SCREENING EXTENDED FAMILIES FOR GENETIC HEMOGLOBIN DISORDERS IN PAKISTAN

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ABSTRACT

Background We have investigated a strategy for identifying and counseling carriers of recessively inherited disorders in developing countries where consanguineous marriage is common. In such communities, gene variants are trapped within extended families, so that an affected child is a marker of a group at high genetic risk.

Methods Fifteen large Pakistani families, 10 with a history of a hemoglobin disorder and 5 without any such history (controls), were screened for β-thalassemia and abnormal hemoglobins. All carriers and married couples consisting of two carriers received counseling, and eight families have been followed for two years.

Results In the control families, no carrier was found among 397 members tested. In the 10 families with an index case, 183 of 591 persons tested (31 percent) were carriers; carriers had a 25 percent risk of being in a marriage at risk for producing an affected child, and 17 of 214 married couples (8 percent) consisted of two carriers. No couple at risk was identified among 10 families with one or no healthy children and prenatal diagnosis. Seven of eight new marriages and engagements are known not to be at risk.

Conclusions Testing of extended families is a feasible way of deploying DNA-based genetic screening in communities in which consanguineous marriage is common. (N Engl J Med 2002;347:1162-8.)

A

S infant mortality declines, congenital and genetic disorders emerge as important causes of early death and chronic disability, and developing countries need strategies for integrating genetic approaches into health care. Pakistan has a population of over 140 million people, 40 percent of whom are younger than 15 years of age; the literacy rate is low, and the average income is $420 per year. The crude birth rate is 29 per 1000 population, and the infant mortality rate is 101 per 1000 live births. The health system is hospital-based, and primary care is practically nonexistent. As in most of North Africa, the Middle East, and South India, there is a strong cultural preference for consanguineous marriage and an associated relatively high prevalence of recessively inherited disorders.

The hemoglobinopathies are major genetic problems in Pakistan. About 5 percent of the Pakistani population carries β-thalassemia, and 0.5 to 1 percent carry hemoglobin S or hemoglobin E. Married couples consisting of two carriers have a 25 percent chance that any child they have will be affected. The estimated rate of birth of affected infants is 1.3 per 1000 live births, and about 5250 infants with β-thalassemia major are born annually. Although only a minority of these cases are diagnosed, charitable thalassemia centers sustain thousands of affected children with monthly blood transfusions. Such transfusions permit an excellent quality of life during childhood but lead to iron overload and death in adolescence or early adult life. The average annual cost of iron-chelation therapy with deferoxamine — $4,400 per patient, or 10 times the average annual income — is prohibitive. The requirements for treating one annual birth cohort of affected children for one year are 90,000 units of blood plus $22 million worth of deferoxamine — more than 4 percent of the current health-related expenditures of the government. As treated children began to survive longer, costs might reach 40 percent of current expenditures for health care.

Screening to identify carriers, genetic counseling, and prenatal diagnosis can greatly reduce the rate of birth of affected infants and improve the prognosis of affected patients. In Pakistan, first-trimester prenatal diagnosis (by chorionic-villus sampling and polymerase chain reaction [PCR]) is not objected to on religious grounds and is acceptable and affordable to...
most families who are at risk. When the fetus is affect-
ed, 89 percent of couples choose to terminate the
pregnancy. However, the weak health care infra-
structure makes it impossible to provide population
screening.

In societies in which most couples are unrelated,
genesis for recessive disorders usually run in families
for many generations without manifesting themselves
through the birth of an affected child. By contrast, in
communities with a cultural preference for consan-
guineous marriage, as in Pakistan, when a gene for
a recessive disorder is present in a kindred, there is
likely to be an affected child in at least one branch of
the extended family. In turn, the diagnosis of disease
in a child serves as a marker of an extended family that
is at increased genetic risk. Therefore, in such commu-
nities, studies of extended families beginning with the
first child with a diagnosis may offer an alternative to
population screening for identifying present and fu-
cuture couples at risk for producing affected children.
Our study was designed to test this hypothesis, using
hemoglobin disorders as a model.

