Clinical Genetics 1988: 33: 11–19

Regular trisomy 21 not accompanied by increased copper-zinc superoxide dismutase (SOD1) activity

ANNA JEZIOROWSKA, LUCJUSZ JAKUBOWSKI[1], JOANNA LACH AND BOGDAN KAŁUŻEWSKI

Department of Medical Genetics, Institute of Endocrinology, and [1]Genetic Councelling Center, Medical Academy of Łódź, Poland

The Cu-Zn superoxide dismutase (SOD1) activity was estimated in red blood cells in children with regular trisomy 21. We report patients displaying typical Down syndrome clinical features and with SOD1 activity in the normal range.

Received 2 July, revised 25 July 1986, accepted for publication 23 August 1987

Key words: Down syndrome; superoxide dismutase (SOD1); trisomy 21

Phenotypic analysis of Down syndrome patients indicates that the presence of an extra copy of all or part (21q22→21qter) of chromosome 21, is primarily responsible for the major clinical features of trisomy 21. The best known gene mapped to chromosome 21 (21q22.1) is cytoplasmatic copper-zinc superoxide dismutase (EC1.15.1.1.,SOD1) (Tan et al. 1973. Levanon et al. 1985). SOD1 is the natural scavenger of superoxide anion (O^-_2) cooperating with catalase and glutathione peroxidase in the intrinsic cellular antioxidant defence system. Numerous subsequent studies have demonstrated a 50% increase in SOD1 activity in Down syndrome patients, thus confirming the gene dosage effect in regular trisomy 21, translocation trisomy 21 and mosaics with the critical region involved (Benson 1975, Eriksson et al. 1975, Frants et al. 1975, Crosti et al. 1976a, Feaster et al. 1977, Garber et al. 1979).

It may be assumed that the mechanism by which the extra chromosome in Down syndrome results in an abnormal phenotype is by overproduction of chromosome 21 specific genes' products (Kurnit 1980). The question is how an increased production of normal gene products causes the developmental anomalies with a broad spectrum of mental retardation ranging from the severe to the mild observed in Down syndrome patients. It was suggested that overproduction of SOD1 may be involved in some of the clinical symptoms in patients with neuro-psychiatric manifestations on account of an unusually rapid elimination of oxygen radicals, thus affecting the oxyradical-dependent biosynthesis of neuromediators (Michelson et al. 1977, Sinet 1982). Increased SOD1 activity has also been reported in schizophrenia, autism and other mental disorders (Michelson 1982, Cohen et al. 1986). Increased lipoperoxidation brain damage was reported by Brooksbank & Balazs 1983. Synaptic abnormalities in Down syndrome have been described in the fetus (Ross et al. 1984) and dendritic tree in pre-

natal and postnatal stages of development (Petit et al. 1984), together with the arrest of neurogenesis and synaptogenesis reported by Wiśniewski et al. (1984).

SOD1 may not directly regulate neuron formation in the developing brain, but may affect the availability of precursors needed to synthesize or insert molecules into membranes at specific stages of neurogenesis and could account for profound changes in neuronal differentiation by altering gene expression, which in the clinical picture would manifest as a lowered mental capacity.

The present paper reports normal SOD1 activity values in some patients with regular trisomy 21 and clinical features of Down syndrome.

Patients and Methods

Routine chromosome analysis was performed on cultured peripheral blood lymphocytes in all the presented cases. In case MT250283 an additional skin fibroblasts culture was set up. The chromosomes were analysed in detail by means of three different banding techniques: GTG, CBG and RHG.

Blood samples were obtained from patients with typical Down syndrome features. Control: 50 healthy children chosen in the appropriate age range served as the control group. In 30 of them a normal diploid karyotype was found; in the other 20, karyotyping was not performed.

Enzyme preparation and assay was performed as described previously (Jeziorowska et al. 1982). Red blood cells lysate was prepared as described by Minami & Yoshikawa (1979).

SOD1 was prepared according to McCord & Fridovich (1969) and the enzymatic activity was determined by its ability to inhibit the autooxidation of colorless adrenaline to colored adrenochrome according to Misra & Fridovich (1972). One unit of SOD1 activity was defined as the concentration which inhibited adrenochrome formation by 50% of control rate. SOD1 activity was expressed in units per mgHb measured by the standard cyanomethemoglobin method.

