9 Consanguineous marriage and recessive disorders

Introduction:

The term “consanguineous” literally means related by blood. A consanguineous marriage is defined as marriage between individuals who have at least one common ancestor usually not more than three generations back and the progeny of a consanguineous marriage is “inbred” (Bittles 1994). All human beings are relatives and our progenitors might even have been one single couple (Vogel and Motulsky 1986). There is some evidence to suggest that a relatively small founder population migrated out of Africa to give rise subsequently to all non African populations (Jones and Rouhani 1986). The long time period since divergence of human populations has helped several intervening mutations to cause genetic variability between different human beings. The relatedness of human beings assumes importance in the context of recessive mutations, as these are expressed only when inherited in a homozygous state. The chances of inheriting two identical genes, including neutral as well as pathological genes, at a particular locus are increased if the parents are close relatives (Bodmer and Cavalli-Sforza 1976).

Genetic effects of consanguineous marriage:

The main genetic consequence of consanguineous marriage is a reduction of genetic variation and an increase in the proportion of homozygotes. Recessive genes, that are unable to express in the heterozygous state, are thus brought to the fore (Bodmer and Cavalli-Sforza 1976). Increase in the proportion of homozygotes should uniformly affect the pathological, neutral as well as the beneficial traits. However, the effects of increased homozygosity for all genes are not distributed evenly about the population mean and a bias towards the pathological end of the spectrum may be observed (Modell and Kuliev 1992). There is no dearth of information on the association of consanguinity and increased incidence of genetic disorders (Neel and Schull 1962; Klingberg et al, 1971; Basu 1975; Naderi 1979; Khlat et al, 1986; Bundey et al, 1991; Teebi 1994). A population inevitably

carries a certain load of detrimental genes originating through mutations. Dominant genes are rapidly eliminated while recessive genes can be hidden and so can accumulate. Studies on the relationship between consanguinity and genetic disorders indicate that on an average an individual may be heterozygous for 1.4 lethal equivalents (Bittles and Neel 1994). The detrimental recessive genes may be unmasked by marriages between close relatives and this risk is directly proportional to the degree of relatedness between the mates (Bittles 1980).

The data on association of consanguineous marriages and harmful traits have almost completely overshadowed the beneficial traits, if any, that might be associated with consanguineous marriages. A prime example of the absence of inbreeding depression can be seen in the genealogies of the Egyptian pharaohs, in which brother-sister mating was practised for many generations without known or reported ill effects (Strickberger 1968). Some studies of intelligence also suggest an association between consanguineous marriages and reduced cognitive performance (Slatis et al, 1961; Bashi 1977). Similarly, there is evidence to suggest higher gross fertility in a consanguineous mating (Bittles 1994).

Inbreeding estimates:

The genetic effects of consanguineous marriage and the resulting inbreeding are measured by a coefficient (*F*), first proposed by Sewall Wright (Strickberger 1968). The coefficient of inbreeding is the probability that an individual receives at a given locus two genes that are identical by descent (copies of a single gene carried by a common ancestor). A closely related coefficient of kinship (Φ) is the probability that a gene taken at random from an individual is identical by descent to a gene at the same locus taken at random from another individual (Vogel and Motulsky 1986). The difference between the two coefficients is that Φ applies to two individuals who have common ancestors and *F* applies to one individual and measures the degree of relationship between his/her parents.

The value of *F* for a first cousin marriage is 0.0625 i.e. 6.25% of the genes are identical by descent. In 1½ cousin and 2nd cousin marriage *F* is 0.0313 and 0.0156 respectively (Bodmer and Cavalli-Sforza 1976). In populations where consanguineous marriage is

customary for several generations the estimates that do not take into account antecedent consanguineous marriages are expected to be less than the actual values (Bittles 1994). When extensive pedigree records are available and it is possible to go back for many more generations the value of *F* may be two or more times as large as obtained through the usual procedures (Bodmer and Cavalli-Sforza 1976).

Inbreeding estimates based on genetic polymorphism:

Genetic polymorphism may be defined as a trait encoded by a piece of DNA (a locus) with two or more alleles (sequence variants), of which at least two occur at a frequency of more than 1% in a given population (Vogel and Motulsky 1986). Blood groups and protein polymorphisms have been used in the past to estimate inbreeding in human populations (Workman and Niswander 1970). The estimates are based on the principle that in a population where mating is not random the heterozygotes are reduced in comparison to Hardy-Weinberg proportions while homozygotes are increased (Bodmer and Cavalli-Sforza 1976). The extent of inbreeding in such a population can be measured by quantifying the departure from Hardy Weinberg proportions of the frequencies of polymorphic genes.

Molecular genetic analysis has revealed that about 2% of the total human genome encodes proteins and the rest does not appear to have any sequence dependent function. A bulk of the intergenic DNA is unique because it consists of tandemly repeating sequences whose length may vary between different individuals in the same population. The polymorphisms created by such elements are termed variable number of tandem repeats (VNTR) for the larger repeats and short tandem repeats (STR) for 1-6 base pair repeats (Krawczak and Schmidtke 1994). VNTRs and STRs follow the same rules of inheritance as the remainder of DNA and therefore are ideal sites as polymorphic genetic markers (Housman 1995).

VNTRs and STRs can be analyzed by the Polymerase Chain Reaction (Horn et al, 1989; Urquhart et al, 1994). The STRs offer a better choice as compared to the VNTR loci because the larger allele of a VNTR system may be missed in PCR based amplification (Newton and Graham 1994). The STRs have an added advantage of accurate sizing of the alleles on automated equipment (Smith 1995). The STR allele frequencies have a potential

for use in estimating the extent of inbreeding in a population. However, there are no published data on the use of STR allele frequencies for inbreeding estimates.

Objectives of the study:

This chapter describes the pattern and frequency of consanguineous marriages and their relationship to autosomal recessive disorders in Pakistani families. The effect that an antecedent consanguineous marriage might have on the inbreeding estimates is also studied. In addition, an attempt is made to calculate the *F* values in a population by using polymorphic STR allele frequencies. The same STR was also studied in the successive generations of two large families to highlight the genetic effects of consanguineous marriage.

Material and methods:

The study families:

A total of 14 large families were studied including nine with a known history of haemoglobin disorder (subjects) and five without such history (controls). The characteristics of the families studied are described in Chapter 5 (Tables: 5.2 and 5.3).

Drawing pedigrees:

Pedigree information on at least the last three generations in each family was collected. Information on relationships of the more remote ancestors was also included where available. The information was obtained from a well informed person in the family. Information about the 2^{nd} and 3^{rd} generations was readily available. However, it was more difficult to get information about the 1^{st} generation or earlier. In the urban families pedigree information had to be collected from multiple sources because the members had infrequent contact with each other. Best efforts were made to ensure the reliability of information.

The pedigree was initially drawn as a sketch on paper. Later, this information was transferred to a computer by using the software package “Cyrillic Version 2.00“ (The Magdalen Centre, Oxford).

Morbidity and mortality in the family:

Information about the births of children with major congenital malformations or symptoms suggestive of a genetic disorder was collected from the person who provided the pedigree information. The result of screening for β-thalassaemia, where carried out, was also included in the pedigree.

Consanguineous marriages:

The couples were classified as double first cousins (F=0.1250), first cousins (F=0.0625), first cousins once removed (1½ cousins) (F=0.0313), and second cousins (F=0.0156).

Coefficient of inbreeding (F) and coefficient of kinship (Φ):

The coefficient of inbreeding of each individual and coefficient of kinship for each couple in a family was calculated using Cyrillic version 2.00. The value of F and Φ for individuals in one of the families was also calculated by the manual method of path coefficient (Vogel and Motulsky 1986). All common ancestors were marked and the mates were connected by all possible pathways leading to the common ancestors. The number of steps in each path was counted. The F values were calculated by the following formula:

$$F = \tfrac{1}{2}\,(2^{-m_1} + 2^{-m_2} \ldots + 2^{-m_r}) = \tfrac{1}{2} \sum_{i=1}^{r} 2^{-m_i}$$

where m denotes the number of steps connecting an individual with the respective common ancestor. The average values of F and Φ in a generation of a family were calculated by the following formulae:

$$F = \sum p_i F_i$$

$$\Phi = \sum p_i \Phi_i$$

The summations go over various types of consanguineous marriages, with p_i , F_i and Φ_i being the relative frequency, coefficient of inbreeding and coefficient of kinship of the i-th type of consanguineous marriage (Vogel and Motulsky 1986).

