rums to FSH (FSH-antiserum S6) and rat antiserum to LH (LH-antiserum S3). Assays were done according to the NIAMDD prescriptions. Each sample was assayed in duplicate or triplicate at different dilutions. Influence of cell suspension medium or culture medium was negligible. The measured FSH and LH values are only relative values; they are expressed in terms of the NIAMDD standard preparations FSH-RP-1 and LH-RP-1 which differ in degree of purity among each other.
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Gene Dose Effect: Regional Mapping of Human Nucleoside Phosphorylase on Chromosome 14

Abstract. *Quantitative analyses of erythrocyte nucleoside phosphorylase in four unrelated cases of partial trisomy 14 indicate that the structural gene for this enzyme maps in the chromosome region 14q11→14q21.*

Determination of the subregional location and linear order of genes on human chromosomes is a first step toward the formulation of a comprehensive human gene map. One approach to intrachromosomal gene mapping utilizes the expected correlation between the number of copies of a given gene (allele) and the amount or activity of gene product detected. Quantitative analyses on cells carrying a well-defined duplication or deletion of a chromosome segment permit the localization of genes within the affected region. Such gene-dose studies have also been used successfully for the detection of carriers for many autosomal recessive diseases (*1*). To date, however, only a few genes have been regionally assigned on the basis of a gene-dose relationship (*2*). Here, we report the use of this approach to define more precisely the intrachromosomal location of the gene for nucleoside phosphorylase (NP). A report concerning a child with a severe defect in T-cell–mediated immunity, whose erythrocytes lack NP activity (*3*), has led to clinical interest in this enzyme.

The enzyme NP (purine-nucleoside: orthophosphate ribosyltransferase, E.C. 2.4.2.1) catalyzes the phosphorolytic cleavage of inosine to hypoxanthine, and has been assigned to chromosome 14 (*4*). Recently, Francke *et al.* (*5*), have presented evidence for the localization of NP to region 14pter→14q21 [that is, the region between the terminus of the short arm (p) and band 21 on the long arm (q)] (*6*) by interspecific hybridization of cells containing a balanced reciprocal X-autosome translocation t(X;14)(p22;q21). In order to obtain additional evidence for this assignment, to define more precisely the chromosomal location of NP, and to get a better understanding of the gene-dose relationship for this enzyme, we have measured red blood cell NP activity in four individuals with a duplication of nonidentical segments of chromosome 14. Partial karyotypes of balanced translocation carriers and affected individuals from four different families are shown in Fig. 1. The extent of the duplicated segment in each case is illustrated in Fig. 2.

Blood collected from 61 males and females, aged 1 day to 52 years, served as the control group. The controls included 39 individuals with a normal karyotype, 8 with a balanced translocation, and 14 with a chromosomal imbalance not involving chromosome 14. The activity of NP was stable in blood samples stored at 4°C in 1 ml of acid citrate dextrose for at least 3 weeks, but most samples were assayed within 7 days of collection. After removal of plasma and buffy coat by centrifugation, the red cells were washed twice in isotonic saline, and lysed by the addition of ten volumes of distilled water; the red cell debris was removed from the hemolyzate by centrifugation at 18,000 rev/min for 20 minutes. For quantitative measurements of NP activity we used a slight modification of the method of Hopkinson *et al.* (*7*). The assay mixture contained 0.05*M* sodium phosphate buffer

