Journal of Medical Genetics (1970). **7**, 367.

Protease Inhibitor (Pi) Phenotypes in Chromosome Aberrations

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Many enzymes and several enzyme inhibitors are found in multiple molecular forms in different human populations. Some of them constitute important polymorphic systems useful for populations genetics, forensic medicine, and chromosome mapping. However, little is known about the distribution of these genetic markers in patients with chromosome aberrations and their parents. The purpose of the present report is to describe the distribution of Pi types in some families of this type.

The Pi system (Fagerhol and Laurell, 1967) comprises the inherited variants of serum α_1-antitrypsin. Some of these variants are associated with chronic obstructive pulmonary disease (Laurell and Eriksson, 1963; Fagerhol and Hauge, 1969) and cirrhosis of the liver in children (Sharp *et al.*, 1969). The pathogenesis of these conditions is poorly understood. Despite the name of this inhibitor, trypsin is probably not the most important enzyme to be inhibited *in vivo*. It is more likely that α_1-antitrypsin is needed to inhibit intracellular enzymes which may be liberated by tissue damage or inflammation.

Proteolytic enzymes are probably active during cell divisions and fertilization. Increased enzyme activity, caused by lack of normal inhibitors, may disturb these events.

Subjects and Methods

The propositi are 32 children with chromosome aberrations examined at the Children's Hospital in Bergen. Serum samples were also taken from all available parents. The sera were stored below $-20°$ C. until tested.

Chromosomal analyses were done by a microtechnique for culturing leucocytes from whole capillary blood. In the XX/XY mosaic cases the mosaic condition was confirmed in bone-marrow cultures.

Pi typing was performed by acid starch gel electrophoresis (Fagerhol, 1968).

Up to now 17 different Pi phenotypes have been observed. A schematic drawing of them is presented in the Figure.

Received 30 January 1970.

Results

The distribution of Pi phenotypes is given in the Table, together with the types of chromosome aberrations and the Pi phenotype frequencies in healthy Norwegians (Fagerhol, 1967).

An exceptional distribution of Pi types was found only in the group of families where sex chromosome mosaic had been observed. About 90% of healthy Norwegians have Pi phenotype MM, while other types (FM, MS, and SS) were found in the patient

TABLE

Pi PHENOTYPES IN FAMILIES WHERE CHROMOSOME ABERRATIONS WERE FOUND

Family No.	Pi Phenotypes			Type of Aberration
	Father	Mother	Propositus	
1	MM	MM	MM	47,XX,21+
2		MS	MS	47,XY,21+
3			MM	47,XY,21+
4	MM	MM	MM	47,XY,21+
5	FM	MM	MM	47,XY,21+
6	MM	MM	MM	47,XY,21+
7	MM	MM	MM	47,XY,21+
8	FM	MM	MM	47,XX,21+
9		MS	MS	47,XY,21+
10			MM	47,XX,21+
11	MM	MM	MM	47,XX,21+
12	MM	MM	MM	47,XX,21+
13		MM	MM	47,XY,21+
14	MM	MM	MM	46,XX,D−,t(DqGq)+
15	MM	MM	MM	46,XX,D−,t(DqGq)+
16	MM	MM	MM	46,XY,18−,t(3?18p)+
17	MM	MM	MM	46,XY,1?−
18	MM	MM	MM	46,XY/47,XY,D+
19	MM	MM	MM	45,X
20	MM	MM	MM	45,X
21	MM	MM	MM	45,X
22	MM	MM	MM	45,X
23	MM	FM	MM	45,X
24			MM	45,X
25		MZ	MZ	45,X
26	MM	MS	MS	45,X/46,XX
27	MM	FM	FM	45,X/46,XY
28		MM	MM	45,X/46,XY
29	MM	MS	MM	45,X/46,XY/46,XX/47,XXY
30	MM	MM	MM	46,XY/46,XX
31	FM	MM	FM	46,XY/46,XX
32	MM	SS	MS	46,XY/46,XX

Pi phenotype frequencies in healthy Norwegians (Fagerhol, 1967): MM: 0·8955, MS: 0·0435, MZ: 0·0297, FM: 0·0252, SS: 0·0005.