Short tandem repeat (STR) analysis:

Allele frequencies for a polymorphic DNA marker, D21S11 (Sharma and Litt 1992), were used to calculate the coefficient of inbreeding in a randomly selected population sample of 132 unrelated individuals with β-thalassaemia trait. They presented at the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi, for screening of thalassaemia. The parental consanguinity of the subjects was also noted. A comparison between the *F* obtained by STR allele frequencies and the conventional method was made.

D21S11 genotypes were determined for the available members of two subject families. A total of 140 individuals from Family No: 1 and 85 individuals from Family No: 2 were studied. The genotypes of 5 dead members in the two families were inferred from their children. The data on genotypes in various generations were used to investigate the effect of genetic drift in the successive generations. The allele frequencies in each generation were calculated by pooling together all individuals in that particular generation because they were the people who would be passing on their alleles to the next generation.

Theoretical considerations:

Gene frequency can be determined by simply counting the alleles and by remembering that every individual has two copies of each gene (Bodmer and Cavalli-Sforza 1976). At a di-allelic locus with 5% carrier rate of β-thalassaemia, for example, the thalassaemia gene frequency will be 0.025 (5 β-thalassaemia genes out of a total of 200 genes at that locus). A multi-allelic system like STRs gives rise to many genotypes. The frequency of each STR allele can also be determined by the method of counting alleles. Each heterozygote represents one gene and the homozygote represents two genes.

In a randomly mating population, the relation between allele and genotype frequencies is simple. If the gene frequencies are known the Hardy-Weinberg law can predict the corresponding genotype frequencies (Vogel and Motulsky 1986). At a di-allelic locus, for example, if the frequency of allele *A* is *p* and that of *B* is *q* then the genotype *AA, AB,* and *BB* will have frequencies of p^2, *2pq,* and q^2 respectively. The same rule can be extended to predict the genotype frequencies at a multi-allelic locus (Committee on DNA technology in forensic science 1992). In a five allele system, for example, if the alleles *A, B, C, D,* and

E have frequencies *p, q, r, s,* and *t* respectively then the 15 possible genotype frequencies (predicted by the formula [n(n+1)/2] where n is the number of alleles), would be p^2, q^2, r^2, s^2, and t^2 for the homozygotes, and *2pq, 2pr, 2ps, 2pt, 2qr, 2qs, 2qt, 2rs, 2rt, 2st* for the heterozygotes.

In a population where mating is not random, the proportion of heterozygotes is reduced in comparison to Hardy-Weinberg proportions by an amount *2Fpq*, while that of each homozygote is increased by *Fpq*. Where *F* is the inbreeding coefficient, and *p* and *q* are the frequencies of the alleles under consideration (Bodmer and Cavalli-Sforza 1976). The genotypic proportions at a single di-allelic locus, say (*AA, AB, BB*) = (*P, H, Q*), where *P*+*H*+*Q*=1, can be represented in terms of the gene frequencies of *A* and *B*, denoted by *p* and *q*, by the formulas:

$$\underset{AA}{P = p^2 + pqF;} \qquad \underset{AB}{H = 2pq\,(1 - F\,);} \qquad \underset{BB}{Q = q^2 + pqF}$$

where $p = P + \frac{1}{2}H$, $q = 1 - p = Q + \frac{1}{2}H$, and $F = 1 - H / 2pq$. Here *F* denotes the deviation from Hardy-Weinberg proportions due to the joint effect of all forces acting on the pattern of genetic variation, including non-random mating, selection, and mutation. When inbreeding is the only factor, *F* is identical with the classical inbreeding coefficient (Workman and Niswander 1970).

Calculation of gene frequencies and coefficient of inbreeding:

The gene frequencies for the D21S11 STR alleles were calculated by the simple method of counting. The *F* value in the subject population was estimated by the following formula:

$$F = 1 - H / 2pq$$

where *H* is the observed proportion of heterozygotes for the allele whose gene frequency is represented by *p*, and *q*=1-*p*.

Results:

Characteristics of the families:

Characteristics of the 14 families studied were described in Chapter 5 (Table: 5.2 and 5.3). There were 9 Punjabi and 5 Pathan families. Family Nos: 1 and 2 were from the urban areas whereas all others were from the rural areas.

Drawing pedigrees:

The individuals from the families were divided into generations and only the last three generations were taken into consideration. The third last generation in a family was called 1st generation and the subsequent generations were labelled 2nd and 3rd. Most pedigrees had complex consanguineous relationships, therefore drawing a pedigree was also complex. The commonest problem was to keep all individuals in a particular generation at the same level. In most situations it was not possible without keeping the individuals in the same generation at split-levels. Another problem was in assigning a generation to the couple and the children of 1½ cousins. The couple were arbitrarily placed (counted) in the generation of the parent from the senior generation. Their children were placed (counted) in the generation of the parent from the younger generation. However, while counting the number of individuals in a generation, each individual was counted in the generation to which it actually belonged. Pedigrees of the 9 subject and 5 control families are presented in Figures 9.4-9.17.

Pattern of marriages:

There were 319 couples in the three generations of 14 families (Table: 9.1). This included 195 couples in the nine subject families and 124 in the five control families. There were 1.3% double first cousins, 33.2% 1st cousins, 4.1% 1½ cousins, and 3.8% 2nd cousins. There were no statistically significant differences between the pattern of marriages in the subject and the control families. In 319 couples 165 (51.7%) were unrelated but from the same Biradri/Tribe and only 19/319 (6.0%) couples were completely unrelated.

It was noted that consanguineous marriages were more frequent in the 2nd generation than the 1st (Table: 9.2). The frequency of 1st cousin marriages had increased from 12% in the 1st generation to 45% in the 2nd generation (p=<0.0001). There were 12 married couples in

the 3rd generation and 7 of them were consanguineous. Although its difference from the previous generation is statistically insignificant due to small numbers but the trend is obvious. Consanguineous marriages have reduced Biradri marriages from 71% in the 1st generation to 40% in the 2nd generation (p=0.003). The frequency of consanguineous and Biradri marriages was similar in the subject as well as the control families. However, in the 2nd generation there were 51% 1st cousin couples in the subject families as compared to 37.5% in the controls, but the difference was statistically insignificant (p=0.25).

Calculation of *F* and *Φ*:

The procedure of calculating *F* and *Φ* by manual method was very complicated and time consuming. On the other hand the use of Cyrillic 2.00 was very efficient in calculating *F* and *Φ* in the complex pedigrees. A complete correlation between the manual and the computerized method was found.

Coefficient of inbreeding (F):

F for the three generations of the subject families is presented in Table: 9.3. A progressive increase for the 1st (0.0046), 2nd (0.0128) and 3rd (0.0352) generation was observed. There were no significant differences between *F* in the three generations of the subject and the control families. There was a wide variation of *F* between the individual families. In the 3rd generation, for example, it varied from 0.000 to 0.0782.

Coefficient of kinship (Φ):

Observed *Φ* of consanguineous couples in the 2nd generation showed a value of 0.1346 for a double first cousin marriage, 0.0718 for a 1st cousin marriage, 0.0330 for a 1½ cousin marriage and 0.0242 for a 2nd cousin marriage (Table: 9.4). It was noted that the differences between the observed and the expected value of *F* for the close and the distant consanguineous relationships was similar.

Genetic/congenital disorders in the study families:

Haemoglobin disorders:

There were 170/571 (30%) carriers in the subject families (Table: 9.5). The number of carriers in each generation varied from 30-34%. In the 2nd generations there were 83/251 (33%) carriers and 30 were unmarried (30% of 101 unmarried individuals). In 150 married

individuals in 2nd generation 53 (35.3%) were carriers. In the 2nd generation 32/150 (21%) carriers were married to a 1st cousin, 3/150 (2%) to a 1½ cousin, 2/150 (1.3%) to a 2nd cousin and the remaining 16/150 (10.7%) to an unrelated Biradri member. In 8/37 (21.6%) carriers married to a cousin the spouse was also a carrier. However, the spouses of 4/16 (25%) carriers married to an unrelated Biradri/Tribe member were also carriers. No carrier was married to a carrier from a different Biradri/Tribe.