METHODS

Study Setting

We conducted the study at the Armed Forces Institute of Pathol-
ogy in Rawalpindi (in the northern region of Pakistan), which sup-
ports several thalassemia centers that provide care for affected chil-
dren, testing to identify carriers, counseling, and prenatal diagnosis.

Testing of Extended Families

Fifteen families of children with a hemoglobin disorder, one fam-
ily of a carrier of β-thalassemia, and eight control families with no
history of a hemoglobin disorder were offered testing to identify
carriers. Criteria for selection were voluntary participation and the
availability of many family members. Genetic counseling was con-
ducted according to internationally accepted guidelines. A meet-
ing was arranged with key members of each family (in affected fam-
ilies, usually the parents of the child with the index case) in order to
explain the importance of testing to identify other carriers. When
family representatives agreed, a three-generation pedigree was drawn
up, and arrangements were made for testing family members at their
homes. Results were given to the carriers themselves (or to the par-
ents when a carrier was less than 15 years old). The meaning of car-
rier status, the importance of knowing whether or not one’s partner
was a carrier, the availability of prenatal diagnosis, and (for those not
yet married) the possibility of taking test results into account when
planning marriage, were explained orally.

Antenatal Screening

For comparison with antenatal screening, testing was offered to
350 consecutive women who were attending a local hospital early
in their pregnancies. They were informed orally of the relevance
of the test and the availability of prenatal diagnosis should they
prove to be at risk. When a carrier was identified, her partner was
offered immediate testing.

Identification of Carriers

When the child with the index case had sickle cell anemia, screen-
ing to identify carriers was performed by cellulose acetate electro-
phoresis; red-cell indexes were not measured, because of technical
limitations. Screening for thalassemia followed a stepwise approach.
First, red-cell indexes (hemoglobin, mean cell volume, and mean cell
hemoglobin) were measured with an electronic counter. When there
was marked microcytosis (mean cell hemoglobin, 125 pg, or mean
cell volume, <75 fl), hemoglobin A2 was measured by cellulose ac-
etate elution; β-thalassemia trait was diagnosed when the percent-
age of hemoglobin A2 was 3.5 percent or higher. The specific thal-
assemia mutations in all affected children and all carriers were
identified by PCR with the use of methods that have been described
elsewhere.

Follow-up of Families

The parents of the child with the index case were asked at inter-
vals about engagements, marriages, and births to couples within the
family who were at risk of having affected children. It was not pos-
sible for investigators to keep track of family members who lived
far away, but when affected children were born, they were brought
to the center; prenatal diagnosis was performed at the center as well.

RESULTS

Study Families

A total of 16 families with an index case of a hem-
oglobin disorder were approached, and 10 requested
testing to identify carriers. The other six families de-
clined for various reasons, including difficulty in get-
ing everyone together, desire to avoid testing because
they were apparently healthy, and concern about pos-
sible problems in arranging marriages or about the
stigma that would be attached to carrier status. Eight
control families with no history of a hemoglobin
disorder were approached, and five of these families
agreed to undergo testing. The other three families
decided because of the difficulty of asking people to
give blood with no obvious benefit to them.

A typical pedigree of a family with an index case
is shown in Figure 1. The 15 study families included
a total of 1455 living members (Table 1). There were
338 married couples, of which 44 percent were con-
sanguineous (second cousins or closer relatives), 49
percent were from the same biradri (tribe or subdi-
vision of a tribe), and 6 percent were completely un-
related. Although there was considerable interfamily
variation in the proportion of marriages that were
consanguineous, there was no major difference in the
mean proportion between the group of index fami-
lies and the control group.

The test results are summarized in Table 2. Testing
was greatly facilitated when it was supported by an
influential family member. It was easiest to undertake
testing in rural areas because most people were readily
available, whereas in urban areas, men and school-age
children were away during the day. Most of those
who were not tested live in remote areas; there is no
indication that they are at lower risk than the rest of
their family.