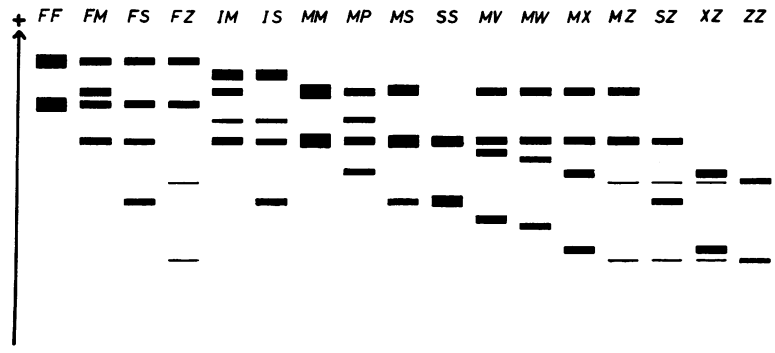

FIG. Schematic drawing of the 17 Pi phenotypes described up to now. On acid starch gel electrophoresis the allele product is a pattern of two major and six minor zones. Only the two major zones in each allele product have been included in this drawing.

and/or the parents in 5 out of 7 families in this group (assuming that the father in family No. 28 has Pi type MM). When the distribution of Pi types in the parents is compared with the expected numbers calculated from the gene frequencies in healthy Norwegians a value of $\chi^2_{(1)} = 9{\cdot}15$, $0{\cdot}001 < p < 0{\cdot}005$ is obtained.

Discussion

α_1-Antitrypsin is a potent protease inhibitor which can inhibit several different enzymes (trypsin, chymotrypsin, plasmin, thrombin, elastase, and leucocyte proteases). Its concentration in normal serum is about 200 mg./100 ml., but a two- to fourfold increase is seen in response to tissue damage or inflammation, during pregnancy, or use of oral contraceptives.

Three Pi phenotypes, FM, MS, and SS, were found in unexpectedly high numbers in families where sex chromosome mosaics occurred. The FM subjects are heterozygous for a fast moving α_1-antitrypsin, while the MS and SS individuals are heterozygous, respectively homozygous, for a slow α_1-antitrypsin. The Pi^S gene results in about 65% of the α_1-antitrypsin concentration in serum as compared with Pi^M. The SS phenotype seems to be associated with pulmonary disease (Fagerhol and Hauge, 1969).

The trypsin inhibitory capacity and the concentration of α_1-antitrypsin (by immunochemical methods) (Fagerhol, 1969) in sera from subjects with Pi types FM and MS fall within the normal range, though they are on the average somewhat lower in MS sera. However, these tests may not be very informative, since trypsin is probably not the most important enzyme to be inhibited and since the immunochemical measurements may not reflect the functional capacity of the proteins. The F and S variants of α_1-antitrypsin may be functionally deficient, or even compete with the normal molecules for the binding site on the enzyme without affecting its proteolytic activity. Most of the variants in the Pi system are probably due to single amino acid substitutions. Up to now nine Pi alleles have been described. Therefore the present MM type must include many variants where amino acids have been exchanged without alteration of the net charge on the molecule. Some of these variants may even be characterized by abnormal reactivity with enzymes.

An abnormal number of chromosomes is usually the result of non-disjunction during the meiotic divisions of gametogenesis. Because the incidence of the autosomal trisomy condition (G_{21}-trisomy, D-trisomy, and E-trisomy) increases with maternal age (Penrose and Smith, 1966; Lenz, Pfeiffer, and Tünte, 1966; Magenis, Hecht, and Milham, 1968), it is likely that the occurrence of non-disjunction in these disorders is more frequent in oogenesis than in spermatogenesis, and that the mechanism leading to non-disjunction is related to ageing of the ovum. In contrast to these autosomal trisomy conditions, 45,X Turner's syndrome is not related to advanced maternal age, and non-disjunction has more often been traced to the paternal than maternal X chromosome (Lindsten *et al.*, 1963; Court Brown, Law, and Smith, 1969).