SOD1 activity was assayed in two blood samples independently: first on the occasion of introductory enzymatic diagnosis and second on the occasion of karyotype analysis. Each SOD1 activity determination was calculated as the mean of a triplicate assay on one blood sample of an individual patient or control. No significant difference in the enzyme level in one blood sample measured in triplicate was observed. In the trisomy 21 ($n = 10$) group as well as in cases of borderline SOD1 levels observed in the control and trisomy 21 ($n = 59$) groups, the enzyme activity was also assayed in a third sample of blood to confirm the estimation and define the intraindividual variation.

Statistical method. The non-parametric median test was employed for statistical analysis. The test value was calculated according to the formula

$$\chi^2 = \sum_i \frac{(n_i - n_{oi})^2}{n_{oi}},$$

where n_i = quantity observed, and n_{oi} = quantity expected. Differences among the means of the tested groups were regarded as statistically significant if the calculated test value was equal to or higher than the value in the χ^2-test table at the probability level $p = 0.05$ and with 1 degree of freedom.

Results

In 59 diagnosed patients displaying Down syndrome features, regular trisomy 21 was

established in peripheral blood lymphocytes (Table 1). Among Down syndrome patients with typical features of the syndrome and regular trisomy 21 diagnosed, 10 cases of regular trisomy 21 not accompanied by the elevated SOD1 activity were found (Table 2). In case MT 250283 an additional skin fibroblasts karyotype was established which confirmed regular trisomy 21. Comparison of the clinical features of the Down syndrome patients with regular trisomy 21 displayed in Tables 1 and 2 shows that the depicted signs of the syndrome are similar in these two groups.

SOD1 activity in trisomy 21 patients ($n=59$) was approximately one and a half times normal enzyme activity (Table 3): trisomy 21 1.48/normal 0.97 U SOD1/mgHb = 1.53.

The scatter plot showing the distribution of SOD1 activities in the control and two Down syndrome groups indicates that no SOD1 elevation similar to the $n=59$ group was observed in the $n=10$ trisomy 21 group. Statistical analysis of the obtained results revealed no significant difference ($\chi^2=1.44; p>0.05$) of the Down syndrome $n=10$ group when compared with the control. Comparison of SOD1 activity of the trisomy 21 $n=59$ group, versus the control group, displayed a statistically significant difference: $\chi^2=84.5$; $p<0.001$. At the same time, a statistically significant difference was found between the trisomy 21 $n=59$ group and the trisomy 21 $n=10$ group: $\chi^2=8.98$; $p<0.01$.

Although the means of the control group and the trisomy 21 $n=59$ group were significantly different, there was an overlap of these two populations. This phenomenon is rather typical of all such variables and has also been shown in other studies (Frants et al. 1975, Burri et al. 1980). The value of SOD1 activity equal to 1.30 U SOD1/mgHb was arbitrarily chosen as cut-off point as this value was the lowest observed in the trisomy 21 $n=59$ group with the overlap with the highest value (1.29 U SOD1/

Table 1

Signs of Down syndrome in patients with regular trisomy 21 and elevated SOD1 activity

	Male (n=25)			Female (n=34)			Both sexes (n=59)		
	+	−	×	+	−	×	+	−	×
1. Hypotonia	22	3	0	28	6	0	50	9	0
2. Slanting eyes	18	4	3	10	10	14	38	14	7
3. Epicanthus in at least one eye	20	5	0	30	2	2	50	7	2
4. Short nose	19	5	1	27	2	5	46	7	6
5. At least one dysplastic ear	16	8	1	25	9	0	41	17	1
6. Wrinkled tongue	4	4	17	6	3	25	10	7	42
7. Tongue protruding	11	8	6	18	7	9	29	15	15
8. Flat face	9	6	10	16	4	14	25	10	24
9. Brachycephaly	15	4	6	19	9	6	34	13	12
10. Arched palate	7	7	11	18	4	12	25	11	23
11. Dental anomalies	9	2	14	15	3	16	24	5	30
12. Short neck	19	6	0	24	6	4	33	12	14
13. Simian crease in at least one hand	15	10	0	20	14	0	35	24	0
14. At least one toe widely spaced	13	12	0	18	16	10	31	18	10
15. Umbilical hernia	13	12	0	13	21	0	26	33	0
16. Cong. heart disease	10	15	0	16	18	0	26	33	0

Other anomalies: a. cryptorchidism in 8 boys (one of them connected with inguinal hernia). b. 1 case of duodenal atresia. c. 2 cases of strabismus (one of them connected with cataract). d. 1 case of polydactyly. e. 1 case of syndactyly. f. 1 case of alopecia.

