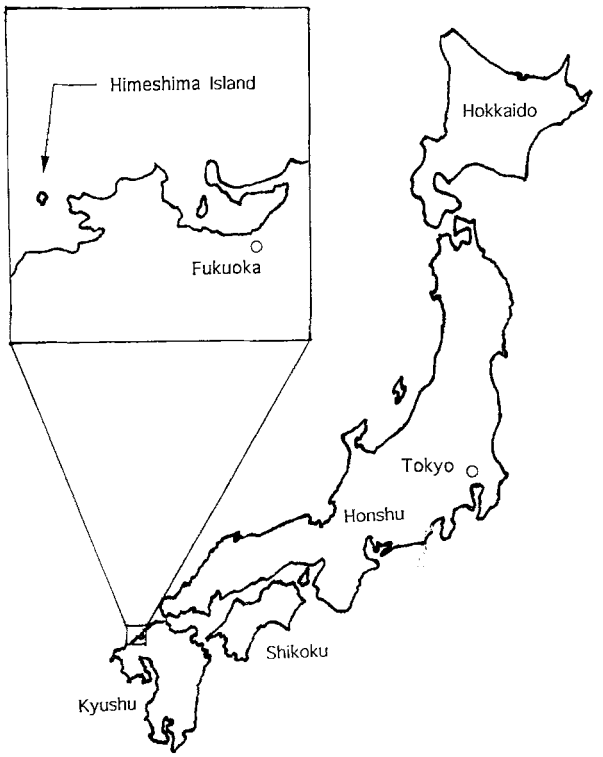

Fig. 1. Location of Himeshima Island. The map represents Japan and the inserted boxed map shows the location of Himeshima Island.

none manifesting anemia or red cell shape abnormalities. The inhabitants of this island have lived there for many generations, and the rate of consanguineous marriage between first cousins or second cousins was 58% (32 cases in 55 couples) by the survey in 1967, which means that the inhabitants are relatively distinguishable from those of the main islands in terms of genetics, in spite of being near the main islands.

Band 3-Memphis was diagnosed by the slow mobility of the chymotrypsin-digested Band 3 on SDS-PAGE. Normal Band 3 gives rise to a single 60 kDa fragment (*MM*), while heterozygous Band 3-Memphis and homozygous Band 3-Memphis produce two bands of 60 kDa and 63 kDa fragments (*Mm*) and a single 63 kDa fragment (*mm*), respectively (Fig. 2A). Family studies demonstrated that the three phenotypes are controlled by two alleles of a single autosomal locus, with no dominance as described before (Palatnik *et al.*, 1990). In two $mm \times Mm$ matings, there were 2 *mm* children and 3 *Mm* children. In one $Mm \times Mm$ mating, there were a *Mm* child and a *MM* child. In 4 $Mm \times MM$ matings, there were 4 *Mm* children and 3 *MM* children. These results are consistent with predictions. A phenotype example of $mm \times Mm$ mating is shown in Fig. 2.

