CHANGES IN INCIDENCE AND SEX RATIO OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY BY POPULATION DRIFT IN TAIWAN

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Abstract. We analyzed data from a single screening center in Taiwan from January 1, 1996 to December 31, 2005 to evaluate the change in incidence and female to male ratio of G6PD deficiency. During the study period, 1,211,632 of 2,667,922 (45.41%) neonates delivered in Taiwan were screened at the National Taiwan University Hospital. Of these, 21,997 neonates (1.82%) were confirmed to have G6PD deficiency. The annual incidence has decreased since 2002, from 1.94% to 1.61%. During this period, the male to female ratio in the screened population was 1.091 (range 1.073-1.098), the incidences in male and female neonates were 2.81% (2.57-3.07%), and 0.7% (0.45-0.95%), respectively. The change in sex ratio of the disease was unrelated to the change in incidence. During 2000-2005, 15-25% of newborns were born from newly immigrated females. G6PD deficiency screening has confirmed a subtle genetic flow in Taiwan. Besides the psychosocial effects, medical issues caused by population movements should be carefully watched in the future in Taiwan.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD), the key regulatory enzyme in the hexose monophosphate shunt, catalyzes the oxidation of G6P to 6-phosphogluconolactone and the production of reducing equivalents in the form of NADPH to meet cellular needs for reductive biosynthesis and maintenance of the cellular redox. G6PD deficiency is the commonest enzymopathy affecting red cell metabolism (Luzzatto *et al*, 2001). The incidence of G6PD deficiency varies among different countries, ranging from 1.3 to 21% or higher. The heterozygote advantage *vis-à-vis* malaria has been invoked to account for the high frequency of the involved alleles in certain populations (Luzzatto *et al*,1969).

Patients with G6PD deficiency are predisposed to a number of diseases including drug- or food-induced acute hemolytic anemia, severe chronic nonspherocytic hemolytic anemia, and neonatal jaundice. Screening of newborn babies for G6PD deficiency to prevent hemolytic anemia has been undertaken since 1981 (Matthay and Mentzer, 1981).

G6PD deficiency occurs in the Chinese population (Du *et al*, 1988a, b). The incidence of G6PD deficiency is as high as 5.5% in Guangdon (Chan *et al*, 1964), and is even higher in minority ethnic groups such as the Li (6.74%) and the Miao (16.67%) (Du *et al*, 1988a). Taiwan is an island located in Southeast China; migration from different areas of China has occurred during different eras. A nation-wide neonatal screening program for

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G6PD deficiency has been in effect since 1985 (Hwu *et al*, 2003). The incidence of G6PD deficiency in Taiwan is estimated to be 3% in males and 0.9% in females (Chiang *et al*, 1999). However, as we describe herein, a review conducted at a single screening center from 1996-2005 presents different findings. In particular, the incidence in females markedly declined, which correlates with immigration from neighboring countries to Taiwan.

MATERIALS AND METHODS

Neonatal screening in Taiwan started as a pilot program in 1981 (Hwu *et al*, 2003). The screening coverage rate increased to 80% in 1990, reached 98% by 1996, and is currently more than 99% (Hwu *et al*, 2003). In this study we analyzed G6PD deficiency data from January 1, 1986 to December 31, 2005 from the Newborn Screening Center at the National Taiwan University Hospital (NTUH). This center routinely randomly screens two-fifths of Taiwanese neonates.

Neonatal blood was spotted and dried on specialized filter paper according to standard procedure. The screening for G6PD deficiency is performed utilizing either a fluorescence spot test (Jiang *et al*, 2003) or, since 2004, an enzyme-linked method (Catalano *et al*, 1975; Huang *et al*, 1982) using the Neonatal G6PD set (PerkinElmer, Wellesley, MA). Neonates revealing a G6PD deficiency were confirmed by a quantitative method conducted in eighteen hospitals throughout Taiwan including NTUH. An external quality assurance program in effect since March, 1999 monitored the performance of the G6PD confirmation test in these referral hospitals (Chiang *et al*, 2003). Final reports were sent to the newborn center in NTUH.