+ = presence of symptom; − = absence of symptom; × = no data.

Table 2

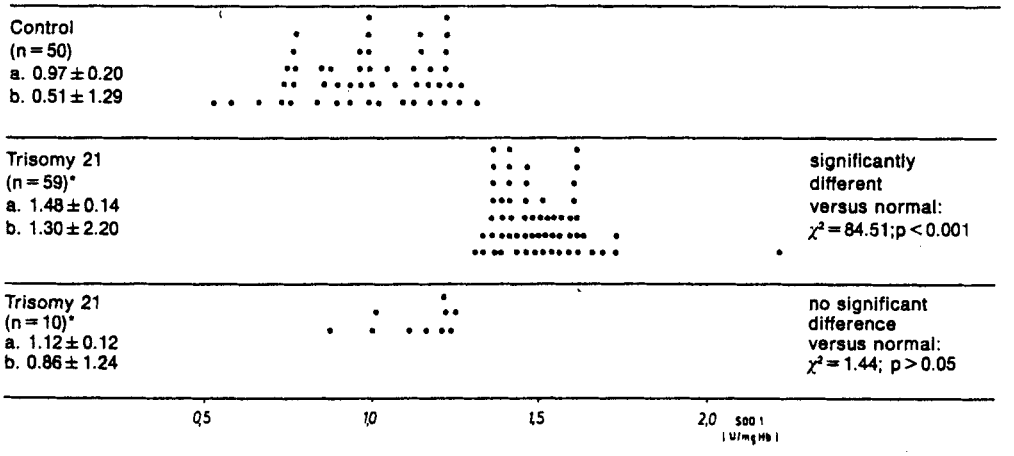
Regular trisomy 21 not accompanied by the elevated SOD1 activity

No.	Patient	Sex	SOD1 U/mgHb mean ± SD (n = 3)	Hypotonia	Slanting	Epicanthus l/r	Short nose	Dysplastic ears l/r	Wrinkled tongue	Tongue protruding	Flat face	Brachycephaly	Arched palate	Dental anomalies	Short neck	Simian crease l/r	Toes widely spaced l/r	Umbilical hernia	Cong. heart disease	Other anomalies	Head circumference at birth
1.	SK090181	M	0.86 ± 0.03	+	+	+/+	+	+/+	×	+	±	+	+	×	+	+/+	−/−	+	+	cr	34
2.	MT250283	M	0.99 ± 0.02	+	±	+/+	+	−/−	+	+	+	+	×	+	±	−/+	+/+	+	+	−	31
3.	KA290280	F	1.00 ± 0.04	+	+	+/−	+	+/+	×	+	+	+	+	+	+	+	+/+	+	+	−	×
4.	PJ170781	M	1.10 ± 0.03	+	±	−/±	−	−/−	−	+	±	±	−	+	+	−/−	+/+	+	−	−	32
5.	KW310879	F	1.15 ± 0.05	−	−	+/+	+	+/+	−	+	+	+	+	+	+	−/+	±/±	−	−	−	×
6.	BO151082	F	1.20 ± 0.02	+	+	+/+	±	+/+	−	+	+	+	+	−	+	+/+	+/+	+	±	−	31
7	TG151082	M	1.21 ± 0.03	+	±	+/+	×	+/+	×	+	±	+	+	×	+	+/+	+/+	±	−	−	33
8.	SB190379	M	1.21 ± 0.02	+	+	+/+	−	+/+	−	−	−	+	±	±	±	−/−	−/−	−	+	−	34
9.	PF160672	M	1.23 ± 0.03	−	+	−/−	+	+/+	×	+	×	±	±	+	+	−/−	−/−	−	+	−	×
10.	IK230484	F	1.24 ± 0.03	+	+	+/+	+	−/+	×	+	+	+	+	+	+	+/+	−/+	−	−	−	33

+ = presence of symptom; ± = symptom weakly marked; − = absence of symptom; × = no data.
l/r = left side/right side; cr = cryptorchidism.