Correlation between the number of carriers, coefficient of kinship and at risk couples:

Table 9.6 summarizes the number of carriers, mean coefficient of kinship, and the number of at risk couples in the 1st and 2nd generations of the subject (index) families. The 3rd generation did not have enough couples so it was not included in the comparison. The proportion of carriers in the 1st generation of most families was lower than in the 2nd generation. Similarly, coefficient of kinship was also lower in the 1st generation. This correlated with the absence of at risk couples in this generation. Family No: 10, however, had 45% carriers and in spite of a low Φ two at risk couples were seen. Both of the couples although unrelated were from the same Tribe. In the 2nd generation the number of at risk couples generally correlated with the number of carriers and the mean Φ for the generation. In most families the carrier rate was above 30% and the kinship coefficient was also high. In Family No: 1 four at risk couples were seen in spite of a relatively low Φ (0.0303). However, the carrier rate in the generation was fairly high (33%) and three non-consanguineous at risk couples were from the same Biradri all of whom also had the same β-thalassaemia mutation (IVSI-1). In Family No: 8, no at risk couple was seen. Although the carrier rate in the individuals tested was fairly high (29%) but the mean kinship coefficient was low (0.0208). In Family No: 9 in spite of a low carrier rate and low Φ one at risk couple was present. The spouses were unrelated but were from the same Tribe. In Family No: 10 carrier rate (22%) as well as Φ (0.0313) were low and there was no at risk couple in this generation.

Couples at risk of genetic disorders:

In the nine subject families 12/195 (6.2%) couples had affected children and 2/195 (1%) were at risk but did not have an affected child (Table: 9.7). Out of the 79 consanguineous couples 11 (14%) had an affected child as compared to 3/116 (2.5%) amongst the non-consanguineous couples (p=0.005). In Family No: 1, four couples were found to have

thalassaemic children. However, the spouse of only one was a first cousin (Φ=0.0869) whereas in the other three spouses were unrelated Biradri members. Mutation analysis revealed that all four couples had the same β-thalassaemia mutation (IVSI-1 (G-T). In Family No: 2, in addition to the index couple (Φ=0.0469), screening for thalassaemia resulted in detection of one more at risk couple (Φ=0.1210). The couple did not have an affected child. In the same family two other consanguineous couples (Φ=0.0820 and 0.0781) had children with congenital deafness. In Family Nos: 5 and 10, one at risk couple each (Φ=0.0781 and 0.000), in addition to the index couples, were identified as a result of screening for a haemoglobin disorder. Both of the couples did not have an affected child.

In the five control families 4/124 (3.2%) couples had children possibly affected by a congenital or genetic disorder (Table: 9.7). All 4 couples were consanguineous and they formed 7% of the 56 consanguineous couples. In contrast, none of the 68 non-consanguineous couples had children possibly affected by a genetic or congenital disorder (p=0.03). In Family No: 11 one consanguineous couple (Φ=0.0781) had three children that died during the first month of life due to unknown causes. In Family No: 13 two consanguineous couples (Φ=0.0625) had several children that died immediately after birth due to unknown causes. In Family No: 14 one couple (Φ=0.1328) was found to have deaths of two children due to multiple congenital abnormalities. In Family No: 15 five consanguineous couples had children who died between 1-5 years of age. The cause of death in all such children was not similar. There was also no visible evidence to suggest that they had any congenital malformation. The possibility of a genetic disorder could not be ruled out.

Table: 9.1. Pattern of marriages in the three generations of the 14 families studied.

Family:	**DIC**	**1st cousins**	**1½ cousins**	**2nd cousins**	**Biradri**	**Non-Biradri**	**Total**
Subject Families:							
No: 1	-	15	2	3	30	2	52
No: 2	-	13	1	1	4	2	21
No: 3	-	7	-	1	5	1	14
No: 4	-	6	-	-	4	2	12
No: 5	-	8	-	-	9	2	19
No: 6	-	5	-	-	9	-	14
No: 8	-	6	2	-	15	5	28
No: 9		5	-	1	14	-	20
No: 10	-	3	-	-	12	-	15
Sub total	-	68 (34.9%)	5 (2.6%)	6 (3.1%)	102 (52.3%)	14 (7.2%)	195 (100%)
Control families:							
No: 11	-	10	2	1	9	-	22
No: 12	-	-	-	-	11	-	11
No: 13	-	6	-	-	16	1	23
No: 14	3	10	3	3	13	-	32
No: 15	1	12	3	2	14	4	36
Sub total	4 (3.2%)	38 (30.6%)	8 (6.6%)	6 (4.8%)	63 (50.8%)	5 (4.0%)	124 (100%)
Grand total	4 (1.3%)	106 (33.2%)	13 (4.1%)	12 (3.8%)	165 (51.7%)	19 (6.0%)	319 (100%)

DIC: double first cousins.

Table: 9.2. Pattern of marriages in the successive generations of the nine subject and five control families.

Family:	**Number of couples**																			
	1st generation							**2nd generation**							**3rd generation**						
	Consanguineous				**Unrelated**		**All**	**Consanguineous**				**Unrelated**		**All**	**Consanguineous**				**Unrelated**		**All**
	DIC	**1st**	**1½**	**2nd**	**Bir**	**NB**		**DIC**	**1st**	**1½**	**2nd**	**Bir**	**NB**		**DIC**	**1st**	**1½**	**2nd**	**Bir**	**NB**	
Subject Families:																					
No: 1	-	1	-	2	11	2	16	-	13	2	1	17	-	33	-	1	-	-	2	-	3
No: 2	-	5	1	-	4	2	12	-	8	-	1	-	-	9	-	-	-	-	-	-	-
No: 3	-	1	-	-	3	1	5	-	6	-	1	2	-	9	-	-	-	-	-	-	-
No: 4	-	-	-	-	4	2	6	-	6	-	-	-	-	6	-	-	-	-	-	-	-
No: 5	-	2	-	-	4	1	7	-	6	-	-	5	1	12	-	-	-	-	-	-	-
No: 6	-	1	-	-	7	-	8	-	4	-	-	2	-	6	-	-	-	-	-	-	-
No: 8	-	-	-	-	5	3	8	-	4	2	-	7	2	15	-	2	-	-	3	-	5
No: 9	-	-	-	-	10	-	10	-	4	-	2	4	-	10	-	-	-	-	-	-	-
No: 10	-		-	-	9	-	9	-	3	-	-	3	-	6	-	-	-	-	-	-	-
Sub total %	-	10 12.3	1 1.3	2 2.5	57 70.3	11 13.5	81 100	-	54 50.9	4 3.8	5 4.7	40 37.7	3 2.8	106 100	-	3	-	-	5	-	8
Control Families:																					
No: 11	-	1	-	-	4	-	5	-	9	2	1	5	-	17	-	-	-	-	-	-	-
No: 12	-	-	-	-	5	-	5	-	-	-	-	6	-	6	-	-	-	-	-	-	-
No: 13	-	-	-	-	6	1	7	-	6	-	-	10	-	16	-	-	-	-	-	-	-
No: 14	-	1	-	2	4	-	7	3	5	3	1	9	-	21	-	4	-	-	-	-	4
No: 15	-	2	2	-	10	2	16	1	10	1	2	4	2	20	-	-	-	-	-	-	-
Sub total %	-	4 10.0	2 5.0	2 5.0	29 72.5	3 7.5	40 100	4 5.0	30 37.5	6 7.5	4 5.0	34 42.5	2 2.5	80 100	-	4	-	-	-	-	4
Grand total %	-	14 11.6	3 2.5	4 3.3	86 71.1	14 11.6	121 100	4 2.1	84 45.2	10 5.4	9 4.8	74 39.8	5 2.7	186 100	-	7 58.3	-	-	5 41.7	-	12 100

DIC: double first cousins; Bir: Biradri member; NB: Non-Biradri member.

Table: 9.3. Coefficient of inbreeding (*F*) in the three generations of the nine subject and five control families.