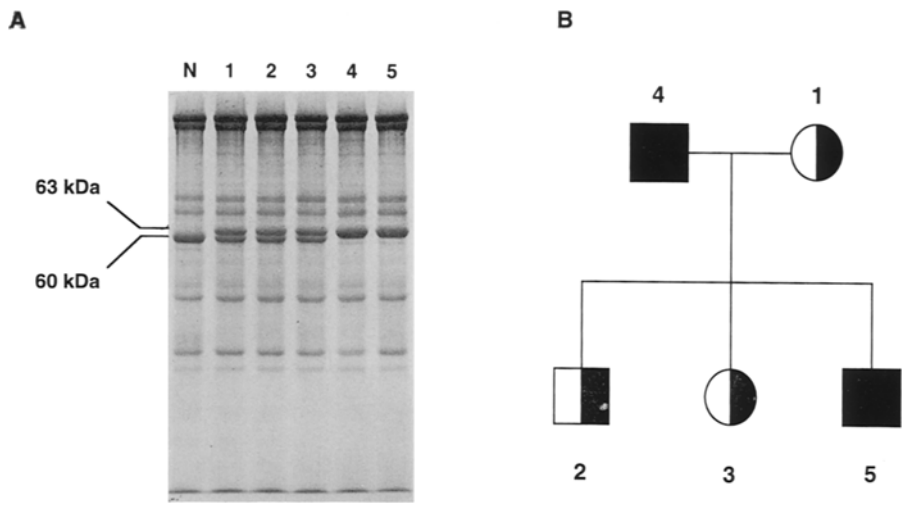

Fig. 2. SDS-PAGE analysis of Band 3 and family study of Band 3-Memphis carriers. Panel A: Chymotrypsin-digested ghosts of the red cell were analyzed by SDS-PAGE. Indicated lines represent 63 kDa fragment (upper) and 60 kDa fragment (down) of Band 3. N, lines 1–3, and lines 4, 5 denote normal Band 3 (*MM*), heterozygous Band 3-Memphis (*Mm*), and homozygous Band 3-Memphis (*mm*), respectively. Panel B: One pedigree of Band 3-Memphis carriers is shown. Square is male and circle is female. ■ and ◨, ◑ represent homozygous Band 3-Memphis (*mm*) and heterozygous Band 3-Memphis (*Mm*), respectively. The SDS-PAGE pattern of each individual in the pedigree is displayed in panel A. The numbers in the panel A correspond with those shown in the panel B.

Table 1. Frequency of Band 3-Memphis in Himeshima Island.

	Normal (*MM*)	Heterozygote (*Mm*)	Homozygote (*mm*)	Total
Male	13 (46.4%)	9 (32.1%)	6 (21.4%)	28
Female	28 (59.6%)	15 (31.9%)	4 (8.5%)	47
Total	41 (54.7%)	24 (32.0%)	10 (13.3%)	75
Expected	38	31	6	75

Determination of three phenotypes was based on the results of SDS-PAGE as described in Fig. 2. The gene frequency of Band 3-Memphis is calculated to be 0.293 (male 0.375, female 0.245). The expected frequency is calculated by Hardy-Weinberg law.

Frequency of *MM* was 54.7% (male 46.4%, female 59.6%), while that of *Mm* was 32.0% (male 32.1%, female 31.9%) and that of *mm* was 13.3% (male 21.4%, female 8.5%) (Table 1). Gene frequency of Band 3-Memphis was calculated to be 0.293 (male 0.375, female 0.245). The observed value of three phenotypes was

distant to the calculated ones by Hardy-Weinberg law ($0.05>p>0.01$). It may be due to the high rate of consanguineous marriage and the small number of the examined samples. The value of 0.293 is significantly higher than those of other populations such as Caucasians (0.04), American Indians (0.098), African Americans (0.091), Chinese (0.072), Filipinos (0.1), and Mexicans (0.083) (Ranney *et al.*, 1990). Ideguchi *et al.* (1992) noted that a Japanese population consisted of 613 patients and 123 healthy workers of the Fukuoka University Hospital showed a high frequency (0.156) compared to other populations; however, still the frequency of the inhabitants in this island is almost twice as that of the Japanese examined by Ideguchi *et al.* Since there was no survey focused on a small population of the Japanese, thus far it is difficult to predict how wide the population carrying Band 3-Memphis is distributed in Japan. Further examinations of the frequency of Band 3-Memphis in other regions of Japan is needed to address this point. The slight difference of the frequency between genders may be explained by the fact that some women come from other areas to this island for marriage and fewer men do so, indicating that the original inhabitants of this island would have higher gene frequency of Band 3-Memphis than the present ones. So far, some analyses of the frequency of Band 3-Memphis in each population have been done, however, our survey is the first evidence that the frequency of Band 3-Memphis in inhabitants who are isolated from those of the main islands in terms of genetics is different from that of the Japanese living in Fukuoka area in Kyushu Island. This result indicates that the frequency of Band 3-Memphis may be a good marker to genetically differentiate small isolated populations.

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