Table 3

Distribution of SOD1 activities in the investigated groups



a. mean ± SD; b. SOD1 U/mg Hb activity range observed. Each dot represents the mean of triplicate assays on an individual patient or control.
* statistically significant difference of SOD1 level was found when two trisomy 21 groups were compared: $\chi^2 = 8.98$; $p < 0.01$.

mgHb) observed in the control group of healthy children.

SOD1 activities in the trisomy 21 n = 10 group, shown as the mean ± SD of three independent assays on individual patients (Table 2), demonstrated very slight intraindividual variation. None of the results obtained in the trisomy 21 n = 10 group ever exceeded the range of SOD1 activity observed for the control group: 0.51–1.29 U SOD1/mgHb. The extreme values of SOD1 activities in the control group as well as in the trisomy 21 n = 59 group were also tested in three independent enzyme determinations and were as follows. Control group: 0.51 ± 0.03; 0.55 ± 0.01; 1.25 ± 0.02; 1.29 ± 0.02. Trisomy 21 (n = 59): 1.30 ± 0.02; 1.32 ± 0.02; 1.72 ± 0.04; 2.20 ± 0.03 U SOD1/mgHb (mean ± SD, n = 3).

It seems that the important distinctive feature of the trisomy 21 n = 10 group in contrast to the trisomy 21 n = 59 group was the presence of SOD1 activity in the normal range. Since the 50% excess of SOD1 activity in regular trisomy 21 patients is considered to be the triple gene-dosage effect, is the trisomy 21 n = 10 group presented above evidence of the lack of a SOD1 gene-dosage effect, or are there other secondary factors, or both?

Discussion

The Down syndrome clinical features of the 10 unusual cases displayed in Table 2 show no specific differences when compared with the features of trisomy 21 cases (n = 59) with elevated SOD1 activity (Table 1). The 10 presented cases of trisomy 21 may provide information on the possible effect of decreased intracellular superoxide radicals in certain features of trisomy 21, as among numerous abnormalities reported in Down syndrome no finding except for the extra

chromosomal material is constant. Even mental deficiency, which is always present, is variable. Bersu (1980) in a comprehensive review noted two generalizations in Down syndrome phenotype analysis. First, no specific features are pathognomonic with regard to trisomy 21. It is a fairly consistent combination of features that allows for the diagnosis of Down syndrome in affected individuals. Second, considerably more variability is observed in the presentation of features in Down syndrome than in the same features in the normal population. Thus the specific feature combination and marked developmental variability are what appear to distinguish Down syndrome phenotypically from normal.

Until now, it appeared that an elevated level of SOD1 was attributed to a gene-dosage effect. Recently this situation has become unclear since cases of Down syndrome phenotype have been reported in which increased SOD1 activity was not observed. Mattei et al. (1981) presented a case study of trisomy 21q22.3 with many of the classical signs of Down syndrome with normal SOD1 activity. Habedank & Rodewald (1982) described three mentally retarded siblings with moderate stigmata of Down syndrome with partial trisomy 21q22.2→qter resulting from maternal translocation t(4q+;21q-), with no excess SOD1, thus providing the hypothesis that neither trisomy of band 21q22.1 nor the increase of SOD1 is the condition *sine qua non* for most of the Down syndrome features, including the mental retardation. To our knowledge, the only case of regular trisomy 21 with SOD1 activity in the normal range was reported in a personal communication by Brinkworth in 1980.

Further studies will probably show whether the SOD1 concentration is influenced by a triple gene dosage only, or by secondary factors such as generally disturbed protein synthesis. As has been reported by Baeteman et al. (1983), the regulation of SOD1 level in lymphocyte subpopulations which appeared to occur with age in the controls was not seen in trisomy 21. According to the gene-dosage effect theory, it would suggest that the control of synthesis of SOD1 acts on transcription and that this control is lacking in trisomy 21.

