An assessment of efficiency in potential screening for Wilson's disease

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SUMMARY The efficiency of screening for Wilson's disease by serum caeruloplasmin determination was assessed by analysing the epidemiologic data of 289 affected families in Japan. The estimated gene frequency was 5.5×10^{-3} . The sensitivity of the screening test was 93% at a proposed cut-off level of 120 mg/l and the specificity was 99.83%. In Japan 1 500 000 children are born every year of whom 50 would be expected to have Wilson's disease. The present analysis of potential screening for all children would grade three of them as false-negatives and identify 2621 as false-positives. An analysis for children only from consanguineous marriages produced a more efficient result, with a much higher predictive value of the positive and case-finding rate. Although the number of patients identified in this latter high-risk screening group was small, it is worth considering as a pilot study.

It is now 70 years since Kinnier Wilson drew attention to the relationship between familial nervous disease and cirrhosis of the liver in the same patients.¹ The condition was later found to be an autosomal recessive disorder.^{2,3} Prevention of this genetic disease by administration of penicillamine to asymptomatic patients was claimed to be successful,^{4,5} and a method of screening by serum caeruloplasmin determination has been considered. A simple and inexpensive way of measuring serum caeruloplasmin using a dried blood spot was recently described.⁶ However, the efficiency of population screening has not previously been fully discussed.⁵

It is the purpose of this paper, therefore, to assess the efficiency of alternative strategies in screening for Wilson's disease in the light of present knowledge based on the analysis of epidemiologic data collected in Japan.

Materials

All articles published between 1965 and 1977 citing cases of the disease in Japan were traced through *Igaku-Chuo-Zasshi (Japania Centra Revuo Medicina)* which summarises briefly the contents of Japanese medical journals. A standard questionnaire was then prepared and sent to each hospital involved in the collection of data for the original article. The hospitals were asked for further details of each

patient and family members. If a questionnaire was not returned, it was filled in by the present author from the original article. Such details were also requested from departments of medicine, paediatrics, and psychiatry in all medical school hospitals in Japan for patients with Wilson's disease who had been seen during the previous 10 or 15 years. The response rate by this latter group was 58%. All data were checked to eliminate any possibility of duplication of registration. A total of 289 families, each with at least one case, was collected.

Methods

The following particulars were extracted, for each family, to provide data for the analysis:

- (1) Consanguinity of parents.
- (2) Number of siblings.
- (3) Age and health status of each family member, and, if death had occurred, cause and age.
- (4) Serum caeruloplasmin concentration (CP) of each person if available.

ESTIMATION OF GENE FREQUENCY AND GENOTYPE FREQUENCY IN THE POPULATION
In this genetic analysis 162 families were used for whom information was complete for the first three items listed above.

To estimate the gene frequency in the population, the following formula was used.⁷ It is an extension of

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Dahlberg's formula using F as the coefficient of inbreeding:

$$q = \frac{c/16 - kF}{(k - c) + (c/16 - kF)}$$

whereby q is the gene frequency in the population to be estimated, c is the proportion of first-cousin marriages in the population, F is the coefficient of inbreeding in the population, and k is the proportion of homozygous recessives (the affected individuals) from first-cousin marriages to those from all marriages.

The term c in the Japanese population at the relevant period was taken to be from 0.040 to 0.045 and the corresponding F value from 0.0031 to 0.0035 judging from published data.^{8,9} This is based on the observation that in the study the years of parents' marriages were on average seven years earlier than the years of patients' births, most of which were between 1945 and 1965. The term k = 0.3245 was provided from the present survey.

The gene frequency in the population, q, was first estimated, and then the genotype frequency was estimated by the following formulae:¹⁰

$$(1 - F) q^2 + Fq \text{ for homozygous recessives} \\ (1 - F) 2pq \text{ for heterozygotes}$$

Table 1 shows the result for the two chosen values of c and F. The gene frequency, q, was estimated to be between 5.22×10^{-3} and 5.96×10^{-3} . The corresponding frequency of the disease in the population ranged from 1 in 17 800 to 1 in 23 100. The rate of consanguineous marriages in Japan has been rapidly decreasing and the proportion of first-cousin marriages, c, in the 1980s will be below 1%.⁸ This will lead to a reduction of the disease frequency in the 1980s, which can be taken as

1 in 30 000 for homozygotes and 1 in 91 for heterozygotes.