Demographic data from the screening center including sex and parental nationality were also collected. We also retrieved data from Department of Household Registration Affairs, Ministry of the Interior, ROC, to compare the eligible population in Taiwan to our study population. Statistical analyses of the information involved Spearman's rank and Mann-Whitney tests.

RESULTS

From January 1, 1996 to December 31, 2005, 1,211,632 of 2,667,922 babies (45.41%) born in Taiwan were screened by the NTUH Neonatal Screening Center. Although the birth rate decreased during this period (Fig 1), the proportion of neonates screened by this center remained high and fairly constant, ranging from 44.02% to 46.13% (Fig 1). Among the 1,211,632 neonates screened, 21,997 were confirmed to have G6PD deficiency, representing a cumulative incidence of 1.82%. The incidence of G6PD deficiency dropped transiently in 1998 and has displayed a downward trend since 2001. During the study period the incidence of G6PD deficiency decreased from 1.94% to 1.61% (Fig 1).

The incidence of G6PD deficiency correlated with both the total birth number (Correlation coefficient =0.851, p=0.002) and number screened by the NTUH center (Correlation coefficient =0.815, p=0.004). However,

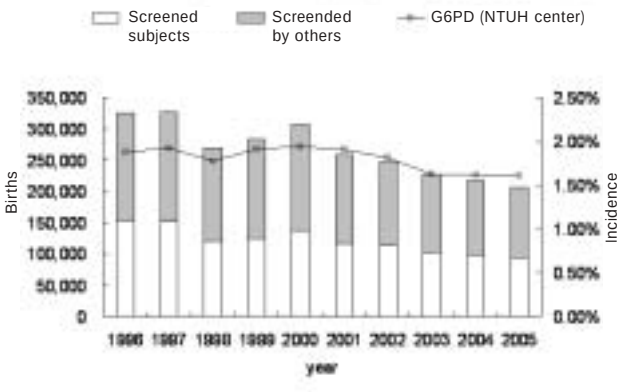

Fig 1–Annual birth rate versus the incidence of G6PD deficiency screened by the National Taiwan University Hospital.

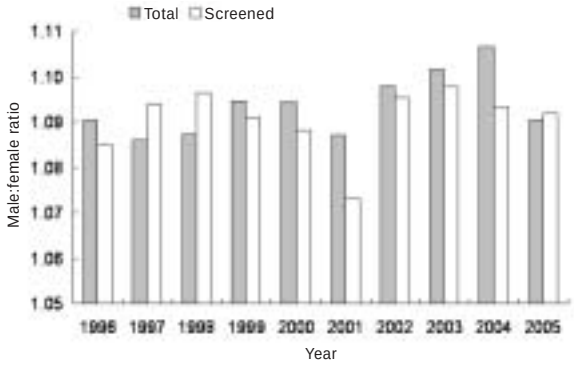

Fig 2–Male to female ratio in the general population and in the screened population.

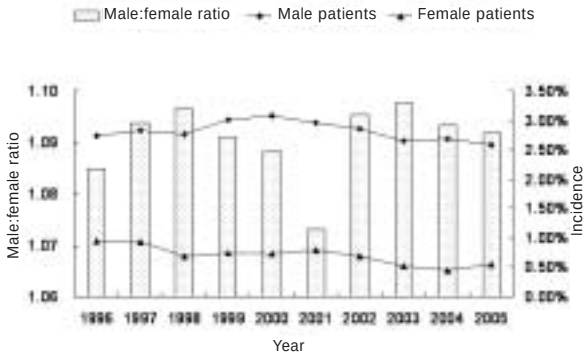

Fig 3–The incidence of G6PD deficiency in male and in female neonates.

since the change in the incidence of G6PD deficiency would not be expected to have a causal relationship with the decrease in birth rate, we analyzed other factors including sex ratio and population drift.

The population screened by our center was composed of 628,485 males (1.09% of the total population, ranging from 1.07-1.10%) and 576,444 females (1.09% of the total population, ranging from 1.09-1.11%) (Fig 2). Despite the small difference, we decided to focus only on the screened population.