The question whether the SOD1 gene has a variable effect in different individuals or different alleles are involved, or both, still remains to be answered. Since the discovery of SOD1 polymorphism by Brewer (1967) numerous publications have appeared on the distribution of these genetic markers in many populations (Weissmann et al. 1982). It has been proposed that two autosomal alleles of CuZn SOD, referred to as SOD^1 and SOD^2, control the phenotype SOD1 common type, SOD2–1 heterozygote and SOD2 rare homozygous type. As has been reported by Beckman et al. (1973) a lower enzymatic activity seems to be associated with the allele SOD2.

However, Crosti et al. (1976b) found that in the heterozygote SOD1–2 the enzyme activity was equal to that of the common SOD1 phenotype. Moreover Marklund et al. (1976) showed that the activity of the purified product of the two alleles did not differ significantly. Since the enzyme activity of the two phenotypes SOD1–2 and SOD1 was in the same range, this may suggest that the allele SOD^2 is selectively neutral (Spedini et al. 1982). Still, little investigation has been carried out yet on the two electrophoretic forms noticed by several authors (Carrico & Deutsch 1970, Beckman et al. 1973, Beckman 1973, Tegelstrom 1975, Marklund et al. 1976, Crosti 1978) of the crude erythrocyte extract as well as of the purified enzyme of the homozygote phenotype SOD1.

In conclusion, Crosti states that the human SOD1 exists in two electrophoretically active forms in erythrocytes and that

these forms have to be considered charge isomers easily permuting one into the other. SOD1 activity changes in erythrocytes were noticed to be dependent on copper status and decreased with copper deficiency in numerous species including man (Okahata et al. 1980, Bettger et al. 1978, Andrewartha & Caple 1980, Néve et al. 1983, Fischer et al. 1984). In another study, human male subjects were fed a low copper diet and no reductions were found in serum copper or ceruloplasmin activity, but erythrocyte SOD1 activity was reduced (Reiser et al. 1985). Thus erythrocyte SOD1 activity has been suggested as a clinical index of tissue copper status since its proportionality to metabolically active copper has been demonstrated (Fischer et al. 1984, L'Abbe & Fischer 1986). Probably further biochemical analysis of enzymatic activities of other enzymes and compounds co-operating with SOD1 in the oxidative defence system, determination of SOD1 polymorphism, their relationship to the copper status in patients described above combined with DNA technology investigations might give results which would be helpful in explaining the phenomenon of regular trisomy 21 cases not accompanied by elevated SOD1.

It is very difficult at present to define precisely the mental retardation differences among the two presented trisomy 21 groups in association with the different SOD1 level observed in them, as the children are young and of various ages. Previous reports have suggested that within the general category of Down syndrome, mosaics are likely to be among the brightest group (Johnson & Abelson 1969), and as has been reported previously, SOD1 activity in the normal range was also found in cases of mosaic trisomy 21 (Jeziorowska et al. 1982).

Records and observations of the mental development under psychological care of the reported cases of regular trisomy 21 with normal SOD1 activity as compared with translocation and regular trisomy 21 with elevated SOD1, and the mosaic trisomy 21 group, are being maintained at the Genetic Counselling Center in order to estimate the rate of psychological development and the intellectual abilities of these Down syndrome children.

Acknowledgements

We thank Irena Szymańska M. Sc. for statistical analysis and Miss Agnieszka Klarecka for excellent secretarial assistance.

References

Andrewartha, K. A. & I. W. Caple (1980). Effects of changes in nutritional copper on erythrocyte superoxide dismutase activity in sheep. *Res. Vet. Sci.* **28**, 101–104.

Baeteman, M. A., A. Baret, A. Coutiere, P. Rebuffel & J. F. Mattei (1983). Immunoreactive Cu-SOD and Mn-SOD in lymphocytes subpopulations from normal and trisomy 21 subjects according to age. *Life Sci* **32**, 895–902.

Beckman, G. (1973). Population studies in Northern Sweden. VI. Polymorphism of superoxide dismutase. *Hereditas* **73**, 305–308.

Beckman, G., E. Lunggren & A. Tarnvik (1973). Superoxide dismutase isozyme in different human tissues, their genetic control and intracellular localization. *Hum. Hered.* **23**, 336–339.

Benson, P. (1975). Gene dosage effect in trisomy 21. *Lancet* **I**, 584.