Index Families

The index person was an affected child in nine in-
dex families and a carrier in one family. The family his-
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tories revealed that there were another 19 affected children, 6 of whom were alive and 13 of whom had
died (Table 2). Of 846 living family members, 591
(70 percent) were tested and 183 (31 percent) were
found to be carriers. Among the 214 married couples
tested, we found 86 carriers, 34 of whom were mar-
tied to another carrier. Nine of the 17 couples who
were at risk were the parents of the child with the
index case in their family; 6 more couples consisting
of two carriers were identified by the family history
(they had already had one or more affected children),
and 2 such couples were identified by testing. All cou-
ples at risk were informed of the availability of pre-
natal diagnosis. A total of 97 unmarried carriers were
identified and informed of their genetic risk and of the
availability of prenatal diagnosis, should their chosen
partner also be a carrier.

In Family 8, a carrier had been identified incident-
ally. Our study showed that a β-thalassemia gene
had entered this family three generations previously
through a marriage to a completely unrelated person.
The gene was present in only one branch of the family,
but Figure 2 shows that the members of the youngest
generation in this branch have a high chance of en-
tering a marriage that would be at risk for producing
affected children. Although six different mutations
were found (Table 2), in 9 of the 10 families we stud-
ied all the carriers had the same mutation.

Control Families

Of 609 living members of the control families,
397 (65 percent) were tested, but no carrier was iden-
tified.

Antenatal Screening

All 350 pregnant women who were approached
agreed to undergo testing, and 17 of them (5 percent)
were found to carry β-thalassemia. All partners of
these 17 women were tested, but no couple was iden-
tified as at risk.

Use of Information on Risk

It has been possible to conduct four years of reg-
ular follow-up through informal visits in 8 of the 10
index families. To date, there have been seven new
marriages and engagements, and family members said
that test results were taken into account when they
were arranged. Four consanguineous couples were
known not to be at risk; in three, one partner was a
known carrier and the other a known noncarrier; and
in one, the man was a known noncarrier, so the wom-
an was not tested. One consanguineous couple who
had not taken part in the study came spontaneously
for premarital testing and was found not to be at risk.

Two men who were carriers and were marrying
more “distant” women (one marrying a second cous-
in, and one marrying within the biradri) married
women who had not been tested. In one case, three
previous engagements had been broken, the marriage
was arranged with difficulty, and the family did not
wish to disclose the problem. In the other case, two
engagements had been broken after the man’s carri-
er status was disclosed; it was not mentioned during
the third engagement, and the bride was found not
to be a carrier after marriage. There have so far been
no reports that a marriage was avoided explicitly be-
cause both partners were carriers.
In these families to date, no couple at risk with two or more healthy children has undertaken a further pregnancy, and seven prenatal diagnoses have been performed for six couples at risk with no, or only one, healthy child. A couple that was prospectively identified as at risk and that had one unaffected child declined prenatal diagnosis for religious reasons and had an affected child.

**DISCUSSION**

We tested the possibility of deploying modern genetic technology appropriately and cost effectively in a developing country. Our hypothesis was that in communities with a cultural preference for consanguineous marriage, gene variants are trapped within extended-family groupings. Hence, an affected child is a marker of a group at high genetic risk, and studies of the extended family may identify many carriers and couples at risk before marriage or reproduction. We tested the hypothesis by offering testing to identify carriers to 16 extended families with a history of a hemoglobin disorder, 8 control families with no such history, and 350 randomly selected pregnant women (for comparison with antenatal screening). About two thirds of the families we approached agreed to undergo testing, and two thirds of their 1455 members were tested.

As would have been expected, 5 percent of the members of families with an affected relative were found to be carriers. No carriers were found among 397 members of families with no known affected relative. The results confirm the extremely heterogeneous distribution of gene variants in such populations.