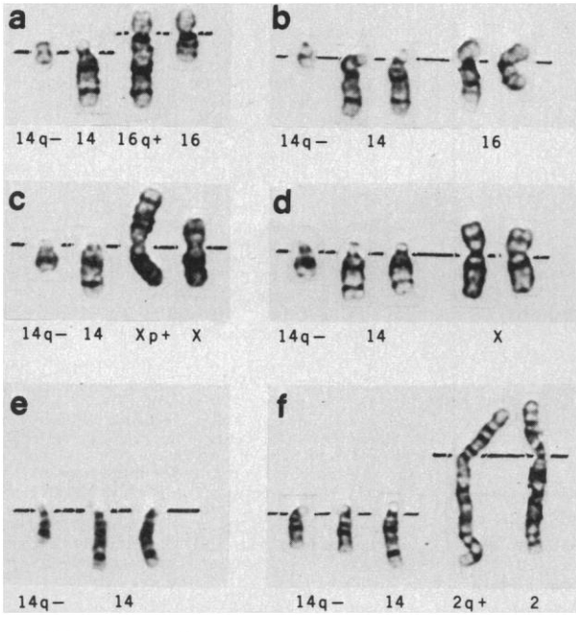

Fig. 1. (a) Balanced reciprocal translocation between long arms of chromosomes 14 and 16, designated t(14;16)(q11;q24) in phenotypically normal mother of subject B.R. (b) Partial karyotype of B.R., a 3-year-old girl with developmental delay and unusual phenotype, who has 47 chromosomes. The extra small metacentric chromosome consists of short arm, centromere, and a minute region of the long arm of chromosome 14 with the telomere band from the long arm of chromosome 16 attached. (c) Balanced reciprocal translocation t(X;14)(p22;q21) between the long arm of chromosome 14 and the short arm of an X chromosome in the phenotypically normal mother of C.M. (d) Partial karyotype of C.M., a 6-year-old girl with severe mental retardation and phenotypic abnormalities, who has 47 chromosomes. The extra small acrocentric chromosome consists of short arm, centromere, and the proximal third of the long arm of chromosome 14 as well as of the telomere region of the short arm of the X chromosome. (e) Partial karyotype of a reported patient (V.J.) with a 47,XX,+(14q−) karyotype. The extra 14q− chromosome is inherited from the t(14;20)(q22;q13) carrier mother and contains a slightly longer portion of the long arm of chromosome 14 than in patient C.M. (*12*). (f) Partial karyotype of S.C., a 5-year-old male who also has the "trisomy 14q− syndrome" with developmental retardation. He has inherited from his father the balanced reciprocal translocation t(2;14)(q37;q24). Due to nondisjunction during meiosis, he has received an additional copy of the 14q− chromosome. Therefore, he has 47 chromosomes with the extra acrocentric chromosome consisting of short arm, centromere, and slightly more than half the proximal long arm of chromosome 14, with the telomere region of the long arm of chromosome 2 attached.

(*p*H 7.5), 0.05 to 0.1 unit of xanthine oxidase (E.C. 1.2.3.2; Boehringer) per milliliter, and 0.2 m*M* inosine in a final volume of 2 ml. The reaction was initiated by the addition of 50 μl of a 1:50 dilution of hemolyzate. The increase in absorbance at 293 nm, caused by the production of uric acid, was monitored for 15 to 20 minutes at 25°C in a Gilford 2400S recording spectrophotometer. A unit of activity is defined as the conversion of 1 μmole of substrate per minute, and the results are expressed in terms of units per gram of hemoglobin. Hemoglobin was determined by the cyanmethemoglobin method (*8*). Red cell adenosine deaminase (E.C. 3.5.4.4) was measured as described by Chen *et al.* (*9*). Adenylate kinase (E.C. 2.7.4.3) and glutathione reductase (E.C. 1.6.4.2) activities were measured as described by Bergmeyer (*10*). Chromosome analysis was performed as previously described (*5*).

The mean red cell NP activity in the control group was 12.66 ± 3.1 units per gram of hemoglobin, with a range of 7.87 to 17.99 (Fig. 3). We found no difference in NP activity based on the age or the sex of the donors. The NP activities of S.C., V.J., and C.M., individuals having a duplication of different regions of chromosome 14 as indicated in Fig. 2, were 21.82, 18.90, and 21.91 units per gram of hemoglobin, respectively. These values represent 172, 149, and 173 percent, respectively, of the mean red cell NP activity of the control group. The activities of adenylate kinase, glutathione reductase, and adenosine deaminase in the red cells were also determined for these three individuals. In contrast to the elevated NP values, the activities of these enzymes, which have been assigned to chromosomes other than 14, were within normal limits. The levels of NP in hemolyzates from three patients with trisomy 21, one with trisomy 18, and eight with a partial duplication of the short arm of chromosome 20, were all close to the mean NP value. Therefore, the elevated NP activity found for S.C., V.J., and C.M. is probably not due to a generalized gene or chromosome imbalance, but it is consistent with the presence of the NP gene in region 14pter→14q21.