Bersu, E. T. (1980). Anatomical analysis of the developmental effects of aneuploidy in man: the Down syndrome. *Am. J. Med. Genet.* **5**, 399–420.

Bettger, W. J., T. J. Fish & B. L. Dell (1978). Effects of copper and zinc status of rats on erythrocyte stability and superoxide dismutase activity. *Proc. Soc. Exp. Biol. Med.* **158**, 279–282.

Brewer, G. J. (1967). Achromatic regions of tetrazolium stained starch gels: inherited electrophoretic variation. *Am. J. Hum. Genet.* **19**, 674–680.

Brooksbank, B. W. L. & R. Balazs (1983). Superoxide dismutase and lipoperoxidation in Down's syndrome fetal brain. Lancet **i**, 881–882.

Burri, B. J., S. G. Chan, A. J. Berry & S. K. Yarnell (1980). Blood levels of superoxide dismutase and glutathione peroxidase in Duchenne Muscular Dystrophy. *Clin. Chim. Acta* **105**, 249–255.

Carrico, R. J. & H. E. Deutsch (1970). The presence of zinc in human cytocuprein and some properties of apoprotein. *J. Biol. Chem.* **245**, 721–725.

Cohen, M. R., V. Sailer, B. McAmis & P. Jenkins (1986). Superoxide dismutase in fibroblasts from patients with schizophrenia. *Biol. Psychiat.* **21**, 322–324.

Crosti, N., A. Serra, A. Rigo & P. Viglino (1976a). Dosage effect of SOD-A gene in trisomy 21 cells. *Hum. Genet.* **31**, 197–203.

Crosti, N., A. Serra, D. Cagiano-Malvezzi & G. Tagliaferri (1976b). The rare allele SOD A^2 in the Italian population. *Ann. Hum. Genet.* **3**, 343–350.

Crosti, N. (1978). On the two electrophoretic forms of the human superoxide dismutase A in the homozygote SOD A1. *Biochem. Genet.* **16**, 739–742.

Eriksson, A. W., R. R. Frants & P. H. Jongbloet (1975). Quantitative immunological studies on cytoplasmatic superoxide dismutase: high concentration in red blood cells of Down syndrome (Abstr.) (1975). *Am. J. Hum. Genet.* **27**, 33A.

Feaster, W. W., L. W. Kwok & C. J. Epstein (1977). Dosage effect of superoxide dismutase-1 in nucleated cells aneuploid for chromosome 21. *Am. J. Hum. Genet.* **29**, 563–570.

Fischer, P. W. F., A. Giroux & M. R. L'Abbe (1984). Effect of zinc supplementation on copper status in adult man. *Am. J. Clin. Nutr.* **40**, 743–746.

Frants, R. R., A. W. Eriksson, P. H. Jongbloet & A. J. Hamers (1975). Superoxide dismutase in Down syndrome. *Lancet* **ii**, 42–43.

Garber, P., P.-M. Sinet, H. Jerome & J. Lejeune (1979). Copper/zinc SOD activity in trisomy 21 by translocation. *Lancet* **ii**, 927.

Habedank, M. & A. Rodewald (1982). Moderate Down's syndrome in three siblings having partial trisomy 21q22.2→qter and therefore no SOD-1 excess. *Hum. Genet.* **60**, 74–77.

Jeziorowska, A., L. Jakubowski, A. Armatys & B. Kałuzewski (1982). Copper/zinc superoxide dismutase (SOD-1) activity in regular trisomy 21, trisomy 21 by translocation and mosaic trisomy 21. *Clin. Genet.* **22**, 160–164.

Johnson, R. C. & R. B. Abelson (1969). Intellectual, behavioral, and physical characteristics associated with trisomy, translocation, and mosaic types of Down's syndrome. *Am. J. Ment. Defic.* **73**, 852–855.

Kurnit, D. M. (1980). A molecular approach to Down syndrome. *Down Syndrome* **3**, 1–2.

L'Abbe, M. R. & P. W. Fischer (1986). An automated method for the determination of Cu, Zn-superoxide dismutase in plasma and erythrocytes using an ABA-200 discrete analyser. *Clin. Biochem.* **19**, 175–178.

