Down's syndrome: changes in protein fractions of blood plasma

J. Kędziora, M. Soszyński, G. Bartosz, H. Witas and W. Leyko[1]

Department of Physiology, Physiological-Biochemical Institute, WAM, Łódź, and Department of Biophysics, Institute of Biochemistry and Biophysics, University of Łódź, PL-90-647 Łódź (Poland), 17 October 1979

Summary. A statistically significant decrease of prealbumin and statistically significant increases of alpha$_2$-macroglobulin and IgA were found in blood plasma of patients with Down's syndrome using quantitative crossed immunoelectrophoresis.

In our previous studies[2,3] we revealed alterations in the paper electrophoretic profile of protein fractions of blood plasma and in the plasma glycoprotein content of children with Down's syndrome as compared with normal controls. The aim of the present work was to investigate selected protein fractions of blood plasma of patients with Down's syndrome using the refined and sensitive technique of quantitative crossed immunoelectrophoresis[4].

Materials and methods. The investigations were performed on 13 subjects aged 14–26 years, divided into 3 groups. One was the control, the 2nd was composed of 6 patients with trisomy G-21 (karyotype 47, XX, +G or 47, XY, +G), and the 3rd was represented by 1 patient with unbalanced translocation G21/22 (karyotype 46, XX, −22, +t (21 q 22 q) mat). A small blood sample was taken into heparin from each subject. Morphotic elements were sedimented by centrifugation.

Horse antiserum against human serum proteins was obtained from Wytwórnia Surowic i Szczepionek, Warsaw. Agarose purchased from Fluka was used for preparation of 1% gel in Tris-veronal buffer, pH 8.6 (24.5 mM veronal, 73 mM Tris, 0.36 mM calcium lactate, 0.2 mM sodium azide). 2 µl of plasma were employed for each separation. Electrophoresis in the 1st dimension was run at a voltage of 10 V cm^{-1} for 1 h. The antiserum was added to the 2nd-dimension gel at a concentration of 3.75 µl of the commercial preparation per cm^2 gel. The gels were stained for protein with Coomassie brilliant blue R[5] and for esterase activity with β-naphthyl acetate and α-naphthyl acetate[6].

Results and discussion. Using the technique of crossed immunoelectrophoresis, 18 precipitates of human blood plasma proteins were obtained, 11 of which were identified (figure 1). From this number, 9 were subjected to quantitative (planimetry) and statistical analysis (figure 2). When

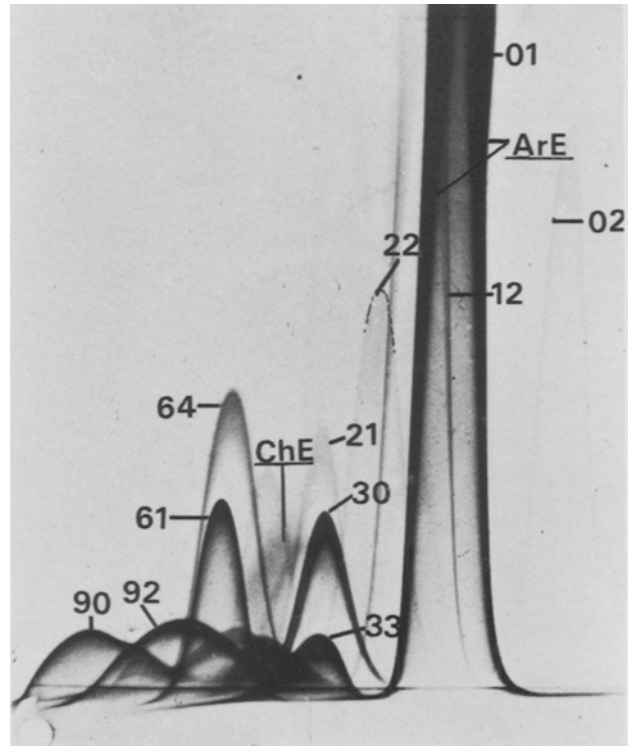

Fig. 1. Crossed immunoelectrophoresis of control human blood plasma. Immunoprecipitates numbered according to Schultze and Heremans[7]: 01, albumin; 02, prealbumin; 12, α-1-antitrypsin; 21, Gc-globulin; 22, α-1-antichymotrypsin; 30, haptoglobin; 33, α-2-macroglobulin; 61, transferrin; 64, hemopexin; 90, IgG; 92, IgA. ChE, cholinesterase; ArE, arylesterase.