SERUM CAERULOPLASMIN CONCENTRATION (CP) IN PATIENTS, HETEROZYGOTES, AND NORMAL SUBJECTS

The distribution of CP in the patients, parents, and healthy siblings of the patients is shown in the Figure. Serum caeruloplasmin concentration reported to the study was measured by various methods and only those expressed in mg/l are selected here. The normal range for this quantity was given as over 150 mg/l.

The distribution of CP in the patients did not fit any of the following distributions: normal, log-normal, Poisson, or square root. There was no statistically significant correlation between CP and age ($p = 0.08$).

The distribution of CP in the parents, who are justifiably taken as heterozygotes, fitted well to a normal distribution with a 20 mg/l interval ($\chi^2_{10} = 10.67$, $0.80 < p < 0.90$). Therefore a normal distribution with a mean of 204 mg/l and a standard deviation of 84 mg/l was used in the analysis for the CP distribution in heterozygotes. There was no significant correlation between CP and age in the parents ($0.5 < p < 0.6$).

In the Figure, the healthy siblings consist of two distinct groups: normal homozygotes without the gene and heterozygotes (carriers). The theoretical ratio of these two groups is 1 : 2 under autosomal recessive inheritance. There was a statistically significant correlation between CP and age ($r = -0.36$, $b = -3.8$, $a = 286$, $0.01 < p < 0.02$; r = correlation coefficient, b = slope of regression line, a = intercept of regression line).

We need three distributions of CP in the analysis—one for patients, one for heterozygotes, and one for homozygotes without the gene. A distribution suitable for the last group was not available in this study; therefore, distributions among

Table 1 Estimation of the gene frequency and the genotype frequency using

$$q = \frac{c/16 - kF}{(k - c) + (c/16 - kF)}$$

k	c	F	q	Homozygote recessive (1-F)q ² +Fq	Homozygote recessive 1 in	Heterozygote (1-F)2pq	Heterozygote 1 in
0.3245	0.040	0.0031	5.22×10^{-3}	4.33×10^{-5}	23100	1.04×10^{-2}	97
0.3245	0.045	0.0035	5.96×10^{-3}	5.63×10^{-5}	17800	1.18×10^{-2}	85

*Where q = the gene frequency in the population

p = 1 - q

F = coefficient of inbreeding

c = proportion of first-cousin marriages in the population

k = proportion of affected children from first-cousin marriages to those from all marriages

Figure Distribution of serum caeruloplasmin concentrations in patients, parents, and healthy siblings of the patients.

'normal' children were obtained from the literature.^{11,12} Almost all 'normal' children must be homozygotes without this gene. None of the 117 children aged between 2 and 14 in Cox's data¹² had a value below 150 mg/l. One of the 100 'normal' children in Arima's data¹¹ had a value between 110 mg/l and 160 mg/l. There is a possibility that this child might have been a heterozygote, as the heterozygote frequency was about 1 in 92 in Japan and 26% of them had values below 150 mg/l according to the present study. From these data it is justifiable to assume that none of the homozygous children without the gene for Wilson's disease should have a CP below 150 mg/l. This was assumed throughout the following analysis.

ANALYSIS

The number of babies born in 1978 was 1.71 million and the birth rate has been decreasing.¹³ Therefore

the number of candidates for screening every year in the 1980s was taken as 1.5 million.

Table 2 shows the sensitivity of the screening test by CP determination at various cut-off levels, derived from the Figure, and the proportion of heterozygotes whose CP value fell below those levels—namely, the proportion of false-positives among heterozygotes, derived from the assumed normal distribution of CP in the parents.

Table 3 was constructed from the data already presented. Indices for screening were calculated¹⁴ when a cut-off level was 120 mg/l. The numbers of patients and heterozygotes were calculated from their estimated frequencies in the population. Forty-seven out of 50 patients are identified in screening because the sensitivity of the test is 8/116. We have three false-negatives. From Table 2 the proportion of false-positives among heterozygotes is 15.9%, which gives 2621 as the number of false-positives. Because of the assumption that none of the normal homozygotes has a CP value less than 150 mg/l, these 2621 heterozygotes are the only false-positives.

The indices at various cut-off levels calculated in the same way are shown in Table 4. For the cut-off levels at 170 mg/l and 200 mg/l, the assumption was made that none of the normal homozygotes had values below those levels.

