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Genetics

Gene dosage: Evidence for assignment of erythrocyte acid phosphatase locus to chromosome 2

(trisomy/translocation/polymorphism/malformations)

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ABSTRACT **A child, trisomic for the distal short arm of chromosome 2 due to a familial 2/18 translocation, has elevated levels of activity of erythrocyte acid phosphatase [orthophosphoric-monoester phosphohydrolase (acid optimum), EC 3.1.3.2] Ferguson-Smith *et al.* [(1973) *Nature New Biol.* 243, 271–274] previously had found decreased levels of activity and loss of expression of an erythrocyte acid phosphatase allele in a subject who lacked one of the two homologous regions containing the distal three bands of chromosome 2. They suggested that the locus for erythrocyte acid phosphatase is located on that segment. Our findings provide further evidence for this assignment and also suggest an *in vivo* gene dosage effect of this autosomal locus, which depends on both the type and number of alleles present.**

The genetic locus for erythrocyte acid phosphatase (orthophosphoric-monoester phosphohydrolase (acid optimum), EC 3.1.3.2.) was provisionally assigned to the short arm of chromosome 2 by Ferguson-Smith *et al.* (1). They studied a child with a chromosome deletion due to a familial reciprocal translocation of the short arm of chromosome 2 and the long arm of chromosome 5. The proband had concurrent loss of a parental acid phosphatase allele, and of the distal three bands of the short arm of chromosome 2. There was also a 45% reduction in acid phosphatase activity. This assignment is consistent with the segregation of acid phosphatase in seven families with structural rearrangements involving chromosome 2 (2). Using spontaneous chromosome rearrangements in human–Chinese hamster somatic cell hybrids, Hamerton *et al.* (3) have recently localized the erythrocyte acid phosphatase gene to the p23 band of chromosome 2. We have studied a child who is trisomic for the region containing the distal three bands of chromosome 2 due to a familial 2/18 translocation.

The patient was born to a 28-year-old mother and a 39-year-old father after a 41-week gestation. Birth weight was 3.7 kg and length was 53 cm. At 6 months of age, unusual facies, physical abnormalities, and delayed development were apparent. At 9 months of age, she weighed 6.8 kg, and measured 63.5 cm in length, and 43 cm in head circumference, all below the third percentile. She had deep set eyes, epicanthal folds, a wide nasal bridge, variable left esotropia, bilateral nasolacrimal duct obstruction, pale optic discs, redundant skin of the posterior neck, widely spaced nipples, a grade II/VI systolic murmur at the left sternal border, long and tapered digits, whorl patterns on all finger tips, elevated skin pads at the base of all fingers, and increased carrying angle at the elbows. She was lethargic and hypotonic, but had a tonic neck reflex, and absence of protective response. She did not reach for objects or follow visual or auditory stimuli with head movement. She could not sit without support. Evaluation with the Denver Developmental Scale (4) indicated that her behavior was at the 4 month level. Her sibship (Fig. 1) includes one normal sibling. She also has one normal half-sibling by a different father. No other family members have developmental or physical anomalies like those described for the proband.

Abbreviations used for the three common alleles at the erythrocyte acid phosphatase locus are: P^A, allele for A genotype; P^B, allele for B genotype; P^C, allele for C genotype.

Cytogenetic methods and results

Metaphase chromosome preparations were obtained from peripheral blood lymphocyte cultures by standard techniques (5). Quinacrine mustard staining (6) was used to identify specific fluorescent banding patterns which characterize each chromosome. Her mother has no cytogenetic abnormality. Extra chromosomal material on the short arm of one chromosome 18 is present in the proband and her father (Fig. 2); he has a balanced translocation of the region containing the distal three bands of the short arm of one chromosome 2 to the short arm of one chromosome 18. There is no evidence from banding analysis for a reciprocal exchange. As Fig. 3 illustrates, the proband is therefore thought to be trisomic for the portion of the short arm of chromosome 2 which contains bands 23, 24, and 25.

Erythrocyte acid phosphatase methods and results

Twenty-six polymorphic genetic markers were determined by conventional methods (7–9). The results for erythrocyte acid phosphatase are informative. Erythrocyte samples were washed in 0.15 M saline and stored at minus 50° after mixing with a 2-mercaptoethanol solution, and erythrocyte acid phosphatase phenotypes were determined by horizontal starch gel electrophoresis as described by Karp and Sutton (10). The proband has a BA phenotype, her father is type BB, and her mother, BA. Since the electrophoretic system

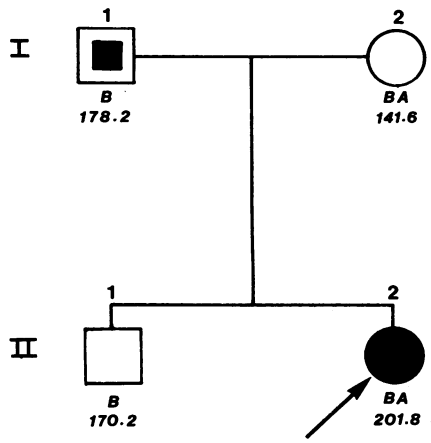

FIG. 1. Family pedigree including translocation carrier father, ■; mother, ○; sibling, □, and trisomic proband, ●.