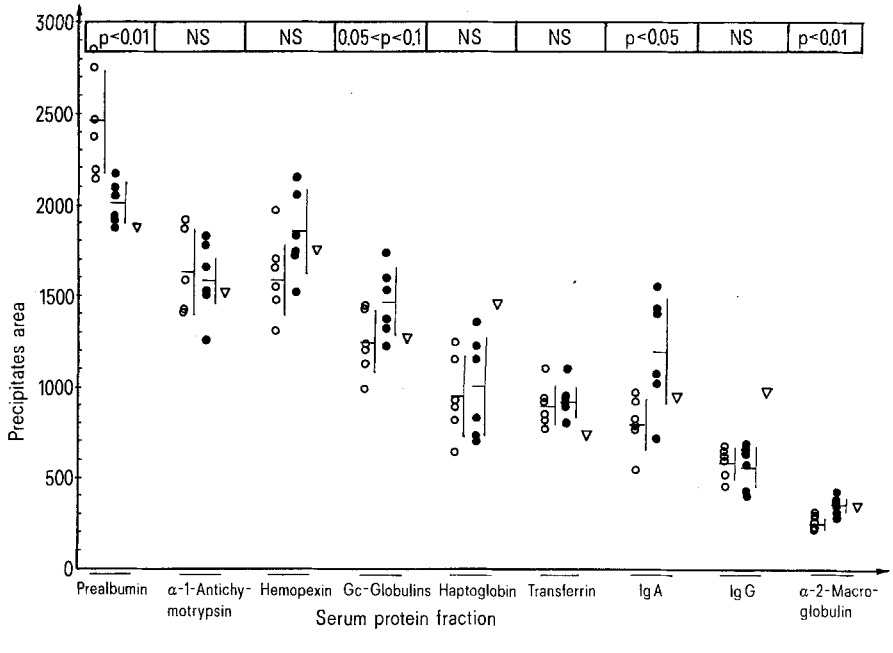

Fig. 2. Surface area below immunoprecipitates of plasma protein fractions (mm^2): ○, control group; ●, trisomy G-21; △, translocation G21/22.

comparing the patients with Down's syndrome and normal controls, a statistically significant decrease of the level of prealbumin and statistically significant increases of the levels of alpha$_2$-macroglobulin and immunoglobulin A were noted. The level of IgA in patients with Down's syndrome is a matter of controversy, different authors finding increased, normal or even decreased levels[7-10]. Our results would be in line especially with those studies which revealed elevated IgA in older groups of patients as contrasted to normal values in young children[10-12].

Staining of the gels with naphthyl acetates yielded a typical pattern of esterase activity contributed by cholinesterase (brownish-red) and arylesterase (orange)[6]. It is shown by this study that the electrophoretic mobilities of both plasma esterase activities are unaltered in Down's syndrome.

The behaviour of the protein fractions studied in the case of translocation G21/22 is noteworthy as it differs in many respect from that typical for trisomy G-21 (figure 2). However, the scarcity of material precludes a broader discussion of this point at present.

1 We are indebted to Prof. T.C. Bøg-Hansen, University of Copenhagen, for the helpful discussion of our results.
2 J. Kędziora and B. Wachowicz, Endokr. pol. *25*, 14 (1974).
3 J. Kędziora, Endokr. pol. *24*, 149 (1973).
4 B. Weeke, Scand. J. Immunol. *2*, suppl. 1, 47 (1973).
5 B. Weeke, Scand. J. Immunol. *2*, suppl. 1, 15 (1973).
6 C.M. Brogren and T.C. Bøg-Hansen, Scand. J. Immunol. *4*, suppl. 2, 37 (1975).
7 A.W. Griffiths, P.E. Sylvester and E.M. Baylis, J. clin. Path. *22*, 76 (1969).
8 K. Reiser, C. Whitcomb, K. Robinson and M.R. MacKenzie, Am. J. menth. Defic. *80*, 613 (1976).
9 A.I. Sutnick, W.T. London, B.S. Blumberg and B.J. Gerstley, J. natl Cancer Inst. *47*, 923 (1971).
10 F. Rosner, P.J. Kozinn and G.A. Jervis, N.Y. St. J. Med. *73*, 672 (1973).
11 E.L. Greene, R.I. Shenker and S. Karelitz, Am. J. Dis. Child. *115*, 599 (1968).
12 H. Dyggue and J. Clausen, Devl Med. Child. Neurol. *12*, 193 (1970).
13 H.E. Schultze and J.F. Heremans, Molecular biology of human proteins, vol. 1. Elsevier, Amsterdam/London/New York 1966.

