

Increased Frequency of the MZ Phenotype of Alpha-1-Protease Inhibitor in Juvenile Chronic Polyarthritis

PHILIPPE ARNAUD, ROBERT M. GALBRAITH, and W. PAGE FAULK, *Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina, Charleston, South Carolina 29401*

BARBARA M. ANSELL, *Clinical Research Centre, Division of Rheumatology, Harrow, Middlesex, England*

ABSTRACT Pi phenotypes of alpha-1-protease inhibitor were determined by isoelectric focusing and print immunofixation in 96 English children suffering from juvenile chronic polyarthritis. A significantly increased frequency of the MZ phenotype (10.41%) was found in comparison with a geographically matched control population (1.6%). The results of this study suggest that the possession of a Z-deficient allele of alpha-1-protease inhibitor could be a predisposing, aggravating, or perpetuating factor in the articular damage occurring in juvenile chronic polyarthritis.

INTRODUCTION

The breakdown of articular collagen is an important feature of arthritic joint damage (1), and this in part appears to be due to an increased activity of synovial collagenases (2, 3). Alpha-1-protease inhibitor (A1Pi,¹ formerly alpha-1-antitrypsin) is known to inhibit a variety of proteases, including human granulocyte collagenase (4) and elastase (5), and it is possible that deficient activity of this protease inhibitor could be a predisposing, aggravating, or perpetuating factor in the pathophysiology of joint damage. The A1Pi

system is polymorphic, and its various phenotypes (Pi types) are determined by two autosomal codominant genes in each individual (6). A certain number of the 25 alleles described to date are associated with decreased serum concentrations of A1Pi. Perhaps the most important of these is the Z allele, the possession of which is associated with A1Pi serum concentrations of only 20% of normal as well as with several clinical disorders such as pulmonary emphysema (7) and liver diseases (8). An increased frequency of Z phenotypes has been reported recently in adults with rheumatoid arthritis (9). We have investigated the hypothesis that deficient alleles may also be more common in children with juvenile chronic polyarthritis (JCP).

METHODS

We studied 96 English Caucasian patients with JCP referred to the British Medical Research Council's Rheumatism Unit at Taplow, England. The criteria for the diagnosis were as defined by Ansell (10): (a) onset before age 16; (b) involvement of four or more joints for at least 3 mo with pain and/or swelling and/or limitation of movements; (c) if fewer joints are involved, histology showing changes compatible with rheumatoid arthritis; and (d) exclusion of other disorders in the 1st yr of disease, including ankylosing spondylitis, psoriasis, ulcerative colitis, and systemic lupus erythematosus. At the time of the blood sample, the mean age of these 96 patients was 5.4 yr (9 mo–15 yr) and the male:female ratio was 2:7 (26 male, 70 female). 20 had presented with a systemic illness going on to polyarthritis, chronic iridocyclitis was present in 35, and amyloidosis in 20. A small number of cases had more than one of these features; i.e., two with chronic iridocyclitis also had amyloidosis. In 58 cases we also examined sera from the patient's father and mother, and parentage in each case was confirmed by Gm, Am, Inv, and HLA typing.

Sera were obtained following informed consent and stored at -20°C until tested. A1Pi phenotypes were determined using analytical thin-layer isoelectric focusing as previously

A preliminary report of this investigation was presented at the Southern Section of the American Federation for Clinical Research, New Orleans, La., 27 January 1977, and has appeared in abstract form (1977. *Clin. Res.* 25: 23A.). This is publication 150 from the Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina.

Dr. Galbraith was the recipient of a Fogarty International Research Fellowship, FO 5 TWO 2309-02.

Received for publication 6 June 1977 and in revised form 11 August 1977.

¹Abbreviations used in this paper: A1Pi, alpha-1-protease inhibitor; JCP, juvenile chronic polyarthritis.

TABLE I
Comparison of A1Pi Distribution in JCP and in Control Population

Subjects	no.	M*	MS	MZ	FM	Others
JCP	96	73.95	14.58	10.41	1.06	—
Controls†	4,565	88.5	8.0	1.6	0.7	1.2

* M variants are listed as M.