Family:	**Coefficient of Inbreeding (*F*):**								
	1st generation:			**2nd generation:**			**3rd generation:**		
	Number:	**Average:**	**Range:**	**Number:**	**Average:**	**Range:**	**Number:**	**Average:**	**Range:**
Subject Families:									
No: 1	32	0.0122	0.000-0.0625	75	0.0153	0.000-0.0664	102	0.0246	0.000-0.0869
No: 2	20	0.0013	0.000-0.0625	48	0.0360	0.000-0.0820	23	0.0782	0.0469-0.1210
No: 3	11	0.0071	0.000-0.0625	28	0.0211	0.000-0.0625	24	0.0533	0.0000-0.0781
No: 4	12	0.000	0.000-0.000	19	0.000	0.000-0.000	20	0.0625	0.0625
No: 5	14	0.0067	0.000-0.0625	31	0.0297	0.000-0.0625	28	0.0441	0.000-0.1094
No: 6	16	0.000	0.000-0000	27	0.0093	0.000-0.0625	20	0.0500	0.000-0.0625
No: 8	13	0.0072	0.000-0.0625	33	0.0009	0.000-0.0625	77*	0.0231	0.000-0.0625
No: 9	21	0.000	0.000-0.000	28	0.000	0.000-0.000	42	0.0245	0.000-0.0625
No: 10	17	0.000	0.000-0.000	42	0.000	0.000-0.000	18	0.0139	0.000-0.0625
Sub total	156	0.0058	0.000-0.0625	331	0.0141	0.000-0.0820	354	0.0343	0.000-1210
Control Families:									
No: 11	12	0.0065	0.000-0.0625	34	0.0165	0.000-0.0625	84	0.0415	0.000-0.0781
No: 12	10	0.000	0.000-0.000	25	0.000	0.000-0.000	34	0.000	0.000-0.000
No: 13	14	0.0011	0.000-0.0156	37	0.0017	0.000-0.0625	74	0.0203	0.000-0.0625
No: 14	14	0.000	0.000-0.000	42	0.0123	0.000-0.0625	100*	0.0237	0.000-0.1328
No: 15	33	0.0019	0.000-0.0313	65	0.0149	0.000-0.0625	74	0.0576	0.000-0.1406
Sub total:	83	0.0019	0.000-0.0625	203	0.0105	0.000-0.0625	366	0.0360	0.000-0.1406
Grand total:	239	0.0046	0.000-0.0625	534	0.0128	0.000-0.0820	720	0.0352	0.000-0.1406

***** combined total of the 3rd and 4th generations

Table: 9.4. Coefficient of kinship (*Φ)* for consanguineous couples in the 2nd generation of the study families.

Family:	**Coefficient of kinship (*Φ*):**											
	Double 1st cousins (0.1250):			**1st cousins (0.0625):**			**1½ cousins (0.0313):**			**2nd cousins (0.0156):**		
	n:	**Average:**	**Range:**	**n:**	**Average:**	**Range:**	**n:**	**Average:**	**Range:**	**n:**	**Average:**	**Range:**
No: 1	-	-	-	13	0.0713	0.0625-0.0869	2	0.0332	0.0322-0.0341	1	0.0176	0.0176
No: 2	-	-	-	8	0.0995	0.0781-0.1210	-	-	-	1	0.0469	0.0469
No: 3	-	-	-	6	0.0729	0.0625-0.0781	-	-	-	1	0.0156	0.0156
No: 4	-	-	-	6	0.0625	0.0625	-	-	-	-	-	-
No: 5	-	-	-	6	0.0833	0.0781-0.1094	-	-	-	-	-	-
No: 6	-	-	-	4	0.0625	0.0625	-	-	-	-	-	-
No: 8	-	-	-	4	0.0625	0.0625	2	0.0313	0.0313	-	-	-
No: 9	-	-	-	4	0.0625	0.0625	-	-	-	1	0.0156	0.0156
No: 10	-	-	-	3	0.0625	0.0625	-	-	-	-	-	-
No: 11	-	-	-	9	0.0729	0.0625-0.0781	2	0.0313	0.0313	1	0.0313	0.0313
No: 12	-	-	-	-	-	-	-	-	-	-	-	-
No: 13	-	-	-	6	0.0625	0.0625	-	-	-	-	-	-
No: 14	3	0.1328	0.1328	5	0.0680	0.0625-0.0781	3	0.0332	0.0332	1	0.0195	0.0195
No: 15	1	0.1406	0.1406	10	0.0758	0.0625-0.0937	1	0.0391	0.0391	2	0.0234	0.0234
Observed *Φ*	4	0.1348	0.1328-0.1406	84	0.0718	0.0625-0.1210	10	0.0330	0.0313-0.0391	8	0.0242	0.0156-0.0469
Expected *Φ*		0.1250			0.0625			0.0313			0.0156	
Obs/Exp *Φ*		1.078			1.149			1.054			1.551	
Obs-Exp *Φ*		0.0098			0.0093			0.0017			0.0086	

n: number of couples

Table: 9.5. Summary of the results of screening for haemoglobin disorders in the available members of the nine subject families with a history of haemoglobin disorder.

Family:	**Members:**		**Carriers:**							
	Alive:	**Tested:**	**1st Generation:**	**2nd Generation:**					**3rd Generation#:**	**Total:**
				Married to:			**Unmarried:**	**Total:**		
				1st C:	**1½ C:**	**2nd C:**	**Biradri:**			
No: 1	199	138 (69%)	2/9	7/47	1/47	-	8/47	2/7	18/54 (33%)	16/75 (21%)	36/138 (26%)
No: 2	85	85 (100%)	4/17	6/19	1/19	1/19	1/19	7/28	16/47 (34%)	6/21 (27%)	26/85 (31%)
No: 3	55	51 (93%)	1/6	3/15	0/15	0/15	0/15	4/9	7/24 (29%)	6/21 (29%)	14/51 (27%)
No: 4	48	41 (85%)	3/6	6/11	0/11	0/11	0/11	2/7	8/18 (44%)	7/17 (41%)	18/41 (44%)
No: 5	69	45 (65%	2/5	5/13	0/13	0/13	1/13	2/6	8/19 (42%)	7/21 (33%)	17/45 (38%)
No: 6	60	42 (70%)	4/9	4/8	0/8	0/8	1/8	5/14	10/22 (46%)	5/11 (46%)	19/42 (45%)
No: 8	98	58 (59%)	1/3	0/14	1/14	0/14	2/14	2/4	5/18 (28%)	6/37 (16%)	12/58 (21%)
No: 9	79	48 (61%)	3/7	0/12	0/12	1/12	2/12	1/2	4/14 (29%)	5/27 (19%)	11/48 (23%)
No: 10	73	63 (86%)	5/11	1/11	0/11	0/11	1/11	5/24	7/35 (21%)	5/17 (29%)	17/63 (27%)
Total:	766	571 (75%)	25/73 (34%)	32/150 (21.3%)	3/150 (2.0%)	2/150 (1.3%)	16/150 (10.7%)	30/101 (29.7%)	83/251 (33%)	63/247 (26%)	170/571 (30%)

almost all of the carriers in the 3rd generation were unmarried

Table: 9.6. Correlation between the number of carriers, coefficient of kinship (Φ) and the number of at risk couples in individuals tested for a haemoglobin disorder in two generations of the families with history of haemoglobin disorder.

Family:	1st Generation (individuals tested):			2nd generation (individuals tested):		
	Carriers:	Φ:	At risk couples:	Carriers:	Φ:	At risk couples:
No:1	2/9	0.0059	None	18/54 (33%)	0.0303	4
No: 2	4/17	0.0300	None	16/47 (34%)	0.0937	2
No: 3	1/6	0.0125	None	7/24 (29%)	0.0503	1
No: 4	3/6	0.000	None	8/18 (44%)	0.0625	1
No: 5	2/5	0.0179	None	8/19 (42%)	0.0416	2
No: 6	4/9	0.0078	None	10/22 (45%)	0.0416	1
No: 8	1/3	0.000	None	5/18 (28%)	0.0208	None
No: 9	3/7	0.000	None	4/14 (29%)	0.0281	1
No: 10	5/11	0.000	2	7/35 (20%)	0.0313	None
Total:	25/73 (34%)	0.0082	2	83/251 (33%)	0.0445	12

Table: 9.7. Genetic/congenital disorders in the 14 study families.