Significant differential gene duplication without ancestral tetraploidy in a genus of mexican fish[1]

B.J. Turner[3], R.R. Miller and E.M. Rasch[4]

Museum of Zoology, University of Michigan, Ann Arbor (MI 48109, USA), and Department of Biology, Marquette University, Milwaukee (WI 53233, USA), 24 September 1979

Summary. A comparison of the protein products of 20–25 structural gene loci among the known species of the goodeid fish genus *Skiffia* suggests that at least 4 loci (16–20%) have undergone species-specific duplications (or, in 1 case, apparent loss) during the evolution of the genus. The species are clearly diploids, and the data therefore indicate that even a large proportion of differentially duplicated loci within a group of related fish species is not critical evidence of common tetraploid ancestry. Differential duplication of structural gene loci may be an important component of the genetic differences that separate congeneric conventional diploid species.

In teleost fishes, gene duplications and species-specific differences in the nature and number of homologous loci have been detected mainly among the genomes of the Palearctic cyprinids[5-8] (barbs and minnows, most notably including the carp)[9], catostomids (suckers)[10], cobitids (loaches)[11] and salmonids (salmons, trouts, and whitefishes)[12,33]. The differentially duplicated homologous loci in all of these families have been attributed to ancestral tetraploidy (usually allotetraploidy) followed by loss of some of the duplicated loci by 'rediploidization'[10,34-40] (fixation of mutations to functional silence, but possibly also including loss of genes by deletion). The little-known fish genus *Skiffia* presents what appears to be a case of significant differential structural gene duplication among congeneric fish species that clearly does not involve ancestral tetraploidy.

The 4 species of the genus (figure 1) are part of an impressive adaptive radiation on the Mexican plateau of cyprinodontoid fishes of the endemic family Goodeidae into habitats and trophic niches usually occupied by several diverse fish families[41]. Cytophotometric data (table) indicate that all 4 species are at the same ploidy level. They are not tetraploids with respect to other goodeids (average DNA content $=2.17\pm0.03$ pg/diploid nucleus, Turner and Rasch, unpublished data) or other cyprinodontoid fishes. For example, the rivuline and funduline killifishes, morphologically generalized and likely to be ancestral to all other new world cyprinodonts[42], have diploid nuclear DNA contents that range from 1.4–3.2 pg, average $=2.5\pm0.3$ pg[43]. The evidence of species-specific structural gene duplications in *Skiffia* is taken from electrophoretic comparisons of the protein products of approximately 20–25 structural gene loci.

1. Hemoglobins. These proteins showed species-specific patterns (figure 2, a) by gel electrophoresis or isoelectric focusing; these patterns differ markedly in the number of components displayed, as well as in their electrophoretic mobility. The number of discernible hemoglobins in each species, and the minimum number of globin chains (= number of globin genes) necessary to form the phenotype of each species, given 2 different models of hemoglobin structure, are presented in the table. The hemoglobins of *S. multipunctata* and *S. francesae* were further analyzed by gel isoelectric focusing of disassociated globin preparations[30] in 8M urea (figure 2, b). The results are consistent with differences in the number of globin chains in the 2 species, postulated in the table.

2. Malate dehydrogenase (MDH, figure 3, a). There are apparently 3 loci that encode malate dehydrogenases in nearly all goodeids (Turner, unpublished data) and all other cyprinodontoid fishes so far studied[44]. 2 of these, MDH-1 and MDH-3, encode cytosolic enzymes; a 3rd, MDH-2 encodes the mitochondrial enzyme. The MDH-1 phenotypes of *S. bilineata* and *S. lermae* were invariant and identical. The most frequent MDH-1 phenotype in *S. multipunctata* was identical to that of the 1st 2 species: 2 specimens of *S. multipunctata* were heterozygous at the MDH-1 locus (genotype MDH-1a/MDH-1b). All specimens of *S. francesae* appeared to be heterozygous at the MDH-1