† Reference 15.

described (11). Modifications of this technique (12) allowed the differentiation of M phenotypes from the recently described M cathodal (Mc) phenotype (13). All results were confirmed by print immunofixation (14). For statistical analysis, chi-square with continuity correction or Fisher's exact test was used as appropriate.

RESULTS

The distribution of A1Pi phenotypes in JCP (Table I) was compared with that found in controls from the same geographical region (15). The frequency of the MZ phenotype was significantly higher in the study group than in controls ($P < 0.001$). The frequency of the MS phenotype was also elevated, although this difference was not statistically significant. The distribution of the other phenotypes did not differ appreciably from control values.

In 58 cases the parental phenotypes of A1Pi were also studied. In the case of S and Mc alleles, inheritance in random fashion equally from mother and father was observed (Table II), supporting the concept of autosomal codominant transmission (6). In contrast, the Z allele appeared to be transmitted to the patient from the father in all but one case (Table II).

DISCUSSION

The striking finding in this study is the increased prevalence of Z phenotypes in JCP. This is an important observation, since A1Pi is known to inhibit the activity of collagenases and neutral proteases, which are found in increased amounts in arthritic joints (2, 16), and levels of A1Pi in synovial fluids appear to be elevated in parallel (17). In patients with deficient phenotypes, the serum level of A1Pi is reduced, and since this level parallels the amount found in synovial

TABLE II
Transmission of the Variant Alleles

Alleles	Patients	Father	Mother
S	9	5	4
Mc	13	6	7
Z	7	6	1

fluid (17), the latter would be expected to be markedly reduced. Since the mechanism of inhibition of proteolytic activity involves formation of complexes between A1Pi and proteases (18–20), low levels of A1Pi could result in increased amounts of protease remaining unbound and active. Furthermore, we have shown that such unbound proteases can act upon formed complexes causing denaturation of A1Pi. This leads to dissociation of the complexes and the release of the previously inactivated protease in a form which is biologically active.² The increase in S allele frequency, although not significant, may have similar significance since this allele is also associated with decreased amounts (20%) of serum A1Pi (21).

The clinical relevance of the increase in Pi-deficient phenotypes is not clear. No differences in distribution of Pi types were apparent when patients were subdivided with respect to clinical complications such as amyloidosis, systemic involvement, and iridocyclitis, although the frequency of S phenotypes was high in patients who had developed amyloid. Similarly, an influence of deficient phenotypes on the age of disease onset was not evident in our study, and we did not find any significant association between Pi alleles and other genetic markers (Gm, Am, Inv, HLA).

Following the previous report by Cox and Huber (9) of adult patients with rheumatoid arthritis, JCP is now the second rheumatic disorder in which an elevated frequency of deficient phenotypes of A1Pi has been reported. Clearly, investigation of other disease groups may reveal additional examples, and it would also be important to investigate persons with deficient phenotypes of A1Pi to determine the frequency of rheumatic disorders. Determination of Pi types may prove in the future to be useful in genetic counseling, as well as in the diagnosis, management, and prognosis of patients with rheumatic disorders, and may provide further information concerning the pathogenesis and transmission of these diseases.

ACKNOWLEDGMENTS

This research was supported in part by a grant from Fondation de l'Industrie Pharmaceutique pour la Recherche, Paris, France, and by U. S. Public Health Service grant HD-09938.

REFERENCES

1. Beard, H. K., W. P. Faulk, L. B. Conochie, and L. E. Glynn. 1977. Some immunological aspects of collagen. *Prog. Allergy*. 22: 45–106.
2. Lazarus, G. S., J. L. Decker, C. H. Oliver, J. R. Daniels, C. V. Multz, and H. M. Fullmer. 1968. Collagenolytic activity of synovium in rheumatoid arthritis. *N. Engl. J. Med.* 279: 914–919.

² Arnaud, P., R. C. Allen, and H. H. Fudenberg. Manuscript in preparation.