Levanon, D., J. Lieman-Hurwitz, N. Dafni, M. Wigderson, L. Sherman, Y. Bernstein, Z. Laver-Rudick, E. Danciger, O. Stein & Y. Groner (1985). Architecture and anatomy of the chromosomal locus in human chromosome 21 encoding the Cu/Zn superoxide dismutase. *EMBO J.* **4**, 77–84.

Marklund, S., G. Beckman & T. Stingbrandt (1976). A comparison between the common type and rare genetic variant of human cuprozinc superoxide dismutase. *Eur. J. Biochem.* **65**, 415–422.

Mattei, J. F., M. G. Mattei, M. A. Baeteman & F. Giraud (1981). Trisomy 21 for region 21q22.3: Identification by high-resolution R-banding patterns. *Hum. Genet.* **56**, 409–411.

McCord, J. M. & J. Fridovich (1969). The utility of superoxide dismutase in studying free radical reactions. *J. Biol. Chem.* **244**, 6056–6063.

Michelson, A. M., K. Puget, P. Durosay & J. C. Bouneau (1977). Clinical aspects of the dosage of erythrocuprein. In *Superoxide and Superoxide Dismutase*. A. M. Michelson, J. M. McCord & I. Fridovich (eds). London, Academic Press, pp. 467–499.

Michelson, A. M. (1982). Clinical use of superoxide dismutase and possible pathological approaches. In *Pathology of Oxygen*, A. P. Autor (ed.) London, Academic Press, pp. 277–302.

Minami, M. & H. Yoshikawa (1979). A simplified assay method of superoxide dismutase activity for clinical use. *Clin. Chim. Acta* **92**, 337–342.

Misra, H. P. & I. Fridovich (1972). The role of O_2^- in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* **247**, 3170–3175.

Néve, J., P. M. Sinet, L. Molle & A. Nicole (1983). Selenium, zinc and copper in Down's syndrome (trisomy 21): blood levels and relations with glutathione peroxidase and superoxide dismutase. *Clin. Chim. Acta* **133**, 209–214.

Okahata, S., S. Nishi, S. Hatano, Y. Kobayashi & T. Usui (1980). Changes in erythrocyte superoxide dismutase in a patient with copper deficiency. *Eur. J. Pediatr.* **134** 121–124.

Petit, T. L., J. C. LeBoutillier, D. P. Alfano & L.

E. Becker (1984). Synaptic development in the human fetus: a morphometric analysis of normal and Down's syndrome neocortex. *Exp. Neurol.* **83**, 13–23.

Reiser, S., J. C. Smith & W. Metz (1985). Indices of copper status in humans consuming a typical American diet containing either fructose or starch. *Am. J. Clin. Nutr.* **42**, 242–251.

Ross, M. H., A. M. Galaburda & T. L. Kemper (1984). Down's syndrome: is there a decreased population of neurons? *Neurology* **34**, 907–916.

Sinet, P. M. (1982). Metabolism of oxygen derivatives in Down's syndrome. *Ann. N. Y. Acad. Sci.* **396**, 83–94.

Spedini, G., E. Capucci, N. Crosti, N. E. Danubio & S. Romagnoli (1982). Erythrocyte glyoxalase and superoxide dismutase polymorphisms in the Mbugu and some other populations of the Central African Republic. *Hum. Hered.* **32**, 253–258.

Tan, Y. H., J. Tischfield & F. H. Ruddle (1973). The linkage of genes for the human interferon-induced antiviral protein and indophenol oxidase-B traits to chromosome G-21. *J. Exp. Med.* **137**, 317–330.

Tegelstrom, H. (1975). Interspecific hybridization *in vitro* of superoxide dismutase from various species. *Hereditas* **81**, 185–189.

Weissmann, J., M. Vollmer & O. Pribilla (1982). Survey of the distribution of adenosine deaminase and superoxide dismutase markers in different populations. *Hum. Hered.* **32**, 344–356.

Wiśniewski, K. E., M. Laure-Kamionowska & H. M. Wiśniewski (1984). Evidence of arrest of neurogenesis in brains of patients with Down's syndrome. *N. Engl. J. Med.* **311**, 1187–1188.

Address:

A. Jeziorowska, D. Sc.
Laboratory of Genetics
Institute of Endocrinology
Medical Academy of Łódź
Dr. Sterling str. 3
91–425 Łódź
Poland