0014-4754/96/090871-03$1.50 + 0.20 © Birkhäuser Verlag Basel, 1996 

# Overexpression of copper-zinc superoxide dismutase in trisomy 21

R. De La Torre[a], A. Casado[a,*], E. López-Fernández[a], D. Carrascosa[a], V. Ramírez[b] and J. Sáez[b]

[a]*Departamento de Fisiopatologia y Genética Molecular Humana, Centro de Investigaciones Biológicas (C.S.I.C.), c/. Velázquez 144, E-28006 Madrid (Spain)*
[b]*Servicio Regional de Salud de la Comunidad Autónoma de Madrid, c/. O'Donnell 60, E-28009 Madrid (Spain)*
*Received 16 April 1996; received after revision 29 May 1996; accepted 11 June 1996*

**Abstract.** Down's syndrome (DS), the most frequent of congenital birth defects, results from the trisomy of chromosome 21 in all cells of affected patients. This disease is characterized by developmental anomalies, mental retardation and features of rapid aging, particularly in the brain, where the occurrence of Alzheimer's disease is observed in trisomy 21 patients over the age of 35. Copper-zinc superoxide dismutase (CuZnSOD) is one of the proteins encoded by chromosome 21 (21q22.1). As a consequence of gene dosage excess, CuZnSOD activity is increased by 50% in all DS tissues. This work reports the SOD activity of a population of DS patients with complete trisomy 21, partial trisomy 21, translocations and mosaicism, in order to confirm the gene dosage effect of SOD on the clinical features of DS, and to help to establish which is the critical region of chromosome 21 in DS. CuZnSOD was measured in red blood cells using the Minami and Yoshikawa method. In the population with complete trisomy 21, SOD activity was increased by 42%; in the population with partial trisomy 21, translocations and mosaicism, SOD activity was normal. In the population diagnosed as DS, but not karyotyped, SOD activity was increased by 28%. No differences between sexes or among ages were found. We conclude that the 21q22.1 segment is not the critical region responsible for DS, as we have found normal SOD activity in patients with the clinical features of DS.
**Key words.** Critical region; CuZnSOD; Down's syndrome; gene dosage; trisomy 21.

Down's syndrome (DS), a genetic abnormality associated with the presence of three copies of chromosome 21, reported by Lejeune [1], is one of the most important human congenital diseases, occurring in 1 of 700–1000 live births [2]. DS patients suffer from a wide range of symptoms. Most obvious among these are morphological defects such as hypotonia in the newborn, short stature and the epicanthic eyefolds which give rise to the eye shape characteristic of the syndrome. Patients are mentally retarded, and those who survive past their mid-thirties usually develop Alzheimer's disease [3]. Premature aging and an increase in the incidence of leukemia and other hematological disorders are common in affected individuals, as are cardiac defects, a high susceptibility to infections and several types of endocrine disorders [3]. Although DS was described more than a century ago, and the relationship between trisomy 21 and the Down's phenotype has been known for over 30 years, very little is known about the way in which the additional chromosome 21 causes the disease. The current concept is that the presence of extra copies of genes that reside in chromosome 21 results in the synthesis of increased amounts of gene products, which creates an imbalance in various biochemical pathways; this in turn causes the physiological defects giving rise to the clinical picture of DS. The over- and underproduction of some enzymes and other proteins is still of uncertain significance with respect to the most remarkable impairments of the syndrome.

The human copper-zinc superoxide dismutase (CuZnSOD) is a key enzyme in the metabolism of oxygen free radicals [4]. It is encoded by a gene residing on chromosome 21 at the chromosomal region 21q22 known to be involved in DS, and elevated levels of the enzyme are commonly found in DS patients [5, 6]. This overexpression of the *CuZnSOD* gene, due to gene dosage, may disturb the steady-state equilibrium of active oxygen species within the cells resulting in oxidative damage to biologically important molecules [7–9]. It has been demostrated that CuZnSOD is able to catalyse hydroxyl radical production from hydrogen peroxide and that elevated levels of SOD enhance the cytotoxicity of active oxygen species. Such processes may in part be responsible for certain clinical symptoms associated with the Down's phenotype [10, 11].