Since G6PD deficiency is an X-linked disease, we calculated and compared the sex-specific incidences. During the study period, the male-to-female ratio in the screened population was 1.09 (range 1.07-1.10). The incidences of G6PD deficiency in male and female neonates were 2.81% (2.57-3.07%) and 0.7% (0.45-0.95%), respectively (Fig 3). However, the changes in incidence were not related to the sex ratio. The results further revealed the incidence in females decreased continuously over the study period, while the incidence in males increased slightly between 1998 and 2000, and decreased from 2000-2005. The change in females constituted the major part in the continuous decrease in G6PD deficiency and the correlation between the incidence of G6PD deficiency and the screened number only held true for females (Correlation coefficient =0.869, p=0.001). Since the screening technology did not change until 2004, the methodology cannot explain the observations made prior to that year.

Under the assumption of the Hardy-Weinberg equilibrium, we calculated the allele frequency based on the incidence of male G6PD deficiency patients (Table 1). Female carriers may present with low G6PD activity so they would be assigned to a “deficiency” status. We proposed a different portion of females with deficient enzyme activity and calculated the female incidence including the deficiency carriers and the homozygous patients. Using this approach, the incidence of female carriers in the years prior to and after 2002 were calculated to be 15% and 10%, respectively.

Factors violating Hardy-Weinberg equilibrium (such as exceptions to random mating) and constant allele frequency (such as genetic drift or genetic flow) were further explored. In recent years, many women from other Asian countries have moved to Taiwan by marrying Taiwanese men. To chart the influence of this influx, we retrieved records of bridal nationality since 2001 from the Ministry of the Interior, ROC. From 2001-2005, the percentage of native (Taiwan-born) brides was 76.2%. During the same period bridal nationality percent-

Table 1
Calculated genotype frequency for 1996-2005 by allele frequency.

Genotype		Annual Incidence									
		1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
Males	X^{G6PD+}	97.26%	97.18%	97.24%	97.00%	96.93%	97.04%	97.15%	97.36%	97.32%	97.43%
	X^{G6PD-}	2.74%	2.82%	2.76%	3.00%	3.07%	2.96%	2.85%	2.64%	2.68%	2.57%
Females	X^{G6PD+}/X^{G6PD+}	94.59%	94.44%	94.55%	94.08%	93.95%	94.17%	94.38%	94.79%	94.70%	94.92%
	X^{G6PD+}/ X^{G6PD-}	5.33%	5.48%	5.37%	5.83%	5.95%	5.74%	5.54%	5.14%	5.23%	5.02%
	X^{G6PD-}/X^{G6PD-}	0.08%	0.08%	0.08%	0.09%	0.09%	0.09%	0.08%	0.07%	0.07%	0.07%
Assuming female patients (homozygous plus X% of carriers)	X=10	0.61%	0.63%	0.61%	0.67%	0.69%	0.66%	0.64%	0.58%	0.59%	0.57%
	X=12	0.71%	0.74%	0.72%	0.79%	0.81%	0.78%	0.75%	0.69%	0.70%	0.67%
	X=15	0.87%	0.90%	0.88%	0.96%	0.99%	0.95%	0.91%	0.84%	0.86%	0.82%
Female incidence by screen		0.95%	0.94%	0.69%	0.74%	0.73%	0.78%	0.69%	0.52%	0.45%	0.55%

ages from Mainland China and Southeastern Asia were 13.8% (range 9.2-18.1%) and 9.8% (range 7.2-13.3%), respectively (Fig 4). Typically, the marriages were soon followed by the birth of a child. Fig 4 displays a plot of G6PD deficiency incidence and bridal nationality. The data suggest that a high percentage of brides from China preceded a drop in male G6PD incidence beginning in 2003.

