Prenatal diagnosis of α_1 antitrypsin deficiency and estimates of fetal risk for disease

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SUMMARY Alpha₁ antitrypsin deficiency is one of the most common metabolic disorders frequently associated with obstructive lung disease and occasionally with childhood liver cirrhosis. Prenatal diagnosis of this deficiency has been accomplished using a DNA polymorphism detected by the restriction enzyme *Ava*II. A unique haplotype of DNA fragments is observed in deficient (PI type ZZ) subjects. Diagnosis is therefore possible directly from fetal tissue, unlike other prenatal diagnoses using linkage of a DNA polymorphism within a specific family. This approach must be modified for rare deficiency alleles of α_1 antitrypsin (*PI**Mmalton, *PI**Mduarte, and *PI**QO or null). Knowledge of risk of severe disease in the fetus is important for the application of prenatal diagnosis. From the limited data available to date, the risk for a given PI ZZ fetus to develop severe liver disease has been estimated at 13% where a previous PI ZZ sib had no liver disease or liver disease which resolved during early childhood, and a risk of 40% where a previous PI ZZ sib had developed severe liver disease.

A deficiency of the plasma protease inhibitor, α_1 antitrypsin (AAT), occurs in about 1 in 7700 white North American people¹ and in some northern European populations it is as frequent as 1 in 2000 to 3000.² The deficiency is usually associated with the genetic type or PI (protease inhibitor) type ZZ, where the Z allele results in production of a variant AAT which is not secreted normally from the liver. Persons with this deficiency are almost certain to develop obstructive lung disease in early adult life if they smoke, but may have a near normal life span if smoking is avoided.^{3,4} Adults have an increased risk for developing liver disease.^{5,6} Based on a prospective study in Sweden,⁷ about 17% of subjects with the deficiency develop clinical signs of liver disease in infancy. In a portion of this group, liver disease progresses to cirrhosis and early death, as first described in 1969.⁸ Because of the fatal liver disease which has occurred in some families, prenatal diagnosis may be desired.

Prenatal diagnosis was first reported by PI typing of fetal blood samples obtained at fetoscopy.⁹ Because of the risk of about 5% for pregnancy loss associated with this procedure,¹⁰ molecular methods which can be carried out on cells obtained from

amniotic fluid or from chorionic villus biopsy offer advantages. Synthetic oligonucleotide probes recognise a single base pair mutation¹¹ and are theoretically a reliable approach when the mutation site has been identified. Specific probes for the normal type M and abnormal type Z proteins have been developed.¹² However, the use of synthetic probes is technically demanding because of the rigid control of hybridisation conditions which must be applied for the detection of a single mismatched base pair.

Using the restriction enzyme *Ava*II, we have identified two polymorphisms, one for each of two genomic probes in the AAT coding and flanking regions.¹³ With one of these probes, the polymorphism appears to show a pattern of DNA fragments unique to persons of PI type ZZ. Reliable prenatal diagnosis is therefore possible, as we have reported briefly.¹⁴ In a collaborative study, we have compared synthetic and genomic probes for prenatal diagnosis of α_1 antitrypsin deficiency,¹⁵ mostly using amniocytes. The more simple method using genomic probes may be preferred in diagnostic laboratories and furthermore can be used for rare deficiency alleles.

In this report, we provide details of the application of these polymorphisms to prenatal diagnosis of AAT deficiency, using the DNA haplotype obtained with *Ava*II. Unusual features of chorionic villus and

discussed. We also review data available to date from our own series and those of other authors to provide risk figures for genetic counselling for parents at risk of having a child of PI type ZZ. These risk figures are crucial for evaluating the appropriateness of prenatal diagnosis in specific families.

Patients and methods

DNA STUDIES

The analysis of DNA polymorphisms in this study was carried out on 25 parents and six sibs of 13 PI ZZ probands, 32 unrelated subjects of PI type ZZ, including the 13 from the above family units, and three subjects with rare types of AAT deficiency; PI types MduarteZ, MmaltonZ, and MnullZ, respectively. Families with a PI type ZZ child are representative of those in which prenatal diagnosis could be carried out. From each of these persons, venous blood samples were collected in EDTA. In family 13, the father was of type ZZ and was not included in the parental analysis. The 'sib' was a fetus for which prenatal testing was carried out on DNA obtained from a 30 mg sample of chorionic villus at 10 weeks' gestation. Data for controls were obtained as outlined previously.¹³

PI typing was carried out on serum or plasma by isoelectric focusing in acrylamide gels, using ampholines of pH 3–6, as described¹⁶ or using Pharmolyte pH 4.2–4.9 (Pharmacia Fine Chemicals). Sera or plasma from all those with AAT deficiency were typed by isoelectric focusing followed by immunofixation¹⁷ to identify rare deficiency variants if present.