SCREENING CHILDREN FROM CONSANGUINEOUS MARRIAGES

Thirty-nine per cent of the study patients were from consanguineous marriages, and it is of interest to consider screening children from these marriages only. For the sake of simplicity, screening children from first-cousin marriages is considered here.

Thirty-two per cent of the patients, namely k in Table 1, were from first-cousin marriages. However, this proportion decreases as the rate of consanguinity decreases. This relationship is shown in Table 5. The proportion in the 1980s will be between 5% and 10% if the rate of first-cousin marriages is between 0.5% and 1%. This means that when the rate of first-cousin marriages is 1%, we would identify approximately 10% of the patients by screening 1% of children born every year. In figures, we would identify five patients by screening 15 000 children at a cut-off level of 120 mg/l. Other indices of screening remain similar

Table 2 Sensitivity of the screening test and the proportion of false-positives among heterozygotes

Cut-off level (mg/l)	200	170	150	140	130	120	110	100
Sensitivity*	113/116	111/116	110/116	109/116	109/116	108/116	108/116	106/116
Proportion of false-positives among heterozygotes** (%)	48.1	34.3	26.0	22.3	18.9	15.9	13.2	10.8

*The proportion of patients whose serum caeruloplasmin level was below these levels in the study

**The proportion of parents whose serum caeruloplasmin level was below these levels. A normal distribution with a mean of 204 mg/l and a standard deviation of 84 mg/l, derived from the CP distribution of the parents in the Figure, was used as a CP distribution of heterozygotes.

Table 3 Sensitivity, specificity, and other indices of screening when a cut-off level is 120 mg/l

Screening test	Wilson's disease		All tests
	+	-	
+	47	2 621	2 668
-	3	1 497 329	1 497 332
All babies	50	1 499 950	1 500 000
Sensitivity	$108/116 (47/50) \times 100 = 93.1\%$		
Specificity	$1497329/1499950 \times 100 = 99.8\%$		
Predictive value of positive	$47/2668 \times 100 = 1.8\%$		
Efficiency	$47+1497329/1500000 \times 100 = 99.8\%$		
Children for referral	$2668/1500000 \times 100 = 0.18\%$		

to those in the screening of all children except for the predictive value of the positive, which is about 17%. A case-finding rate (defined here as the ratio of the number of cases identified by screening to the number of children screened) is 0.033% (5/15 000) in this type of screening compared with 0.003% (47/1 500 000) in population screening.

Discussion

Screening by CP measurement is best carried out on children aged about 2 or 3. This is supported by the following observations:

(1) None of the 308 cases in this study developed recognisable symptoms or signs before the age of 4.

(2) The pathological changes in the liver are minimal in asymptomatic patients aged under 4,^{15 16} and prevention of the development of the disease is very satisfactory if treatment is started early.^{4 5}

(3) Although the CP in normal subjects is quite low in early infancy, which is therefore not an appropriate time for screening, it has already reached almost its highest level by this age.^{6 12} Therefore false-positives are least likely to occur around this age. (Another advantage of this age is that children would be accessible for screening if it were combined with regular immunisation or other preventive measures).

A cut-off level for screening placed at around 120 mg/l would be most useful, judging from Table 4. If it is higher, false-positives increase without decreasing false-negatives; if it is lower, the

false-negatives increase. It is wise not to place the cut-off level above 150 mg/l because the chances of normal homozygotes having a CP below this level become high, thus dramatically increasing false-positives.

In the analysis of screening, several assumptions were made, some explicit and others implicit. The following are the most important:

(1) The proportion of first-cousin marriages in the study period was taken to be between 4% and 4.5%, and in the 1980s to be between 0.5% and 1%.

(2) The distribution of the CP of patients aged about 2 would be similar to that of patients from the study data.

(3) The distribution of the CP of heterozygotes aged about 2 would be similar to that of the parents from the study data.

(4) No healthy children of homozygotes without the gene would have CP values less than 150 mg/l.

The relevance to the analysis of these assumptions needs further consideration.

ASSUMPTION 1

The proportion of first-cousin marriages in the population has a substantial influence on the estimation of the gene frequency and hence of the genotype frequency. If the proportion *c* is around 3%, which would be the lowest possible value for the whole of Japan at that time, the frequency of the gene becomes about 3.7×10^{-3} . The highest possible *c* value would be 6% and in this case it is around 8.3×10^{-3} . The estimate of 5.5×10^{-3} used in the analysis, however, is very similar to an estimated value from 3.3×10^{-3} to 6.6×10^{-3} by Arima *et al* in Japan about 15 years before the present survey.³ When *c* and *F* change and *F* is larger than 0.001 the gene frequency *q* also changes over time.¹⁷ But the change takes place over more than decades. Therefore the gene frequency used in the analysis through the 1940s to the 1980s should also be reasonable.