Mosaicism generally arises from errors in the early mitotic divisions after fertilization and might be explained as result of non-disjunction or anaphase lag. Anaphase lag refers to the observation that the sex chromosomes tend to be among the last chromosomes to reach the poles of the mitotic spindle during anaphase movement. Occasionally, one

chromosome might lag so far behind that the cell plate closes between the two poles before it can reach its destination. The result is that the chromosome may be included in the wrong daughter cell or lost. Mosaics may arise from either normal or abnormal zygotes. Some autosomal mosaics and some of the more complicated sex chromosome mosaics probably originate from zygotes which are primarily abnormal because of meiotic non-disjunction, and these mosaic conditions may be related to advanced maternal age.

45,X/46,XX and 45,X/46,XY mosaicisms are best explained by anaphase lag and loss of an X or Y chromosome during an early mitotic division of a normal XX or XY zygote, respectively. The occurrence of these types of mosaicism is not dependent upon maternal age (Court Brown *et al.*, 1969). The most likely explanation for the origin of a 46,XX/46,XY mosaic is dispermic fertilization of an ovum and its polar body, or two ova by an X-bearing and a Y-bearing spermatozoon (Ford, 1969). So far, only a few patients with 46,XX/46,XY mosaicism have been reported, and dispermic fertilization is probably a very rare event in humans.

It appears that a single general aetiology is not sufficient to account for the various chromosome aberrations. Apart from the predisposing effect of advanced maternal age, the aetiology remains obscure.

The finding of the more rare Pi types in two of the three families with an 46,XX/46,XY propositus is noteworthy, since it has been demonstrated that proteolytic enzymes are involved in the fertilization process (Lundblad, 1954). Experimental studies with sea urchin eggs have shown that both trypsin and trypsin inhibitor may interfere with several of the mechanisms preventing the egg from being fertilized by more than one spermatozoon, the so-called blocking mechanism to polyspermy. Pretreatment of the unfertilized egg with relatively high concentrations of trypsin inhibits the formation of the fertilization membrane and increases the tendency towards polyspermy (Hagstrøm, 1961; Lönning, 1967). Moreover, it has been shown that the cortical reaction following insemination is both delayed and inhibited in the presence of trypsin inhibitor, resulting in the formation of an abnormal incomplete fertilization membrane and increased susceptibility to polyspermy (Hagstrøm, 1957; Lönning, 1967). Proteolytic enzymes may also be involved in the phenomenon of anaphase lag, since it has been shown that the first cell divisions after fertilization are considerably accelerated by trypsin (Hagstrøm and Lønning, 1963). Anaphase lag has a relation to the 45,X/46,XY/46,XX/47,XXY, 45,X/46,XX and 45,X/46,XY cases, and it will be noted that the rarer forms of Pi types were found in the propositi and/or mother in three of the four families in this group.

We are fully aware that the limited data in this report do not allow definite conclusions. However, we feel that the observations are so exceptional and their possible implications of such importance that similar studies should be extended to a larger number of patients than that available to us.

Summary

Pi phenotypes (inherited variants of serum α_1-antitrypsin) were examined in 32 Norwegian families where chromosome aberrations had been found. Pi phenotypes other than the most common MM type were found in the patient and/or the parents in 5 out of 7 families with sex chromosome mosaics. This differs significantly from the distribution of Pi types in healthy Norwegians.

The results are discussed in relation to possible mechanisms involved in the origin of chromosome aberrations and to observations on the fertilization and development of sea urchin eggs treated with trypsin and trypsin inhibitors.

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