Buffy coats were aspirated from EDTA whole blood, contaminating red cells were lysed, white cells were saline washed and lysed with SDS in EDTA containing buffer, and DNA was extracted with phenol chloroform (method of D Hoar, 1983, personal communication). Digestion with *Ava*II was carried out as recommended by the manufacturer on 3 μ g DNA. The restriction digest was electrophoresed on 0.8 to 1% agarose gel, transferred to Biodyne (Pall®) or more recently Hybond-N® (Amersham), and hybridised to ³²P labelled DNA probes as described.¹⁸ Two DNA genomic probes, provided by S L C Woo, were used: a 4.6 kb *Eco*RI genomic fragment, extending from the 5' flanking region into the first intron of the AAT gene, and a 6.5 kb *Bam*HI fragment extending from within exon II to the 3' flanking region of the AAT gene, both having been subcloned from α T35.¹⁹ Studies with the 4.6 kb probe have been described.¹³

FAMILY DATA FOR ASSESSMENT OF RISK

Families of probands with AAT deficiency have

been studied at The Hospital for Sick Children since 1971, mostly by Drs James Weber and Andrew Sass-Kortsak. During that time, 40 probands and their first degree relatives have been ascertained. PI typing was carried out on all probands, their parents, and sibs. Twelve sibs were found to be of PI type ZZ. Only those probands and PI ZZ sibs who have been followed to at least three years of age have been included. Studies on some of these probands have previously indicated that clinical assessment and tests of liver function by two to three years of age usually indicate whether the early evidence of liver abnormalities has resolved to normal, or whether liver deterioration and cirrhosis will result.²⁰ These early observations have been borne out by later follow up studies of these same patients at our hospital; these original patients have been followed for up to 10 to 15 years.²¹ A further more detailed follow up study has been initiated.

Published sibships in which there has been a proband affected with liver disease and one or more sibs have been reviewed. Only those series presenting a number of families have been reviewed, in an attempt to avoid families featured in case reports and selected because of the occurrence of multiple affected sibs. Only those studies in which the clinical status of all PI ZZ sibs has been given are included.

Results

STUDIES OF DNA POLYMORPHISMS

The 6.5 kb AAT probe is particularly useful for prenatal diagnosis. Patterns of DNA fragments in typical families, obtained by using the 6.5 kb AAT probe on *Ava*II digested DNA, are shown in fig 1B. DNA fragments observed range in size from 0.48 to 2.7 kb. Autoradiograph bands of fragments which show polymorphisms have been numbered from 1 to 7, as described,¹³ except that there is probably no polymorphic band, only a constant band, in position 6. Allele frequencies have been calculated from 47 non-Z PI types. In summary, there are three constant DNA fragments between polymorphic bands 1 and 2. Band 2 (not shown) is rare. Another less intense constant band lies in the same position as polymorphic band 2. Alternate alleles show presence or absence of band 1; the alternate allele, based on further studies, appears to be band 4. Genotype 14 is shown in fig 1B, lane 7. In normal non-Z haplotypes, the allele frequency for presence of band 1 is estimated at 0.70 and presence of band 4 is 0.30 for non-Z PI types. Bands 5 and 7 are alleles with frequencies of 0.70 and 0.30 respectively. A unique pattern of DNA fragments is observed in all 32 subjects of PI type ZZ, representing 64 chromosomes. This pattern is characterised by an absence