In the present work we have studied the levels of SOD activity in a population of DS patients showing complete trisomy 21, partial trisomy 21, translocations and mosaicism in order to confirm the gene dosage effect in these patients.

* Corresponding author.

## Material and methods

SOD activity was determined in a total of 260 DS patients (123 females and 137 males), aged between 2 and 45 years. One hundred and eight patients had been karyotyped, showing in 57 patients a complete trisomy 21, and in 51 patients partial trisomy 21, translocations and mosaicisms. One hundred and fifty-two DS patients had not been karyotyped. The patients were residents of 27 institutions for the mentally retarded in Madrid. SOD activity was also determined in a control healthy population of matched ages.

SOD activity was measured in red blood cells; 0.1 ml of blood was hemolysed by 0.9 ml of ice-cold water. Hemoglobin was removed by adding 0.25 ml of chloroform and 0.5 ml of ethanol followed by vigorous mixing. The mixture was centrifuged at 18,000 *g* for 60 min. The clear supernatant was used for SOD assay. This assay was performed using the method of Minami and Yoshikawa [12], which is based on the inhibition by SOD of the nitro blue tetrazolium reduction produced by the superoxide radical generated by the autooxidation of pyrogallol. The rate of inhibition of the superoxide reaction by SOD was calculated according to the definition of McCord and Fridovich [13].

All data were expressed as mean $\pm$ standard deviation (SD). Tests of significance for group differences were performed by analysis of variance (ANOVA). Differences were considered significant if the p value was smaller than 0.05.

## Results and discussion

As can be seen in table 1 and in figure 1, in the population with complete trisomy 21 analysed, SOD activity was $6.38 \pm 0.76$ U/ml of blood, 42% more than the SOD activity observed in the normal population. In the population with partial trisomy 21, translocations and mosaicisms, SOD activity was $5.01 \pm 1.2$ U/ml of blood, which is not significantly different than that observed in the normal population. Finally, in the DS population that had not been karyotyped, SOD activity was $5.75 \pm 1.04$ U/ml of blood, 28% more than that observed in the normal population. No differences between sexes and among different ages were found.

In complete trisomy 21, an increase of 42% in SOD activity was found. This increase is lower than the increase in gene dosage (50%). It is possible that a compensatory mechanism tries to lower this high activity in order to avoid the oxidative damage that an excess of SOD activity produces. The results obtained in the non-karyotyped DS patients suggest that is a population with a majority of complete trisomy 21. The SOD activity observed in the DS patients with partial trisomy 21, translocations and mosaicisms seems to confirm the finding that the distal region 21q22.3 is the critical region for DS.

After Lejeune [1] correlated trisomy 21 and DS, the entire chromosome 21 was held responsible for the clinical picture observed. In 1973, Aula [14] suggested that the clinical picture of trisomy 21 was essentially due to the trisomy for the single distal segment of the chromosome long arm. After the precise localization of the *CuZnSOD* gene in 21q22.1, Sinet [15] put forward the hypothesis that the chromosome segment containing the *CuZnSOD* gene is also responsible for the clinical features of trisomy 21. The observations of Hagemeijer [16] seemed to confirm the role of the 21q22 segment in the clinical picture of trisomy 21. However, Kedziora [17] reported the cases of three trisomy 21 subjects without any increase in SOD activity, and in 1981, Mattei [18] also reported the observation of a patient with clinical features of DS but with a karyotype reveal-