Another type of question concerning the estimation of the gene and genotype frequency is whether the use of these formulae is justifiable for the whole Japanese population considered as a single

Table 4 Indices for screening at various cut-off levels

Cut-off level (mg/l)	200	170	150	140	130	120	110	100
Sensitivity	97.4	95.7	94.8	94.0	94.0	93.1	93.1	91.4
False-negative (/50)	1	2	3	3	3	3	3	4
False-positive (/1 500 000)	7929	5654	4333	3676	3115	2621	2176	1780
Specificity (%)	99.47	99.62	99.71	99.75	99.79	99.83	99.85	99.88
Test-positive (referral)* (%)	0.53	0.38	0.29	0.25	0.21	0.18	0.15	0.12
Predictive value of positive (%)	0.61	0.84	1.08	1.26	1.49	1.76	2.11	2.52
Efficiency (%)	99.47	99.62	99.71	99.75	99.79	99.83	99.85	99.88

*The proportion of test-positive children among those screened.

Table 5 Proportion of the affected children from first-cousin marriages to those from all marriages at various levels of *c* and *F*, estimated by

$$k = \frac{c(1 + 15q)^*}{16 \{ (1 - F)q + F \}}$$

q	c	F	0.005	0.01	0.04	0.05
			0.00044	0.00078	0.0031	0.0039
0.0050	k	0.062	0.116	0.332	0.378	
0.0055	k	0.057	0.108	0.315	0.361	
0.0060	k	0.053	0.101	0.300	0.345	

*This formula is an algebraic rearrangement of the formula in Table 1 solving for *k*.

inbreeding unity. It is very likely that in some parts of the country the gene frequency is low and in others it is high, namely, that there exist genetic isolates. In such circumstances the application of the genetic formulae to a whole population leads to a discrepancy between the theoretical and the observed frequency.¹⁸ The extent of the discrepancy in this analysis is, however, difficult to estimate and the answer would be obtained from the results of screening.

ASSUMPTION 2

It has been shown that asymptomatic patients show very low CP levels even under the age of 4 and some asymptomatic patients were actually found because of their low CP values.⁵ Moreover, no statistically significant correlation was observed between CP and age among patients. Even if the correlation was statistically significant, possibly with a larger sample size, the slope of the regression line would be around -1.0 and this would change the age-adjusted distribution of CP very little. It is not unreasonable, therefore, to assume that the majority of asymptomatic patients would show levels below 100 mg/l. The more relevant question, however, is whether the proportion of asymptomatic patients aged about 2 whose CP values are above the cut-off level is similar to that derived from the Figure. Some authors have indicated that patients with high CP levels tend to have abnormal liver function, particularly those with severe hepatic failure.¹⁹ This observation was partly supported in the present study. Sternlieb's data,²⁰ however, showed that even some asymptomatic patients had high CP values, although the proportion was not given. From these observations it could be said that the proportion of patients with CP values above cut-off level would not be high but could well be lower than that used in the analysis. This would decrease the number of false-negatives in screening. For reference, among patients in the present study the proportion with

normal CP concentration was similar to the proportions reported in other studies.^{11 21}

Another aspect of this question is the variability of CP within patients over time. Those whose CP values are high at a time of hepatic failure tend to show lower values after they have recovered from acute failure.¹⁹ There seem to be some fluctuations of values during the course of the disease, but in most patients the values are fairly constant over time.^{22 23} Therefore variations of CP within individuals would have little effect on the proportion of false-negatives.

ASSUMPTION 3

The use of the CP distribution in parents as data for heterozygotes aged about 2 requires special attention.