FIG 1 Autoradiograms of leucocyte DNA digested with *Ava*II and probed with the AAT 4.6 kb probe (A) and 6.5 kb probe (B and C). Sizes of λ *Hind*III marker fragments are indicated to the right of each set. Fragment numbers are indicated on the left. Anode is at the bottom. DNA haplotypes are as follows, PI types in parentheses: (A) 1, + -; 2 and 4, + +; 3, - -; (B) 5, 335714 (M1M1); 6, 337711 (ZZ); 7, 337714 (M1Z); 8, 337711 (ZZ); 9, 337714 (M1Z); (C) 10, 335711 (MmaltonZ); 11, 337711 (ZZ); 12, 335711 (MduarteZ). Arrow indicates trace fragment between fragments 4 and 5 observed rarely with PI ZZ DNA. DNA Biodyne transfer membrane was used.

of bands 4 and 5 and the presence of bands 1 and 7 (fig 1B, lanes 6 and 8). The haplotype, or combination of restriction sites, associated with the *PI**Z allele can be indicated as 371. Fragment 1 is placed at the end of the haplotype because we know from the sequence of the AAT gene that the band must lie in the 3' flanking region of the AAT gene. This pattern is not observed in 32 normal controls or 25 MZ parents of PI ZZ probands. The results using this probe for normal and PI ZZ AAT deficient subjects are summarised in table 1. Results for the 13 families of PI ZZ probands are shown in table 2, indicating that all PI ZZ children have the unique DNA haplotype with an *Ava*II digest and the 6.5 kb

probe. The fetus in family 13 is also predicted to be of PI type ZZ.

Rarely in DNA fragment patterns from PI ZZ

AvaII polymorphisms		Controls (n=32)	Parents PI MZ (n=25)	Probands PI ZZ (n=32)
Probe	DNA fragment pattern			
4.6	+	12 (37.5)†	18 (72.0)	32 (100)
6.5	3 7 1	0	0	32 (100)

*Homozygous for corresponding fragments.
†Percentage is given in parentheses.

TABLE 2 Extended haplotype (DNA haplotype* and PI type) in families of PI ZZ probands.

Family no	Father	Mother	Sib	Proband
1	+374M1+371Z	+374M1+371Z		+371Z+371Z
2	-351M2+371Z	+351S+371Z		+371Z+371Z
3	+374M1+371Z	-351M2+371Z	+371Z+371Z	+371Z+371Z
4	+351M2+371Z	+374M1+371Z		+371Z+371Z
5	+354M1+371Z	+351M1+371Z	+351M1+371Z	+371Z+371Z
6	+374M1+371Z	-351M1+371Z		+371Z+371Z
7	-351M2+371Z	+374M1+371Z	-351M2+371Z	+371Z+371Z
8	-351M1+371Z	+354M1+371Z		+371Z+371Z
9	-351M1+371Z	+351S+371Z		+371Z+371Z
10	-351M1+371Z	+374M1+371Z	+371Z+371Z	+371Z+371Z
11	+354M3+371Z	+351M1+371Z		+371Z+371Z
12	-351M3+371Z	+351M1+371Z	-351M3+371Z	+371Z+371Z
13†	+371M1+371Z	+351M1+371Z	+371Z+371Z	+371Z+371Z‡

*AvaII digest with 4.6 kb probe results indicated first (+ or -) followed by results with 6.5 kb probe.

†Family for which prenatal diagnosis was carried out.

‡PI type ZZ predicted in fetus; one band was reduced in intensity as is common in chorionic DNA, therefore presence of band 1 in both haplotypes has been inferred.

subjects, a weakly hybridising band is observed between bands 4 and 5 (fig 1B, lane 8). The marked difference in intensity (less than 10% intensity of band 4) and position between bands 4 and 5 makes this band readily identifiable, when present, in leucocyte or amniocyte DNA.

A difference in intensity of certain fragments is observed between genomic DNA from the chorionic villus and leucocyte or amniocyte DNA. This difference is noted in the results for family 13, as shown in fig 2. The chorionic villus DNA (lane 2) has a 'Z specific' band (between 4 and 5), which is almost equal in intensity to that of band 6. Furthermore, band 6 is widened on its leading edge, suggesting the presence of an additional fragment somewhat shorter than fragment 6. The adjacent sample (lane 1) has bands 4, 5, and 7 present and is an important control. In this family, the fetus can be only PI type MZ or ZZ. The fetus has clearly not inherited band 5 from the mother (lane 3). DNA from the father (lane 5) is present in excess and shows a visible 'Z specific' band. However, the proband (lane 6) shows only a trace of this band. At increased intensity, even the mother shows evidence of the 'Z specific' band (lane 8). This enhancement of the 'Z band' is consistently noted in chorionic villus, particularly with Hybond-N transfer membrane. Good separation of the fragments and use of an adjacent control DNA, having bands 4 and 5, is particularly important for chorionic DNA. In this family, the diagnosis in the fetus was further confirmed by the absence of a rare *TaqI* fragment²² segregating with the mother's M1 haplotype.