Family:	**Couples:**										
	All:			**With a genetic/congenital disorder:**				**Prospectively found at risk:**			
	Con:	**NC:**	**Total:**	**Con:**	**NC:**	**Total:**	**Condition:**	**Con:**	**NC:**	**Total:**	**Condition:**
Subject (index) families:											
No: 1	20	32	52	1	3	4	Thalassaemia	-	-		-
No: 2	15	6	21	1 2	- -	1 2	Thalassaemia Congenital deafness	1	-	1	Thalassaemia
No: 3	8	6	14	1	-	1	Thalassaemia	-	-	-	-
No: 4	6	6	12	1	-	1	Thalassaemia	-	-	-	-
N0: 5	8	11	19	2	-	2	Thalassaemia	-	-	-	-
No: 6	5	9	14	1	-	1	Thalassaemia	-	-	-	-
No: 8	8	20	28	-	-	-	-	-	-	-	-
No: 9	6	14	20	1	-	1	Sickle	-	-	-	-
No: 10	3	12	15	1	-	1	Sickle	-	1	1	Sickle
Subtotal:	79	116	195	11/79 13.9%	3/116 2.5%	14/195 7.2%	-	1	1	2	-
Control families:											
No: 11	13	9	22	1	-	1	Unknown causes of deaths during the first month of life.	-	-	-	
No: 12	-	11	11	-	-	-		-	-	-	-
No: 13	6	17	23	2	-	2	Unknown causes of deaths at the time of birth.	-	-		
No: 14	19	13	32	1	-	1	Multiple congenital malformations.	-	-		
No: 15	18	18	36	??	-	??	??	-	-		-
Subtotal:	56	68	124	4/56 7.1%	0/68 0%	4/124 3.2%	-	-	-		
Grand total:	135	184	319	15/135 11.1%	3/184 1.6%	18/319 5.6%	Various	2	-		-

Con: Consanguineous; NC: Non-consanguineous

Short tandem repeat (STR) analysis:

D21S11 allele frequencies:

Ten alleles were seen at the D21S11 locus (Fig 9.1a). The alleles were named according to the size in base pairs as determined by an automated fragment size analyzer. Sharma and Litt (1992) showed that the alleles at D21S11 locus are four base pair repeats. It was observed that, in addition to the four base pair repeats, alleles varying by two base pairs were also present. Similar observations have also been made by other workers (Kimpton et al, 1993; Urquhart et al, 1994).

Table: 9.8 shows the overall genotype frequencies in 132 unrelated individuals. There were 36 different genotypes as compared to the theoretically predicted 55. The observed number of homozygotes for all alleles was 26. The observed frequencies for the common alleles (Table: 9.8) in the descending order were 234 (0.193), 220 (0.174), 224 (0.159), 216 (0.152), and 230 (0.144).

Calculation of F by the STR allele frequencies:

The *F* value, calculated by parental consanguinity of the 132 subjects, was found to be 0.0257 (Table: 9.9). The values of *F* as calculated by the frequency of the D21S11 alleles, for which a homozygote was also observed, are presented in Table: 9.10. The values ranged from 0.0231 with the commonest allele (234) to 0.0313 for the less common allele (230). The value of *F* calculated from the uncommon allele frequencies were widely outside the acceptable range (0.0567 and 0.1285 for the allele 238 and 228 respectively). The average *F* calculated from the five common alleles was 0.0272.

D21S11 analysis in the two subject families:

The results of D21S11 allele frequencies in the last three generations of Families No: 1 and 2 are shown in Table: 9.11 and 9.12 respectively. A considerable fluctuation in the frequency of different alleles in various generations was observed. The frequency of allele 220 in Family No: 1, for example, had progressively gone down from 0.20 to 0.120 to 0.052 in the last generation. The allele 224 in the same family, on the other hand, had increased in frequency from 0.05 to 0.102 to 0.132 in the last generation. Similar fluctuations of the allele frequencies in various generations of Family No: 2 were also

observed. The allele 220 had increased from 0.125 to 0.149 to 0.214 in the three successive generations. The allele 224 showed a progressive decline in its frequency from 0.20 to 0.149 to 0.095. Similarly the allele 230 was reduced from 0.10 to a complete extinction in the last generation.

Table: 9.8. D21S11 observed genotype frequencies in 132 unrelated individuals.

212	-									
216	-	3 (0.0227)								
220	-	8 (0.0606)	5 (0.0378)							
224	2 (0.0151)	10 (0.0757)	6 (0.0454)	4 (0.0303)						
226	-	3 (0.0227)	-	1 (0.0075)	-					
228	-	1 (0.0075)	2 (0.0151)	2 (0.0151)	-	1 (0.0075)				
230	-	5 (0.0378)	8 (0.0606)	4 (0.0303)	1 (0.0075)	-	4 (0.0303)			
234	-	4 (0.0303)	7 (0.0530)	6 (0.0454)	1 (0.0075)	2 (0.0151)	10 (0.0757)	7 (0.0530)		
238	1 (0.0075)	3 (0.0227)	5 (0.0378)	2 (0.0151)	1 (0.0075)	1 (0.0075)	-	5 (0.0378)	2 (0.0151)	
242	-	-	-	1 (0.0075)	-	-	2 (0.0151)	2 (0.0151)	-	-
	212	**216**	**220**	**224**	**226**	**228**	**230**	**234**	**238**	**242**

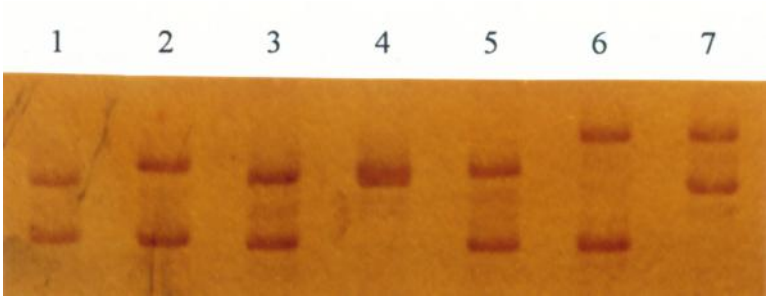

Fig: 9.1a . Results of the PCR amplification of the STR at D21S11 locus after electrophoresis on 6% denaturing polyacrylamide gel and silver staining. Lane 1 shows allele 216/226, lane 2: 216/228, lane 3: 216/226, lane 4: 226/226, lane 5: 216/228, lane 6: 216/234, and lane 7: 226/234. (Note: The same samples were initially run on ALF for allele sizing).

Table: 9.9. Coefficient of inbreeding of the 132 subjects calculated by the standard methods.

Consanguinity:	**Number:**	**Percent:**	**Coefficient of Inbreeding:**
1st Cousins:	45	34.1%	0.0212
1½ Cousins:	15	11.4%	0.0035
2nd Cousins:	9	6.8%	0.0010
Unrelated:	63	47.7%	0.0000
Total:	132	100%	0.0257

Table: 9.10. Calculation of *F* values from the D21S11 allele frequencies in a sample of 132 unrelated individuals.

Allele (n):	***p***	***q*=1-*p***	***H:***	**1-*H:***	***2pq:***	***F*=1-*H* /*2pq***
212 (3)	0.011	-	-	-	-	-
216 (40)	0.152	0.848	34 (0.2575)	0.7425	0.2578	0.0288
220 (46)	0.174	0.826	36 (0.2727)	0.7273	0.2874	0.0253
224 (42)	0.159	0.841	34 (0.2575)	0.7425	0.2674	0.0277
226 (7)	0.027	-	-	-	-	-
228 (10)	0.038	0.962	8 (0.0606)	0.9394	0.0731	0.1285
230 (38)	0.144	0.856	30 (0.2272)	0.7728	0.2465	0.0313
234 (51)	0.193	0.807	37 (0.2803)	0.7197	0.3115	0.0231
238 (22)	0.083	0.917	18 (0.1363)	0.8637	0.1522	0.0567
242 (5)	0.019	-	-	-	-	-
All (264)	1.00	-	-	-	-	-

p= allele frequency; *H*= observed number of heterozygotes

Table: 9.11. D21S11 allele frequencies in the successive generations of Family No: 1.