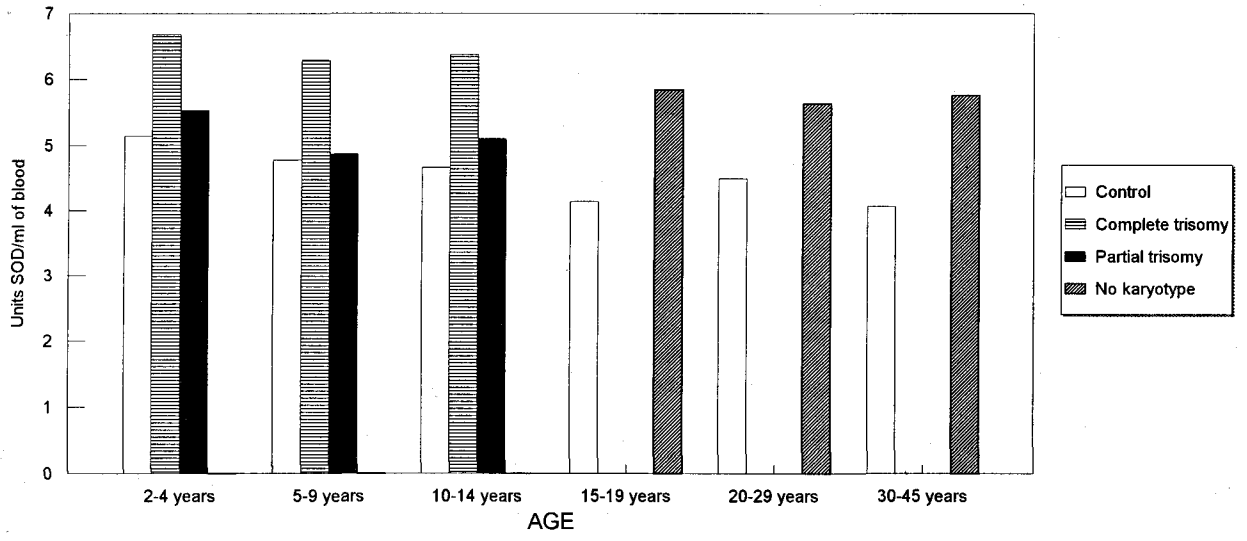


Figure 1. Superoxide dismutase activity levels in the population with Down's syndrome.

Table 1. SOD activity in DS patients expressed in U/ml of blood.

| | Control* | | | Complete trisomy 21 | | | Partial trisomy 21 | | | Not karyotyped | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | no. | X | SD | no. | X | SD | no. | X | SD | no. | X | SD |
| Total | 2139 | 4.50 | 0.99 | 57 | 6.38 | 0.76 | 51 | 5.08 | 1.20 | 152 | 5.75 | 1.04 |
| 2–4 years | 255 | 5.14 | 1.08 | 6 | 6.68 | 1.18 | 7 | 5.53 | 2.13 | – | – | – |
| 5–9 years | 206 | 4.78 | 1.01 | 13 | 6.29 | 0.92 | 16 | 4.87 | 0.64 | – | – | – |
| 10–14 years | 96 | 4.66 | 1.13 | 38 | 6.37 | 0.63 | 28 | 5.10 | 1.16 | – | – | – |
| 15–19 years | 369 | 4.14 | 0.79 | – | – | – | – | – | – | 76 | 5.84 | 1.12 |
| 20–29 years | 756 | 4.49 | 0.92 | – | – | – | – | – | – | 53 | 5.63 | 0.88 |
| 30–45 years | 457 | 4.07 | 1.01 | – | – | – | – | – | – | 23 | 5.76 | 1.10 |

X = mean, SD = standard deviation, * unpubl. results.

ing a duplication involving only the 21q22.3 region, CuZnSOD activity being normal. Other observations by Miyazaki [19] and Jeziorowska [20] seemed to confirm the existence of clinical features of trisomy 21 with normal CuZnSOD. Thus we concluded that the 21q22.1 segment is not the critical region responsible for DS. This finding is important, in that it will direct research away from an unfruitful area.

Acknowledgements. This study was supported in part by a grant from the Plan Regional de Investigación de la Comunidad Autonoma de Madrid (CAM).