The unique DNA haplotype is associated specifically with the *PI**Z deficiency allele. Heterozygotes carrying one rare deficiency allele (*PI**Mmalton or *PI**Mduarte) are shown in fig 1C to differ by the

presence of a band 5. This fragment was also associated with the one *PI**QO (PI null) allele tested (not shown).

Because a unique fragment pattern occurs in PI ZZ subjects, this probe and *AvaII* digestion can be used alone for prenatal diagnosis. Although the PI ZZ DNA haplotype is unique, DNA from an affected PI ZZ sib, when available, and from parents is important to verify that the expected Z haplotype is present. For chorionic villus DNA particularly, a confirming polymorphism is useful. The same Biodyne blots of *AvaII* digests were reprobbed using the 5' 4.6 kb AAT probe, shown in fig 1A. This is one of several polymorphisms described in this DNA region.¹³ Briefly, one set of two fragments shows variability between subjects due to two alleles. In 32 normal controls (13 spouses plus 19 random normals)¹³ and 27 MZ first degree relatives including all parents in table 2, for whom only the fragment shown by family studies not to segregate with the Z allele was included, the frequency for presence of the restriction site (0.9 kb fragment, band 2) was 0.68 and for absence of site (1.0 kb fragment, band 1) 0.32. Among 32 normal unrelated subjects not carrying the PI Z allele (that is, PI type M or MS), the 0.9 kb fragment only was observed in 12 subjects, while this pattern was observed in all of 32 subjects of PI type ZZ, including those in table 1.

The extended haplotype for DNA digested with *AvaII* and probed with both 6.5 and 4.6 kb probes with the PI allele present is shown in table 2. In eight of the 13 families, the *AvaII* digest, reprobbed with the 4.6 kb probe, could identify a non-PI ZZ fetus (that is, either PI type MZ or MM) because of the absence of the restriction site in at least one chromosome of the fetus (+-) where the PI Z allele

FIG 2 Autoradiograms of DNA digested with *AvaII* and probed with the 6.5 kb probe. Band designations are as shown in fig 1B and C. All lanes contain leucocyte DNA, with the exception of lane 2. *AvaII* DNA haplotypes and PI types are as follows: 1, 374-351 (M1M1). 2, 371-371 (predicted ZZ, DNA from chorionic villus in family 13). 3, 351-371 (M1Z, mother in family 13). 4, 371-371 (ZZ). 5, 371-371 (ZZ, father in family 13, 6 µg DNA). 6, 371-371 (ZZ, proband in family 13). 7, 371-371 (ZZ). 8, as in lane 3, 6 µg DNA. 9, 351-371 (M1Z). All lanes have about 3 µg DNA unless specified. Arrow indicates 'Z specific' band. Hybrid-N transfer membrane was used.

is associated only with presence of the restriction site (table 2). This polymorphism will further confirm the diagnosis in these cases. However, a homozygous '+' result in these families would have an equal chance of being PI type MZ or ZZ. Specific DNA haplotypes are associated with specific PI types (D W Cox, unpublished data). PI typing with reliable M subtyping, in combination with the *AvaII* 6.5 results for both parents, can be helpful in predicting which of the other DNA polymorphisms we have described¹³ could be useful in a specific family for additional confirmation.

FAMILY STUDIES FOR ASSESSMENT OF RISK

Of 38 probands of PI type ZZ who have been patients at The Hospital for Sick Children between 1974 and 1984 and who are currently three years of age or more, five have died of their liver disease and two have died of unrelated defects. These other defects were severe combined immune deficiency (one patient) and congenital heart disease (one patient). At least eight patients in the series of Udall *et al*²³ also had additional diseases or abnormalities. This is probably because the deficiency will be picked up incidentally only in patients in hospital.