Alleles:	**1st generation: (*F*=0.0122)**	**2nd generation: (*F*=0.0153)**	**3rd generation: (*F*=0.0246)**
212	1 (0.05)	1 (0.009)	-
216	4 (0.20)	30 (0.278)	39 (0.257)
220	4 (0.20)	13 (0.120)	8 (0.052)
224	1 (0.05)	11 (0.102)	20 (0.132)
228	1 (0.05)	6 (0.056)	11 (0.072)
230	5 (0.25)	30 (0.278)	45 (0.296)
234	4 (0.20)	16 (0.148)	28 (0.184)
238	-	1 (0.009)	1 (0.007)
Total:	20 (1.00)	108 (1.00)	152 (1.00)

Table: 9.12. D21S11 allele frequencies in the successive generations of Family No: 2.

Alleles:	**1st generation: (*F*=0.0125)**	**2nd generation: (*F*=0.0360)**	**3rd generation: (*F*=0.0782)**
216	8 (0.20)	23 (0.245)	10 (0.238)
220	5 (0.125)	14 (0.149)	9 (0.214)
224	8 (0.20)	14 (0.149)	4 (0.095)
226	5 (0.125)	7 (0.075)	8 (0.190)
230	4 (0.10)	10 (0.106)	-
234	9 (0.225)	22 (0.255)	11 (0.117)
238	1 (0.025)	2 (0.021)	-
Total:	40 (1.00)	94 (1.00)	42 (1.00)

Discussion:

The origin of consanguineous marriage on the Indian Subcontinent is not clear. No one knows what the conventions of the original population of India were, but the lower castes and tribal populations may well have favoured consanguineous marriage (Reddy 1994). The population of North India, however, was originally exogamous as much of North India is today (Sinclair 1972). Islam came to the Subcontinent with the arrival of Arabs and Turks in the 7th and 8th century A.D (Wolpert 1977). The practice of consanguineous marriages amongst the Muslims might be related to adoption of Islam. As it was predominantly the lower castes who were attracted by the Islamic concept of human equality, some converts might already have favoured consanguineous marriage (Modell and Kuliev 1992).

Frequency and trends of consanguineous marriages:

Studies on consanguineous marriages in Pakistan show that up to 50% of the marriages are between close relatives and another 35% take place between Biradri relatives (Bittle 1994). In this study consanguineous marriage was as popular among urban as among the rural families. The practice varied between the individual families i.e. some favouring it more while others did not favour it at all.

Studies on consanguineous marriages are mostly based on a cross-sectional view of the population (Shami and Zahida 1980; Wahab and Ahmad 1996). The present study is unique because it provides an opportunity to study individual families and to retrospectively examine changes in the pattern of marriages with time. The information derived from almost all families clearly indicates that the frequency of consanguineous marriages has increased significantly in the youngest generation of couples as compared to their predecessors (Fig: 9.1b). The changes were similar in the subject (index) as well as control families. This is contrary to the possibility of finding greater numbers of consanguineous couples amongst index families because they formed a selected group with a recessive disorder.

A significant decline in the frequency of consanguineous marriages was observed in the recent generations of Western Europe, North America, and Japan (Coleman 1980; Lebel 1983; Imaizumi 1986). This is attributed to industrialization, greater population mobility, a decline in family size, and higher literacy rates. A similar change would also be expected in other populations. Consanguineous marriage still remains popular without any sign of decline in its frequency in a vast majority of the population of Middle East, Central Asia, North Africa, and the Indian Subcontinent (Bittles 1990). Modell and Kuliev (1992) have anticipated that the absolute number of consanguineous marriages may increase in future because of a high population growth-rate and a fall in the infant mortality leading to survival of greater number of children to reproductive age.

Darr and Modell (1988) noted an actual increase in the 1st cousin marriage in British Pakistanis and it was tentatively attributed to the effect of migration. Wahab and Ahmad (1996) also observed a similar change in Pakistan. The results of this study also suggest that consanguineous marriages have significantly increased in the recent past. The first choice for marriage in Pakistan is mostly confined to a close circle of near relatives or to a wider circle of kinsmen called Biradri. A marriage outside this circle is considered only when a suitable match is not available (Punjabi 1976). An increasing trend in consanguineous marriages observed in the recent generations of the study families, which in fact is a change from marriages within the Biradri to a closer circle of relatives, may well be due to decreased infant mortality and greater availability of cousins. If this is true then, at least in the immediate future, the trend will increase still further.

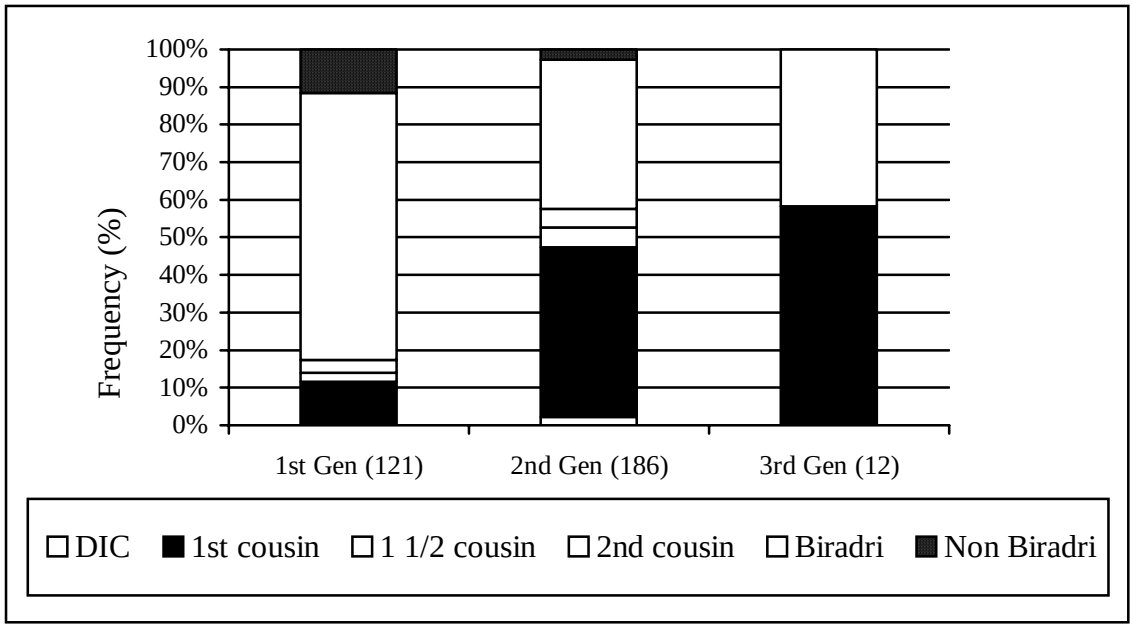

Fig: 9.1b. Pattern of marriages in the three generations of the study families.

No published data are available on the frequency of consanguineous marriages in the previous generations of Pakistan. Before the partition of the Subcontinent the population of present Pakistan was an admixture of Hindus and Muslims. Hindus of Northern India are mostly exogamous (Sinclair 1972) and consanguineous marriage amongst the Muslims of the present Northern India is around 33% (Basu 1975). Contemporary figures from the adjacent part of Punjab in Pakistan show about 50% consanguineous marriages (Shami and Zahida 1980). Compliance of marriage pattern with the local social pressures may cause variation in marriage preferences (Malhotra 1979). The marriage pattern amongst Muslims of Northern India may well have been influenced by a general lack of preference for a similar trend in the Hindu community. After the partition of the Subcontinent the Muslims in Pakistan have been relieved from the social pressure which the Muslims in the Northern India are still experiencing. It is likely that the frequency of consanguineous marriages amongst the Muslims of pre-partition India were not different from the Muslims of present Northern India. An increasing tendency of consanguineous marriages seen in this study may partly be due to a change in the social circumstances.

The changing pattern of marriages is also reflected in the coefficient of inbreeding for the last three generations of the study families. The average coefficient of inbreeding by generation showed a progressive rise with time (Table: 9.13). The pattern was similar in the subject as well as the control families. For example the value in the youngest generation was 0.0352, which is approximately 25% higher than the estimated 0.0280 for the Pakistani population (Bittles 1994). An extrapolation of the value of *F* when plotted against time (Fig: 9.2) shows that with the current pattern of consanguineous marriages the value of *F* by the year 2015 will be something like 0.0575.

Table: 9.13. Coefficient of inbreeding by generation in the study families. A similar pattern was observed in the subject as well as the control families.