Maternal nationality information from the NTUH screening center has only been available since the beginning of 2004. Of a total 149,989 fathers, 97.85% were Taiwanese, 306 (0.2%) were from China, and 413 (0.28%) were from Southeast Asian countries, including Vietnam, Indonesia, Thailand, Philippines, Burma, and Cambodia. There were others who were either from other countries (n=543, 0.36%) or who refused to reveal their nationality (n=1,956, 1.30%). Of a total of 150,087 mothers, 86.27% were Taiwanese, 7,853 (5.20%) were from China, and 12,472 (8.31%) were from Southeast Asian countries, including those listed above. There were 252 (0.17%) from other countries and 23 (0.02%) who refused to reveal their nationality (Fig 5A). Mothers from China and Vietnam represented 38.12% and 45.88% of the total foreign mothers, respectively. Fig 5B shows the incidences of G6PD deficiency with respect to maternal nationality. Babies born to mothers from China and Vietnam had a lower incidence than those born to native mothers, while babies born to mothers from other countries had a higher risk of G6PD deficiency. Data from the fathers was not revealing, because of the small number of fathers of foreign nationality.

DISCUSSION

The prevalence of G6PD deficiency varies greatly throughout the world. In some Asian populations where malaria is widespread (Luzzatto *et al*, 1969), G6PD deficiency has a high frequency (Panich, 1981). Malaria has

been largely controlled in some countries in Asia and the selection advantage for heterozygotes of G6PD eficiency no longer exist for some countries. Without this selection pressure, the incidence of G6PD deficiency should gradually decrease. A more rapid decrease would not be favorable for population health in the event of a recurrence of malaria. We observed a decrease in the incidence of G6PD deficiency in neonatal screening, especially since 2001. This change is not likely due to the natural loss of selection of the disease.

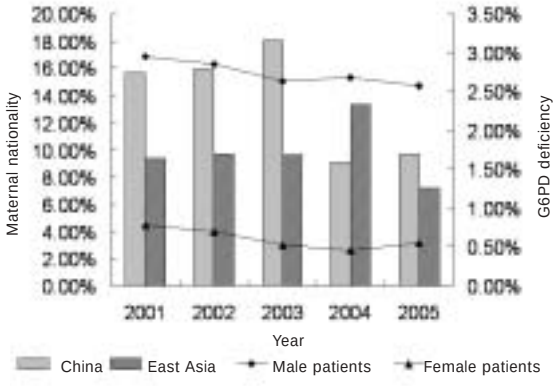

Fig 4–Bridal nationality versus the incidence of G6PD deficiency in male and female neonates.

Population migration creates political, social, and medical issues worldwide. In Taiwan, partly due to the high male-to-female ratio, an increasing number of males experience difficulty in finding a suitable companion. As a consequence, the immigration of females from neighboring countries has increased. This specific type of immigration has raised serious concerns to the Taiwan government, including a change in health status due to differences in genetic background. The current study supports this view, but from a positive viewpoint, namely a decrease in G6PD deficiency.

The population of Taiwan originated mainly from China in different eras, and the old immigrants who came several hundred years ago are often regarded as ethnic Taiwanese. Consequently, the prevalence of G6PD deficiency differs among the different groups. A previous study documented prevalence rates of 4.52% in Hakkanese, 1.57% in mainland Chinese, 3.3% in Taiwanese, 3.5% in the aboriginal Ami tribe, and 0.3% in aboriginies from other tribes (Lee *et al*, 1963). An initial rise in the incidence of G6PD deficiency with the influx of new immigrants would be expected. However, many of the new immigrants were from China, where G6PD deficiency is less common than in Taiwan. For