PI typing of all sibs of probands was carried out for the HSC series. In 10 families where parents are heterozygotes (PI MZ, SZ) there was more than one PI ZZ child. The clinical status of all PI ZZ children born in the sibships studied is indicated in table 3 on 1 January 1987. The first born PI ZZ child has been classified as 'normal' if there has been no evidence of liver disease at any time, 'resolved' if there was neonatal evidence of liver abnormalities with apparent resolution by two to three years of age and no present evidence of liver abnormality, or as having 'severe liver disease', that is, deteriorating liver function frequently with cirrhosis.

In addition to data from our own series, there have been four published reports which have described the clinical status of all PI ZZ children within the sibship. This includes two studies from the USA,^{8, 23} one study from Norway,²⁴ and one study from Great Britain.²⁵ From the latter study selected cases in their table 3 and case 47 were included. Families never coming to medical attention for liver symptoms were omitted. A group in which patients had 'persisting liver disease' was also omitted: in these cases, there was an increased concentration of serum AST (aspartate aminotransferase) as the only abnormality. However, there was no indication to what degree the levels of this enzyme were raised in the children involved. In our

TABLE 3 Status of all PI ZZ sibs of proband (born before and after proband).

No of families	Proband status*	Liver status in sib		Source and reference
		Normal or resolved	Severe	
3	S	5	0	Present data
7	N,R	7	0	Present data
2	S	1	1	USA ⁸
4	S	5	0	USA ²³
1	S	2	0	Norway ²⁴
8	S	3	5	Great Britain
7	N,R	5	2	Great Britain

*S=severe liver disease.

N=normal, no liver disease.

R=resolved liver disease; normal or slight rise in liver enzymes.

TABLE 4 Risk in PI ZZ sib after proband with resolved or no liver disease.

No of families	No of sibs (ZZ)	Clinical state of sib		Reference
		Severe liver disease	Normal	
7	7	2	5	25 Present data
8	8	0	8	
15	15	2 (13%)	13 (87%)	

TABLE 5 Risk in PI ZZ sib after proband with severe liver disease.

No of families	No of sibs (ZZ)	Clinical state of sib		Reference
		Severe liver disease	Normal*	
2	2	2	0	8
4	5	0	5	23
1	1	0	1	24
8	9	6	3	25
2	3	0	3	Present data
17	20	8 (40%)	12 (60%)	

*Includes resolved liver disease.

TABLE 6 A priori risk for heterozygous parents having a PI ZZ child with severe liver disease (mean estimates).

Previous normal or resolved liver disease	$(0.25 \times 0.13) = 3\%$
Previous severe liver disease	$(0.25 \times 0.40) = 10\%$

experience, a modest rise of serum glutamyl oxalo-transaminase (SGOT), about two or three times normal concentration, if it is the only abnormal liver function test, has been associated with resolved liver disease. However, a SGOT concentration greater than about three times the normal limit has been associated with subsequent liver deterioration.²⁰

The clinical status of children born after the first PI ZZ child in a sibship is given in tables 3 and 4, both for our HSC series and for those in published reports. Table 4 includes those children born after a child with normal or resolved liver disease and indicates an average risk of about 13% for having a PI ZZ child who develops severe liver disease. Table 5 includes those children born after a child with severe liver disease, and indicates an average risk of about 40% for a similarly affected child. Table 6 shows a priori risks for an affected child before prenatal test results are considered.

Discussion

Using the restriction enzyme *Ava*II and a genomic probe for the coding region and 3' flanking region of

the AAT gene, we have shown the presence of a unique series of restriction sites, or haplotype, found only with the *PI*Z* deficiency allele to date. This unique haplotype should allow reliable prenatal diagnosis. Although this unique haplotype has not been found with non-*PI*Z* alleles to date, the possibility exists that when larger numbers of subjects are studied, this haplotype may occur with non-*Z* alleles and, conversely, that other haplotypes may be associated with the *Z* allele. Therefore, parents or an affected sib must always be tested. If a PI MZ parent is found to have the *Z* haplotype with both *M* and *Z* alleles, or if a different haplotype is found with the *Z* allele, prenatal diagnosis using *Ava*II and the 6.5 probe would not be reliable. However, extending the DNA haplotype by using the 4.6 kb probe on the *Ava*II digested DNA, and other DNA polymorphisms we have described,¹³ might prove useful in distinguishing the *M* from the *Z* allele in parent and fetus. In about half of the families we have tested, use of the 4.6 kb probe on *Ava*II digested DNA could discriminate a non-ZZ fetus.