Families:	**Coefficient of inbreeding (*F*):**		
	1st generation	**2nd generation**	**3rd generation**
Subjects:	0.0058	0.0141	0.0343
Controls:	0.0019	0.0105	0.0360
All:	0.0046	0.0128	0.0352

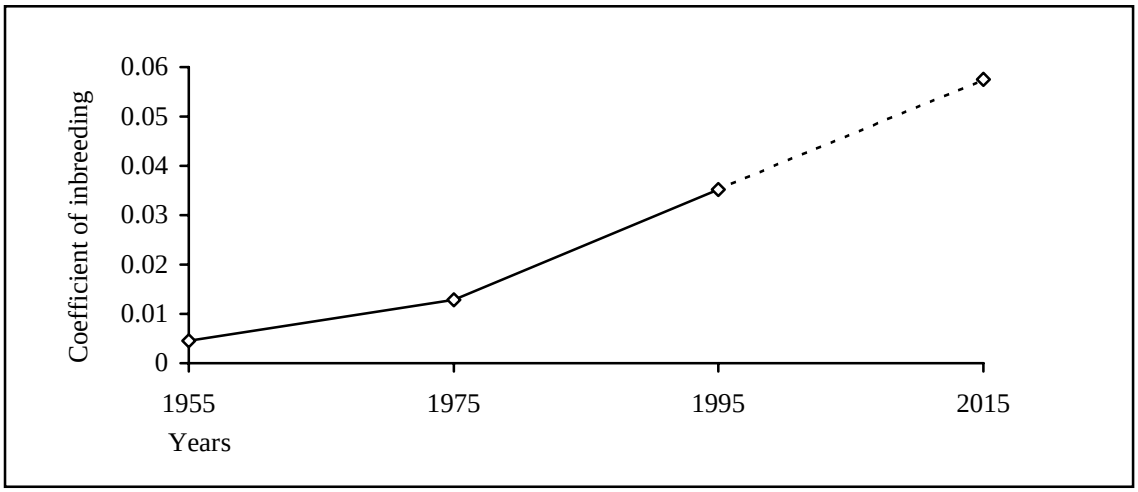

Fig: 9.2. Change in the coefficient of inbreeding over time. Extrapolation of the line to the year 2015 shows that with the current pattern of marriages the *F* may increase to 0.0575.

Antecedent consanguineous marriages and inbreeding estimates:

The results of this study clearly show that antecedent consanguineous marriages significantly affect the inbreeding estimates. For example the kinship coefficient for a 1st cousin marriage was found to be approximately 15% higher than the theoretically predicted (Table 9.14). Similarly values for the other consanguineous relations were also higher. The results also indicate that the increment in *F* due to antecedent consanguinity was similar for all degrees of relationship. This suggests that antecedent consanguinity may be more significant in increasing genetic risk for more distantly related couples. Normally consanguineous relations more than 2nd cousins are considered insignificant (Bittles 1994). But this may not be so in Pakistan where distant relatives or even Biradri relatives may be at a significant risk. The results of screening for thalassaemia in the families investigated showed that unrelated Biradri spouses of four carriers had the same thalassaemia mutation. But no carrier was married to another carrier from a different Biradri/Tribe.

A revised calculation of *F* in different ethnic groups of Pakistan based on the kinship coefficient calculated in this study shows that the predicted value in Punjabis are closer to the mean coefficient of inbreeding seen in the youngest generation of the study families (Table: 9.15).

Table: 9.14. Coefficient of kinship for a consanguineous relationship in the most recent generation of the study families.

Coefficient of kinship (Φ):				
	DIC	**1st cousin**	**1½ cousin**	**2nd cousin**
Expected:	0.1250	0.0625	0.0313	0.0156
Observed:	0.1348	0.0718	0.0330	0.0242
% increase:	8%	15%	5.5%	55%

DIC: Double first cousin

DNA based estimate of inbreeding in a randomly selected sample investigated in this study also showed that the estimates were generally higher than those calculated by the conventional methods. However, these were significantly lower than the estimates

obtained by pedigree analysis. It appears that pedigree analysis may be more suitable for inbreeding estimates. It was also observed that the values obtained for different alleles in the same sample varied considerably. For a more realistic picture it might have been appropriate to use a STR locus with fewer number of alleles or to investigate a larger sample size.

An important factor that is not taken into consideration when making inbreeding estimates is the practice of marriages within a circle of Biradri. Bittles (1994) has shown that up to 85% of the marriages in Pakistan take place within a Biradri circle. In this study an even stronger tendency (94%) of marriages within the Biradri or Tribe was observed. The term Biradri literally means “brotherhood”. It is a community of large-scale descendent group whose members are related either closely or distantly by blood or by marriage so that the members think of each other as kinsmen (Kolenda 1978). Family members of the spouses arrange a typical Pakistani marriage. The Biradri or Tribe members are given priority and a marriage outside this circle remains under criticism for the rest of the life of those contracting such a relationship (Punjabi 1976). The Biradri circle can be seen as a relatively isolated population. In one of the families investigated in this study it was observed that three out of four couples who had children affected by thalassaemia major were non- consanguineous but were from the same Biradri. Incidentally all of these couples had the same β-thalassaemia mutation i.e. IVSI-1, a mutation that is seen in 5% of the general population. These findings highlight the genetic consequences that a marriage within a Biradri circle can bring.

The usual methods of calculating inbreeding coefficients, even by drawing extensive pedigrees, would be unable to incorporate the contributions made by Biradri relationships. The use of a polymorphic DNA marker on a large number of unrelated Biradri members may be of help in solving this problem. In the two study families, in whom STR analysis was carried out, only 12 unrelated Biradri members in Family No: 1 and 4 in Family No: 2 were available for DNA analysis. This number was too small to draw conclusions about the inbreeding estimates.

Table: 9.15. Observed and predicted coefficient of inbreeding in the five major ethnic groups of Pakistan.

Ethnic Group (n):	**Proportion of couples and their contribution for the *F* based on the figures observed in this study:**						**Coefficient of inbreeding:**		
	DIC: (0.1348)	**1st Cousin: (0.0718)**	**1½ Cousin: (0.0330)**	**2nd Cousin: (0.0242)**	**Biradri Member:**	**Unrelated:**	**Observed:**	**Predicted:**	**Reference:**
Punjabi (9,520):	0.9% 0.00122	37.1% 0.0266	11.7% 0.00386	0.6% 0.00014	33.9% 0.000	15.8% 0.000	0.0280	0.0318	Bittles 1994
Pathan (2,037):	-	22.0% 0.0158	5.3% 0.00175	6.8% 0.00165	8.3% 0.000	57.6% 0.000	0.0164	0.0192	Wahab and Ahmad 1996
Sindhi (202):	5.0% 0.00674	55.4% 0.0398	5.9% 0.00185	6.9% 0.00167	21.3% 0.000	5.4% 0.000	0.0437	0.0501	Dr. Rafique Memmon Personal communication.
Baluchi (189):	-	84.1% 0.0604	-	2.6% 0.0006	? 0.000	13.2% 0.000	0.0532	0.0610	Dr. Jaleel Anwar personal communication.
Mohajir (120):	0.8% 0.00011	24.2% 0.0174	8.3% 0.00274	14.2% 0.00344	? 0.000	52.5% 0.000	0.0209	0.02095	Mr. Mohammad Iqbal personal communication

DIC: Double 1st cousin

Genetic effects of consanguineous marriage:

Thalassaemia, a recessive disorder whose carriers can be identified by simple blood tests, can be used as an example to investigate the factors that may determine the number of at risk couples for a recessive disorder in families where marriages are predominantly consanguineous. In this study two main factors were identified that correlate with the number of at risk couples. These include (1) the number of carriers in a generation and (2) the coefficient of kinship (Φ) for the generation. In most families at least 30% carriers were present in a generation where at risk couple was seen.

The situation in Family No: 8 was interesting because the thalassaemia gene was first introduced in this family by an unrelated Biradri member from the 1st generation (Fig: 9.10). In the 2nd and 3rd generations the number of carriers had increased but it was not enough to allow marriage between two carriers. If the current pattern of marriages continues and no efforts are made to avoid marriages between carriers, it is expected that over the next 2-3 generations a couple at risk might be seen. This suggests that in the presence of about 45% consanguineous marriages it may take 4-5 generations before a recessive gene can manifest itself. Spontaneous recessive mutations may also take a similar number of generations to manifest in such families.