In theory, when the prevalence of carriers is 5 percent in the general population and 31 percent in families with an affected member, a person with an affected relative would have an approximately 1.6 percent chance of entering a marriage at risk for producing affected children (31 percent of 5 percent). The index families included 214 couples, 126 of whom were tested. Seventeen couples were found to be at risk — 13 percent of those tested, or at least 8 percent of all couples. Thus, in these families, about 10 percent of couples are probably at risk, and carriers have a 20 to 30 percent chance of entering a marriage at risk for producing affected children.

Of the 17 couples found to be at risk, 11 were closely consanguineous and 6 were related only through the biradri. This finding suggests that variant genes are often trapped not only within extended families, but also in the larger biradri groupings from which many partners are drawn. The proposal that consanguineous marriage should be discouraged in such communities on genetic grounds is ethically unacceptable; it is also unrealistic, because more than 90 percent of marriag-
**Table 2. Affected Children, Carriers, Marriages between Carriers, and DNA Data, According to the Family Studies.**

<table>
<thead>
<tr>
<th>FAMILY NO.</th>
<th>CONDITION IN INDEX CASE</th>
<th>AFFECTED CHILDREN</th>
<th>FAMILY MEMBERS TESTED</th>
<th>CARRIERS</th>
<th>CARRIERS MARRIED TO CARRIERS</th>
<th>MUTATIONS FOUND ON DNA TESTING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NO.  ALIVE  NO.  DEAD  TOTAL NO.</td>
<td>NO. UN-MARRIED (%)</td>
<td>NO. MARRIED</td>
<td>TOTAL NO. (%)</td>
<td>OF TESTED FAMILY MEMBERS)</td>
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<td>Index families</td>
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<tr>
<td>1</td>
<td>β-Thalassemia major</td>
<td>4 3 7</td>
<td>138 (69)</td>
<td>75</td>
<td>63</td>
<td>36 (26)</td>
</tr>
<tr>
<td>2</td>
<td>β-Thalassemia major</td>
<td>1 0 1</td>
<td>85 (100)</td>
<td>48</td>
<td>37</td>
<td>26 (31)</td>
</tr>
<tr>
<td>3</td>
<td>β-Thalassemia major</td>
<td>1 2 3</td>
<td>51 (93)</td>
<td>29</td>
<td>22</td>
<td>14 (27)</td>
</tr>
<tr>
<td>4</td>
<td>β-Thalassemia major</td>
<td>1 0 1</td>
<td>41 (85)</td>
<td>22</td>
<td>19</td>
<td>17 (41)</td>
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<tr>
<td>5</td>
<td>β-Thalassemia major</td>
<td>1 1 2</td>
<td>45 (65)</td>
<td>25</td>
<td>20</td>
<td>17 (38)</td>
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<tr>
<td>6</td>
<td>β-Thalassemia major</td>
<td>1 1 2</td>
<td>42 (70)</td>
<td>29</td>
<td>13</td>
<td>19 (45)</td>
</tr>
<tr>
<td>7</td>
<td>β-Thalassemia major</td>
<td>2 4 6</td>
<td>20 (25)</td>
<td>8</td>
<td>12</td>
<td>14 (70)</td>
</tr>
<tr>
<td>8</td>
<td>β-Thalassemia trait</td>
<td>0 0 0</td>
<td>58 (59)</td>
<td>38</td>
<td>20</td>
<td>12 (21)</td>
</tr>
<tr>
<td>9</td>
<td>Sickle cell anemia</td>
<td>3 2 5</td>
<td>48 (61)</td>
<td>25</td>
<td>23</td>
<td>11 (23)</td>
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<tr>
<td>10</td>
<td>Sickle cell anemia</td>
<td>1 0 1</td>
<td>63 (86)</td>
<td>40</td>
<td>23</td>
<td>17 (27)</td>
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<td>Control families</td>
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<td>Total in index families</td>
<td>15 13 28</td>
<td>591 (70)</td>
<td>339 252</td>
<td>183 (31)</td>
<td>97 86 34 (40) 17 11 6</td>
<td>Frame shift 41–42</td>
</tr>
<tr>
<td>Total in control families</td>
<td>0 0 0</td>
<td>397 (65)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Total in all families</td>
<td>15 13 28</td>
<td>988 (68)</td>
<td>339 252</td>
<td>183</td>
<td>97 86 34        17 11 6</td>
<td>Hemoglobin S</td>
</tr>
</tbody>
</table>