1 Lejeune J., Gautier M. and Turpin R. (1959) Etude des chromosomes somatiques de 9 enfants mongoliens. C. R. Acad. Sci. Paris **248:** 1721–1728
2 Hook E. B. (1981) Down's syndrome-frequency in human populations and factors pertinent to variation in rates. In: Trisomy 21 (Down's Syndrome): Research Perspectives, pp. 3–68, de la Cruz F. F. and Gerarld P. S. (eds), University Park Press, Baltimore
3 Epstein C. J. (1986) The Neurobiology of Down's Syndrome, Raven Press, New York
4 Fridovich I. (1986) Advances in enzymology and related areas of molecular biology. In Superoxide Dismutases, vol. 58, pp. 61–97, Meister A. (ed.), John Wiley & Sons Inc., New York
5 Francke U. (1981) Gene dosage studies in Down's syndrome: a review. In: Trisomy 21 (Down's Syndrome): Research Perspectives, pp. 237–251, de la Cruz F. F. and Gerarld P. S. (eds), University Park Press, Baltimore
6 Sherman L., Dafni N., Lieman-Hurwitz J. and Groner Y. (1983) Nucleotide sequence and expression of human chromosome 21-encoded superoxide dismutase mRNA. Proc. Natl Acad. Sci. USA **80:** 5465–5469
7 Badwey J. A. and Karnovsky L. M. (1980) Active oxygen species and the functions of phagocytic leukocytes. Ann. Rev. Biochem. **49:** 695–726
8 Cerutti P. A. (1985): Prooxidant states and tumor promotion. Science **227:** 375–381
9 Halliwell B. and Gutteridge J. M. C. (1985) Free radicals in biology and medicine, Clarendon Press, Oxford
10 Balazs R. and Brooksbank B. W. L. (1985) Neurochemical approaches to the pathogenesis of Down's syndrome. J. Ment. Defic. Res. **29:** 1–14
11 Groner Y., Lieman-Hurwitz J., Dafni N., Sherman L., Levanon D., Bernstein Y., Danciger E. and Elroy-Stein O. (1986) Molecular structure and expression of the gene locus on chromosome 21 encoding the Cu/Zn-superoxide dismutase and its relevance to Down's syndrome. Ann. N. Y. Acad. Sci. **450:** 133–156
12 Minami M. and Yoshikawa H. (1979) A simplified assay method of superoxide dismutase activity for clinical use. Clin. Chim. Acta **92:** 337–342
13 McCord J. M. and Fridovich I. (1969) Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. **244:** 6049–6055
14 Aula P., Leisti J. and Von Koskull H. (1973) Partial trisomy 21. Clin. Genet. **4:** 241–251
15 Sinet P. M., Couturier J., Dutrillaux B., Poissonier M., Raoul O., Rethore M. O., Allard D., Lejeune J. and Jerome H. (1976) Trisomie 21 et superoxide dismutase 1 (IPO-A): tentative de localisation sur la sousbande 21q221. Exp. Cell. Res. **97:** 47 55
16 Hagemeijer A. and Smit E. M. E. (1977) Partial trisomy 21: further evidence that trisomy of band 21q22 is essential for Down's phenotype. Hum. Genet. **38:** 15–23
17 Kedziora J., Bartorz G., Keyko W. and Rosynkawa D. (1979) Dismutase activity in traslocation trisomy. Lancet **1:** 105
18 Mattei J. F., Mattei M. G., Baeteman M. A. and Giraud F. (1981) Trisomy 21 for the region 21q223: identification by high resolution R banding patterns. Hum. Genet. **56:** 409–411
19 Miyazaki K., Yamanaka T. and Ogasawara N. (1987) A boy with Down's syndrome having recombinant chromosome 21 but no SOD-1 excess. Clin. Genet. **32:** 383–387
20 Jeziorowska A., Jakubowski L., Lach J. and Kaluzewski B. (1988) Regular trisomy 21 not accompanied by increased copper-zinc superoxide dismutase (SOD-1) activity. Clin. Genet. **33:** 11–19