Most of the at risk couples were present in the 2nd generation of the index families. In the same generation approximately 30% of the unmarried individuals were also carriers. If a thalassaemia carrier in Pakistan chooses to marry a close family relative then there is a theoretical possibility that in about 30% his or her spouse will also be a carrier as compared to only 5% if he or she marries an unrelated person. The observed risk of marrying a close cousin was slightly lower than expected which may be due to the small numbers investigated. It was observed that 25% of the carriers were married to a close cousin and in about 22% of the carriers married to a cousin the spouse was also a carrier. In contrast, none of the carriers was married to a carrier from another Biradri/Tribe. In 25% of the carriers married to a Biradri/Tribe member the spouses were also carriers. The actual risk of thalassaemia in a consanguineous marriage in Pakistan would be approximately four times higher than a completely unrelated marriage. The risk in a

consanguineous marriage for the rare recessives, that collectively may be more common than thalassaemia, would be even more marked (Modell and Kuliev 1992).

The relationship between kinship coefficient and the number of at risk couples in a generation for the common consanguineous relations is straightforward. It is more complicated for Biradri/Tribe members. Normally inbreeding estimates do not take into consideration the contribution made by a Biradri or a Tribe member. The data from this study suggest that, at least for a common recessive disorder, a marriage within the Biradri or a Tribe circle may also be at a higher risk.

DNA polymorphism data from the two study families indicate that genetic drift, due to a small number of the family members, may also contribute towards fluctuation in the carrier rate and the number of at risk couples. Fig: 9.3 shows how random drift can influence gene frequencies between generations. The allele frequency can vary from one generation to another and some alleles may change significantly to a higher or a lower level within the span of relatively few generations. A typical Pakistani family can be seen as a small population isolate. The size of this isolate is maintained and partitioned from the rest of the population due to frequent consanguineous marriages. Consanguineous marriage in it self does not affect gene frequencies (Modell and Kuliev 1992). However, it may act indirectly by maintaining the finite size of the population. In a small population group like this, drift may help to cause fluctuation in the frequency of a particular gene (Bodmer and Cavalli-Sforza 1976).

In the five control families, 4/56 (7%) of the consanguineous couples had deaths of children due to causes that were possibly genetic in origin. In contrast, none of the 68 non-consanguineous couples from these families had deaths of children due to genetic causes (p=0.03). However, there is only circumstantial evidence that the children of the consanguineous couples had a genetic disorder. The studies on association of consanguineous marriages and increased mortality carried out in less developed regions face a major problem in diagnosing cause of death and under such circumstances it is difficult to partition mortality into genetic and non genetic components with any confidence (Bittles 1994). Interaction between consanguinity and social variables can also complicate to a significant extent assessment of the genetic effects of consanguinity.

Consanguineous marriage is more likely to be favoured by the poorest and least educated families, whose children are at greatest risk of elevated rates of infant and childhood mortality from the effects of infections and nutritional deficiencies. Failure to control for socio-economic differentials can readily lead to biased estimates of the adverse effects of consanguinity (Bittles 1995). A recent study from Pakistan shows that the frequency of both consanguinity and birth defects were related with the socio-economic levels of the study groups, but there was no association between inbreeding and birth defects (Yaqoob et al, 1993). The apparent lack of association, when correction for other confounding factors is applied, may be due to elimination of the deleterious recessive genes over time from a community where consanguineous marriages are customary for several generations (Sanghvi 1966). The deleterious recessives, however, cannot be completely eliminated because of new mutations, reproductive compensation and heterozygote advantage (Bittles 1980). Studies on consanguinity and genetic disorders indicate that the average mortality in the progeny of a 1st cousin marriage is 4.4% higher than an unrelated marriage. The excess mortality is due to the presence of about 1.4 recessive genes per person that are lethal in the homozygous state (Bittles and Neel 1994). The results from this study support the later observations because the affected and the unaffected couples from the families, especially the controls, were living under identical socio-economic conditions that largely rules out the possibility of involvement of confounding variables.

The subject families were a selected group because of a history of recessive disorder in the family whereas the controls were randomly selected families. A comparison between the number of affected couples in the two groups showed that in the subjects there were 11/79 (14%) consanguineous couples who had children affected by a genetic disorder and in the controls there were 4/56 (7.1%) couples with affected children. The difference between the two groups was statistically insignificant (p=0.26).

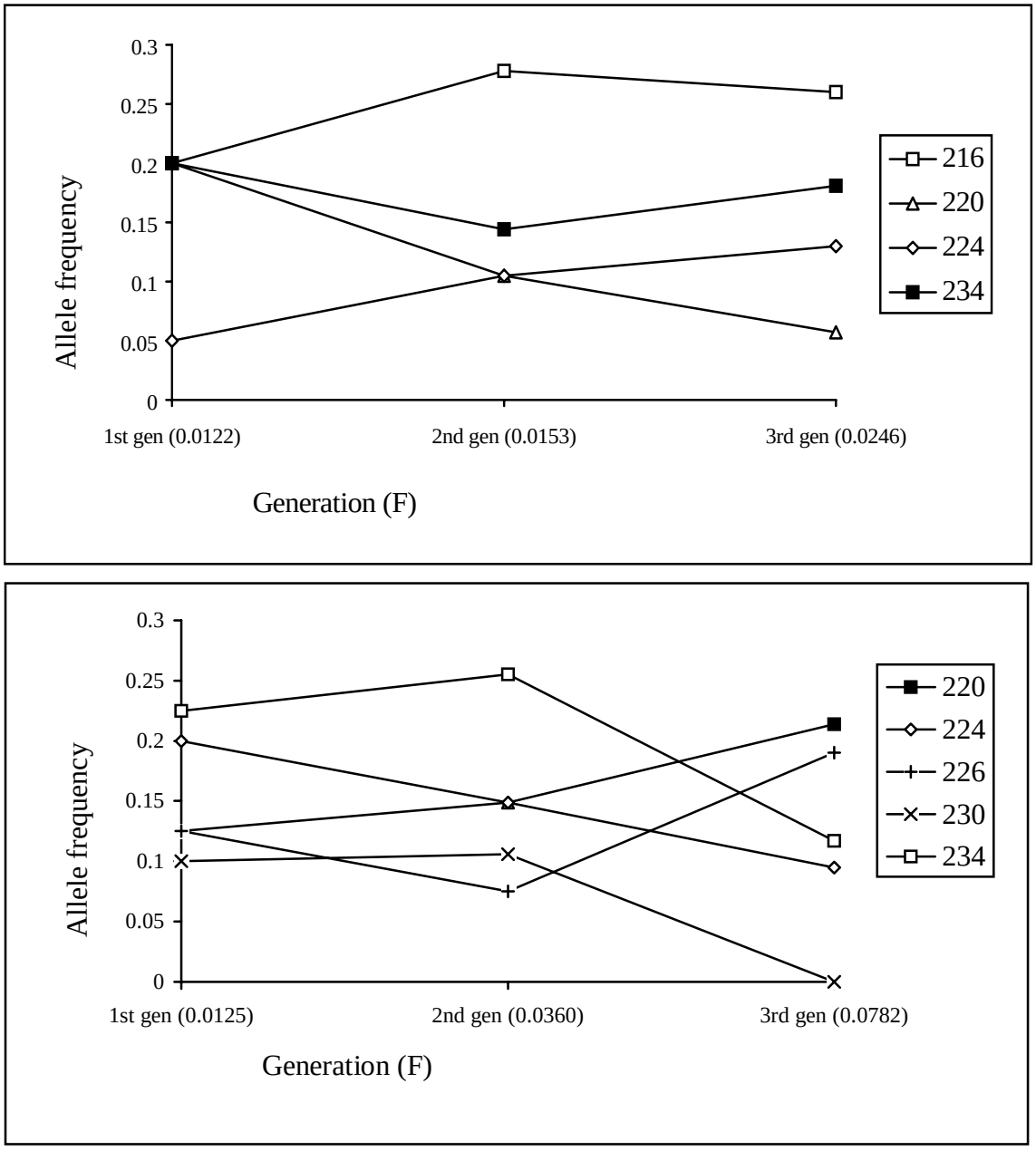

Fig: 9.3. Variation in D21S11 allele frequencies in the three generations of Family No: 1 (top) and Family No: 2 (bottom).