*Since no carriers were identified in the control families, we did not calculate the numbers of married and unmarried family members tested.*
es occur within the biradri and logically would have to be discouraged. Such a wholesale attack on social structure has no chance of success. Our study shows that a real option for providing genetic counseling in a way that is compatible with the social mores and kinship structure of the Pakistani population involves accurate testing to identify carriers and the provision of precise information on the presence of risk.

The effectiveness of studies of families is compared with that of population screening in Table 3. Family studies provide a highly effective approach to risk detection in Pakistani society. Population screening is less powerful, but follow-up of carriers can identify increased risk before the birth of any affected child (as it did in the case of Family 8). Therefore, the ideal policy is to provide both family studies and premarital or antenatal screening for the relatives of affected children. This combined approach is a long-term strategy, and its effects are likely to increase with time — assuming that ways are found to help families to retain genetic information (which they tend to forget) and to ensure that each new generation is offered testing to identify carriers.

It is worth determining whether persons are at risk only if they wish to know about it and can use the information provided. The main choices open to those who are at risk are avoiding marriage to another carrier or using prenatal diagnosis. Information on carrier status has little effect on the choice of partners in Mediterranean countries. This generalization may or may not be applicable in communities in which many marriages are arranged, but there is very little systematic understanding of how such marriages come about — for example, who suggests them, at what age possible partners are identified, and what part is played by the preferences of the young persons themselves. Our observations suggest that close relatives accept marriage with a carrier more easily than unrelated persons do. In the latter situation, it may be particularly difficult for the parents of a woman who is a carrier to ask that a potential partner be tested; most family members said that their preferred solution in such a situation is to postpone testing until after marriage and then to use prenatal diagnosis if required.

As has been reported in Western studies, most couples in our study who were found to be at risk and who had two or more healthy children avoided further pregnancy; many with one healthy child or none requested prenatal diagnosis; and some avoided any intervention in pregnancy for religious reasons. Our observations to date do not permit any firm conclusions other than that many families use information about risk to avoid problems as far as possible.

Our study confirms that in communities in which consanguineous marriage is common, an approach targeting the extended family is useful because it produces a high yield of information on carriers and couples at risk; family members often understand the condition because they have had contact with an affected child; and usually only one gene variant is present in a given family, simplifying and reducing the cost of DNA-based diagnosis. The approach also overcomes problems such as a weak health care infrastructure (because the family study can be undertaken at the center where the index case of the disorder is diagnosed and treated) and a low level of literacy (because information is communicated directly between families with affected children who are attending the center). It is
also an equitable approach that can be built into services as they are developed and so reach the gradually growing proportion of the population with access to services. In developed countries where labor costs are high, the fact that extended family studies are labor-intensive might be a detraction. Since labor costs are proportionately low in developing countries, where the primary difficulty is the high cost of reagents that must be purchased abroad.

Our study has more than local relevance; the conclusions are applicable for all recessive disorders in populations where consanguineous marriage is common. It is estimated that about 10 percent of congenital and genetic disorders worldwide are associated with customary consanguineous marriage; in most of the Middle East, the proportion is 30 percent, and in Pakistan, it is 40 percent. The growing number of facilities providing DNA-based diagnostic services for hemoglobin disorders provides a basis for far broader clinical molecular-genetics services capable of applying many new techniques for the accurate diagnosis of these disorders. It will take a long time to implement the approach in developing countries; it should be possible to deploy it more rapidly in the sizable communities favoring consanguineous marriage that reside, for example, in the United Kingdom and Scandinavia, where resources for genetic testing are comparatively abundant.

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REFERENCES


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