3. Baver, E. A., A. Z. Eisen, and J. J. Jeffrey. 1971. Studies on purified rheumatoid synovial collagenase *in vitro* and *in vivo*. *J. Clin. Invest.* **50**: 2056-2064.
4. Ohlsson, K., and I. Olsson. 1977. Neutral proteases of human granulocytes. IV. Interaction between human granulocyte collagenase and plasma protease inhibitors. *J. Lab. Clin. Med.* **89**: 269-277.
5. Ohlsson, K., and I. Olsson. 1976. Neutral proteases of human granulocytes. III. Interactions between human granulocyte elastase and plasma protease inhibitors. *Scand. J. Clin. Lab. Invest.* **34**: 349-355.
6. Fagerhol, M. K., and T. Gedde-Dahl, Jr. 1969. Genetics of the Pi serum types. *Hum. Hered.* **19**: 354-359.
7. Eriksson, S. 1965. Studies in alpha-1-antitrypsin deficiency. *Acta Med. Scand.* **177**(Suppl. 432): 1-85.
8. Feldman, G., J. Bignon, and P. Chahinian. 1974. The liver in alpha-1-antitrypsin deficiency. *Digestion.* **10**: 162-174.
9. Cox, D. W., and O. Huber. 1976. Rheumatoid arthritis and alpha-1-antitrypsin. *Lancet.* **I**: 1216-1217.
10. Ansell, B. M. 1976. Differing patterns in juvenile chronic polyarthritis. *Mod. Top. Immunol.* **8**: 65-72.
11. Arnaud, P., C. Chapuis-Cellier, and R. Creyssel. 1975. The Pi system: Its study by means of thin-layer electrofocusing in polyacrylamide gels. In *Protides of Biological Fluids*. H. Peeters, editor. Pergamon Press, Inc., Elmsford, N. Y. 515-520.
12. Arnaud, P., C. Chapuis-Cellier, G. B. Wilson, J. Koistinen, R. C. Allen, and H. H. Fudenberg. 1977. Polyacrylamide gel isoelectric focusing (PAGIF) applied to alpha-1-protease inhibitor (A1Pi) phenotyping. II. Immunochemical procedures. In *Electrofocusing and Isotachopheresis*. B. J. Radola and D. Graesslin, editors. Walter De Gruyter & Co., Berlin. 265-272.
13. Arnaud, P., C. Chapuis-Cellier, R. Creyssel, and H. H. Fudenberg. 1977. Pi M Cathodal (Mc): A new allele of serum alpha-1-antitrypsin (AAT). *Clin. Res.* **25**: 20A. (Abstr.)
14. Arnaud, P., G. B. Wilson, J. Koistinen, and H. H. Fudenberg. 1977. Immunofixation after electrofocusing: Improved method for specific detection of serum proteins with determination of isoelectric points. I. Immunofixation print technique for detection of alpha-1-protease inhibitor. *J. Immunol. Methods.* **16**: 221-231.
15. Cook, P. J. L. 1975. The genetics of alpha-1-antitrypsin: A family study in England and Scotland. *Ann. Hum. Genet.* **38**: 275-287.
16. Janoff, A. 1975. At least three human neutrophil lysosomal proteases are capable of degrading joint connective tissues. *Ann. N. Y. Acad. Sci.* **256**: 402-408.
17. Swedlund, M. A., G. G. Hunter, and G. J. Gleich. 1969. Alpha-1-antitrypsin increased in serum and synovial fluid in rheumatoid arthritis. *Arthritis Rheum.* **12**: 337A. (Abstr.)
18. Shtacher, G., R. Maayan, and G. Feinstein. 1973. Proteinase inhibitors in human synovial fluid. *Biochim. Biophys. Acta.* **303**: 138-147.
19. Erhlich, M. G., M. J. Mankin, M. Jones, R. Wright, C. Crispen, and G. Vigliani. 1977. Collagenase and collagenase inhibitors in osteoarthritic and normal human cartilage. *J. Clin. Invest.* **59**: 226-233.
20. Ohlsson, K. 1975. Alpha-1-antitrypsin and alpha-2-macroglobulin: Interactions with human neutrophil collagenase and elastase. *Ann. N. Y. Acad. Sci.* **256**: 409-419.
21. Fagerhol, M. K. 1969. Quantitative studies on the inherited variants of serum alpha-1-antitrypsin. *Scand. J. Clin. Lab. Invest.* **23**: 97-103.