The existence of the correlation between age and CP value among healthy siblings is largely dependent upon the higher values found in the younger siblings. If these higher values were to consist principally of homozygotes, the correlation with age would disappear. The data on 'normal' subjects indicated^{6 12 24} that there was a correlation between CP and age, and their mean CP value at the age of about 2 was higher than that of adults. The difference ranged from 40 to 100 mg/l. If we assume that there is an inverse relationship between age and CP among heterozygotes and that the difference in the mean CP level between heterozygous children aged 2 and adults is similar to that among 'normal' subjects, then the proportion of false-positives among heterozygotes ranges from 7% to 1.4% at the cut-off level of 120 mg/l instead of 15.9% used in the analysis. The corresponding number of false-positives ranges from 1154 to 230 instead of 2621. This was obtained by shifting the normal distribution used as the distribution of CP among heterozygotes towards a higher value by 40 and 100 mg/l respectively. Even if there is actually a difference in the mean CP between heterozygous children and heterozygous adults, it would be small. In any event, it would reduce false-positives.

In this connection it should be said that the proportion of heterozygotes with CP values below the normal range was higher in this study than in previously published papers.^{11 16}

ASSUMPTION 4

It is not impossible that a certain fraction of normal homozygotes have a CP value below 150 mg/l, but the fraction must be very small. If the value of 120 mg/l is taken as a cut-off level, the proportion of normal homozygotes below this level must be smaller still. The answer could be found only from a large-scale survey, which has not been done on these children.

The fact that a low CP was observed in patients with other disease^{19 25} may also challenge this assumption. In the present analysis this possibility was not considered. In the screening process, however, only apparently 'healthy' children aged about 2 are screened, and the majority of children with these diseases would be easily recognised beforehand. Therefore it is not likely that these diseases would increase the proportion of false-positives among normal homozygotes so greatly as to jeopardise the present analysis.

Menkes's kinky hair syndrome is another disorder with very low CP. The frequency of the disease was estimated to be 1 in 35 000 in Australia.²⁶ The patients were identifiable by CP determination.⁶ It is therefore of interest to consider screening for this disease in combination with screening for Wilson's disease. One of the problems, however, is the age at screening. Most patients with Menkes's syndrome develop the disease in infancy, and pathological changes occur quite early in infancy,²⁶ so screening needs to be done at that time, which poses a dilemma. A large overlap of CP value between affected and normal children is expected in screening for Wilson's disease and possibly for Menkes's disease. It would be premature to discuss the possibility of combined screening without gaining further evidence.

Also, the accuracy and precision of whichever screening test is used need to be assessed.

Apart from the assumptions discussed above, some other questions need to be considered.

The most important question in screening is how to identify the asymptomatic patients among the referrals. There are several ways of distinguishing between genotypes but unfortunately none of them seems to be completely efficient. The clinical test using radio copper seems to be quite effective^{20 27} but not complete, and ethical questions remain, even though the dosage of radio copper is claimed to be within acceptable limits.²⁸ For patients with some symptoms and signs, making the definitive diagnosis presents some problems.²⁹ Making the diagnosis or finding cases among referrals in screening is a difficult one. It was possible to distinguish between three genotypes by the use of radiocopper even among asymptomatic patients.³⁰ However, that study did not include the very young. There are not yet enough data on the efficient discrimination of asymptomatic patients in children under 4 to enable this question to be discussed. Again, a reliable answer could be obtained either by the accumulation of appropriate data through the process of making the diagnosis or through screening. The rules for dealing with referrals remain to be formulated.

Screening children from consanguineous marriages seems to give worthwhile results. The

predictive value of the positive and the case-finding rate are much higher than in the general population. However, one drawback is that only a proportion of the patients we have every year are identified; the proportion depends upon the rate of consanguinity in the 1980s. Nevertheless, it may be worthwhile to do it as a pilot study. It offers useful information, such as the efficiency of screening and the gene frequencies in different geographical areas, which will be useful for future screening in high-risk areas.

The information on consanguinity will be obtained from the *Maternal and child health record book* handed to every pregnant woman in Japan, or by asking parents at the time of immunisation or regular health screening of infants.

Recent reports on neonatal screening for congenital metabolic disorders in Japan revealed the following incidence of each disease³⁰; phenylketonuria 1/59900; maple syrup urine disease 1/671300; histidinemia 1/8100; homocystinuria 1/145900; galactosemia 1/139800; and congenital hypothyroidism 1/8200. Wilson's disease seems to be comparable to these disorders judging from the principles of screening laid out by Wilson and Junger.³¹ Cost and benefit of the screening need to be assessed further.

In summary, the conclusions of this analysis are as follows. It would be worth screening children from consanguineous marriages for Wilson's disease at the age of about 2 as a pilot study. A cut-off level for screening placed at around 120 mg/l would be most useful.

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