This type of prenatal diagnosis with the observation of a specific *Z* DNA haplotype is appropriate only where the affected fetus is PI ZZ. We have observed a different *Ava*II 6.5 haplotype with the PI deficiency alleles *Mmalton*, *Mduarte*, and *null* (*QO*), each of which is about one hundred times less frequent than the *Z* allele.²⁶ When these rare deficiency alleles are present, identification of an AAT deficient fetus should be possible using the usual approach of identifying the PI/DNA haplotypes of parents and affected proband and grandparents where necessary. The specific mutations in other rare deficiency alleles have not been identified, so diagnosis using synthetic probes would not be possible. It should be pointed out that risk for liver disease has not been established for rare alleles.

Using genomic probes as in the present study, 2 to 3 μ g DNA is adequate, allowing the direct use of DNA from uncultured chorionic villus samples. When chorionic villus is cultured, there is a risk of contamination by maternal cells, which may lead to an erroneous diagnosis. When the prenatal diagnosis is carried out by amniocentesis, a single assay might be possible directly on uncultured amniocytes, although cultured amniocytes are more frequently used. Care must be taken with chorionic villus DNA to have excellent separation of fragments in the 0.6 kb region and to use appropriate controls. We prefer to confirm with the 4.6 kb probe or another enzyme.

For appropriate genetic counselling and use of prenatal diagnosis, accurate risk figures are impor-

tant. Because of the small number of follow up studies carried out to date on AAT deficient persons and their sibs, little information is available. However, we have attempted to summarise our own data and relevant published reports. While those of PI type ZZ have an increased risk for the development of obstructive lung disease in adult life, such lung disease can be considerably delayed by the avoidance of smoking.^{3,4} Treatment with injected AAT is being attempted and there is a rationale for providing AAT to prevent elastase destruction in the lung.²⁷ The cause of the liver disease in childhood is unknown and liver transplant is the only known therapy. It is therefore probable that prenatal diagnosis will be considered only because of the risk for severe childhood liver disease. There is suggestive evidence from the reports summarised that the risk for having a child with severe liver disease may be increased if a previous PI ZZ child has severe liver disease (40%) compared with the risk when the previous child of PI type ZZ does not have liver disease (13%). However, the reported results vary considerably. For example, the greatest risk of recurrence of severe liver disease has been reported in studies from Great Britain, where at least 67% (six out of nine, excluding 'persisting liver disease') of subjects have been reported to be affected after the birth of a PI ZZ child who develops severe liver disease. At present, there are inadequate data to determine the precise difference in risk for sibs born after a child with severe liver disease in comparison with a birth after a child with normal or resolved liver disease. Furthermore, the differences in apparent risk figures between reports may be due to biases of ascertainment for various countries, or could reflect genetic or environmental influences which vary between geographical locations.

The overall risk for heterozygotes to have a child with severe liver disease can be compared with the risk of 7% for any PI ZZ child to develop severe liver disease. These figures were obtained from a Swedish study in which the PI ZZ children ascertained at birth⁷ have been followed to four years of age. Of the 120 children ascertained, three had developed cirrhosis (two living and one dead at the time of the report), one had hepatomegaly, and three had an increase of greater than three times the normal of their liver enzymes.²⁸ More data will be needed to determine if the 13% risk after a child with resolved or no liver disease is really different from the 7% risk for any PI ZZ child of developing severe liver disease.

AAT deficiency has now been recognised for more than 15 years. Follow up studies of children with liver disease, and of their sibs, is now possible

over a considerable period of time. When such follow up studies are carried out, more precise risk figures should be available. In the meantime, the figures reported here should help parents to make informed decisions.

Note added in proof

A polymorphism in *MaeIII* is useful for prenatal diagnosis (*Lancet* 1986;ii:741-2). This will be particularly helpful for confirmation with chorionic villus DNA. We have successfully used a combination of the two enzymes for three recent prenatal diagnoses.

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