To further define the location of NP, we measured the red cell activity of this enzyme in case B.R., who has a duplication of region 14pter→14q11. The NP value for B.R. is 9.4 units per gram of hemoglobin, a value well within the normal range. Therefore, NP is probably not located in the duplicated region 14pter→14q11. Creagan *et al.* (*11*), using clones of mouse × human hybrid cells involving a t(14q22q) translocation chromosome, found concordant segregation of human NP expression and the translocation chromosome. They concluded that the gene for NP is on the long arm of chromosome 14. However, the presence or absence of a normal human chromosome 14 in these clones was not reported. Our finding that nucleoside phosphorylase activity is not elevated in hemolyzates from patient B.R. provides additional evidence for the assignment of NP to the long arm of chromosome 14.

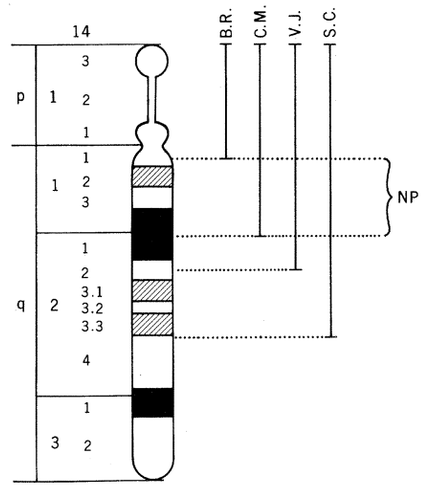

Fig. 2. Diagrammatic representation of the major and minor G bands of chromosome 14 [modified from (*6*)]. The extent of the duplicated segments in the four patients (B.R., C.M., V.J., and S.C.) is indicated by the vertical lines. The probable regional location of NP, based on results from this study, is also shown.

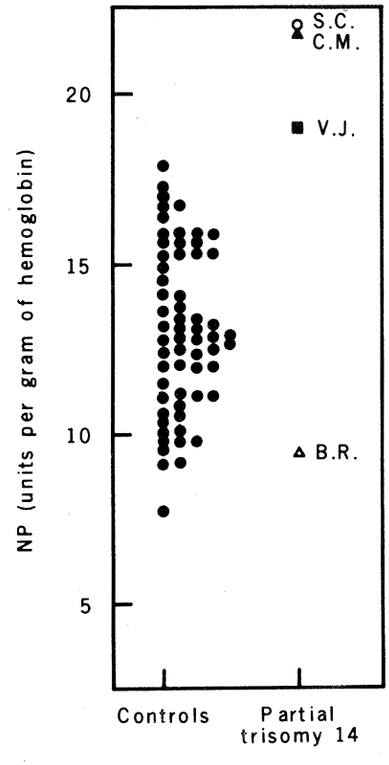

Fig. 3. Erythrocyte NP activities in controls and four individuals with trisomy 14q−.

Therefore, the quantitative NP measurements presented here, together with the data of Francke *et al.* (*5*) from studies of Chinese hamster × human somatic cell hybrids, indicate that human NP is located in region 14q11→14q21. Further, we have found that S.C., V.J., and C.M. have red blood cell NP values corresponding to approximately 150 to 170 percent of the normal mean. Giblett *et al.* (*3*) have reported that the parents of a child whose erythrocytes lacked detectable NP activity, and who are presumably heterozygous for a "silent" allele at the NP structural gene locus, have red cell NP activity below 50 percent of the normal mean. Together, these findings indicate that, at least for NP activity expressed in red blood cells, the amount of enzyme activity is essentially proportional to the number of alleles present.

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