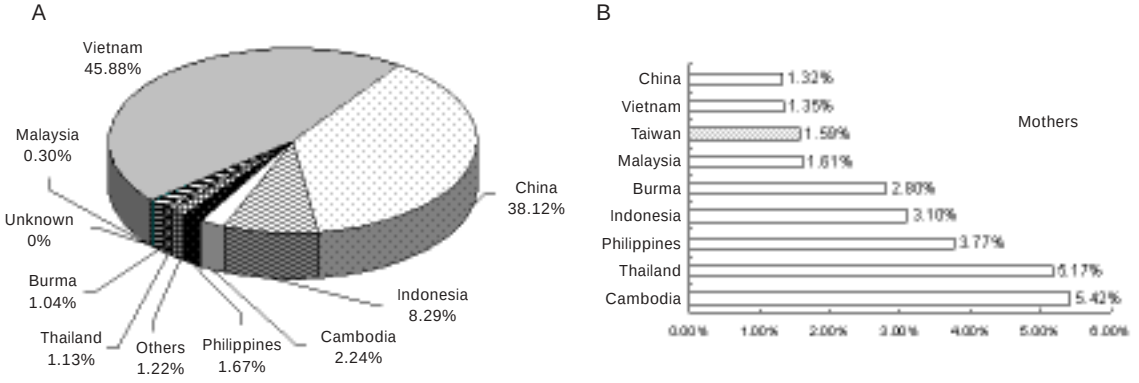

Fig 5–National distribution of immigrant mothers (A), and the incidence of G6PD deficiency in their children (B).

those who came from Southeast Asia where G6PD deficiency is prevalent, most of the new immigrants were from Vietnam, where the prevalence of G6PD is also low. This may well explain the decreasing trend in G6PD deficiency observed among Taiwanese neonates.

Data from the current study reveal a change in the incidences of G6PD deficiency among males and females. The transient increase in the incidence in males from 1998-2000 cannot be explained, as data about nationality for those years is lacking. In comparison, the decrease in incidence in females occurred earlier and was more consistent. Our data reveals that the majority of the recent new foreign mothers are from China and Vietnam. The reported incidence of G6PD deficiency in South Vietnamese of 1.31% (Panich *et al*, 1980) is similar to that reported presently. The most common variants in Vietnam are G6PD Mahidol and Canton (Panich *et al*, 1980; Toncheva, 1986). G6PD Mahidol as well as Viangchan are common Southeast Asian variants (Laosombat *et al*, 2005) and both belong to class 3 World Health Organization mutations (Vulliamy *et al*, 1989; Beutler *et al*, 1991). On the other hand, the Taiwan Hakka, Canton, and Taipei G6PD variants frequently found in Taiwan are all class 2 variants (Stevens *et al*, 1990; Chiu *et al*, 1991). Class 2 mutations indicate a severe enzyme deficiency (less than 10%) while class 3 mutations indicate a moderate to mild enzyme deficiency (10-60%). It is possible that females who carry a class 3 G6PD mutation are less likely to have low G6PD activity, and so are more likely to be reported as normal. The flow of class 3 mutations into the Taiwanese population probably caused the early and continuous decline of female G6PD deficiency observed in the present study.

The influence of screening technology on the changing incidence values cannot be disregarded. The fluorescence spot test was replaced by an enzyme-linked assay in 2003. This short period of time should not affect the data. The confirmation test performed in a number of screening centers has remained the same throughout the study period. Therefore, it is doubtful that technology has appreciably influenced the data.

The clinical presentation of G6PD deficiency is heterogeneous, and sometimes cannot be correlated with G6PD activity. Additional factors, such as drug metabolism and UDP-gluconosyl-transferase activity, affect both drug-induced favism and neonatal jaundice (Huang *et al*, 2002). Accumulating evidence supports the suggestion that G6PD deficiency may not only be a hematologic disease. This enzyme is also important to other systems, so patients with G6PD deficiency may have a higher risk for infection (Mallouh *et al*, 1987), diabetes mellitus (Wan *et al*, 2002), cataracts (Orzalesi *et al*, 1981), tumors (Cocco *et al*, 1998), or preeclampsia (Abdulhadi, 2004). Since female heterozygotes can also develop neonatal hyperbilirubinemia (Meloni *et al*, 1983) or severe hemolytic episodes, it is of the utmost importance to classify them correctly by screening during the neonatal period (Reclos *et al*, 2000). A previous report identified the incidence in Taiwan as 2.8% in male neonates and 2.5% in female neonates (Tang *et al*, 1992), which is different from another report about neonatal screening stating the incidence as 0.9% in female infants (Chiang *et al*, 1999). It may be necessary to adjust the cut-off value in the screening test, and change the definition of G6PD deficiency in the confirmation test in Taiwan in order to include more females with partial G6PD deficiency. The flow of new G6PD alleles to Taiwan may also alter the phenotype of the disease.

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