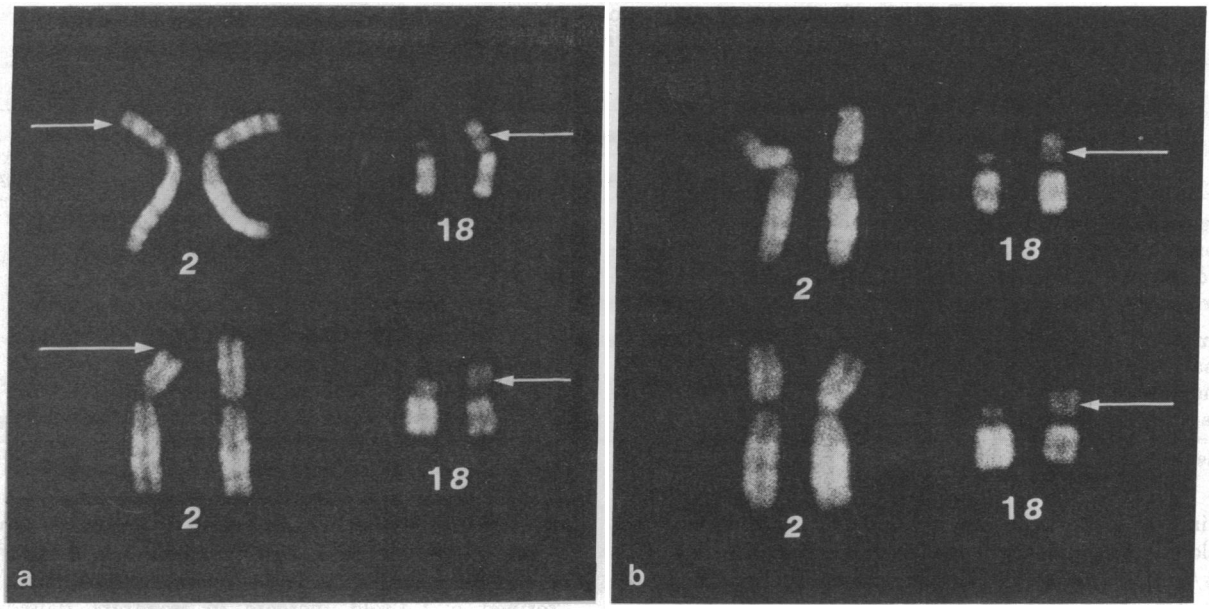

FIG. 2. Representative chromosomes 2 and 18 stained with quinacrine mustard: (a) from the balanced carrier father, (b) from the trisomic proband.

for distinguishing acid phosphatase phenotypes does not rule out the possibility that the proband is BAB, quantitative measurements of this enzyme were undertaken.

Blood samples were collected in heparin or acid-citrate-dextrose, and washed three times in phosphate buffered 0.15 M saline, pH 7.4. The acid phosphatase activities of washed erythrocytes stored at 4° in acid-citrate-dextrose did not change for at least 6 weeks and all results were obtained from samples stored for less than this time. A 0.1 ml volume of packed erythrocytes was added to 2.4 ml of hemolyzing reagent (1 part 0.05 M triethylamine at pH 7.4, 2 parts of 0.15 M saline, and two drops of Nonionox per 50 ml). Erythrocyte acid phosphatase activity was measured by a method modified after that described by Bessey *et al.* (11) and by Hopkinson *et al.* (12). Equal amounts of 0.1 M citrate buffer at pH 6.0, 1 mM in $MgCl_2$, and of 0.01 M disodium *p*-nitrophenylphosphate in 1 mM HCl were mixed and placed in ice. A 0.5 ml volume of this substrate mixture and 0.05 ml of hemolysate, water, or *p*-nitrophenyl standard (Sigma, St. Louis, Mo.) were incubated for 30 min at 37°. The reaction was then stopped by adding 0.5 ml of 10% trichloroacetic acid and placing the tubes in ice. Five milliliters of cold 1 N NaOH were added to each tube, and the optical density was read at 415 nm using the water tube as a blank. After addition of three drops of concentrated HCl, the optical density at the same wave length was determined. The difference in optical density was converted to μM of *p*-nitrophenyl liberated and expressed as units of activity per gram of hemoglobin. Standard hematologic parameters (13) for all family members including hemoglobin, erythrocyte count, hematocrit, and reticulocyte count were determined and found to be normal.

In Table 1 our quantitative erythrocyte acid phosphatase results for the proband, her parents, 15 age matched controls, and 19 adult controls are compared with the results from two published series. The age matched controls include eight subjects who are trisomic for chromosome 21. Our results for age matched controls are slightly lower than the adult controls, which are comparable to the previously published values (14, 15). Within the age matched group, those with trisomy for chromosome 21 do not differ from euploid subjects with the same acid phosphatase phenotype. The proband, who is trisomic for the distal part of the short arm of chromosome 2, has a BA phenotype, and an acid phosphatase activity more than five standard deviations higher than both adult and age matched controls.

Hopkinson *et al.* (12) have presented evidence that the P^A, P^B, and P^C alleles at the acid phosphatase locus account for additive activities in a ratio of 2:3:4. Our results are consistent with this observation, and would assign 58 and 80 units of acid phosphatase activity per gram of hemoglobin to the P^A and P^B alleles, respectively. A BAB phenotype, ac-

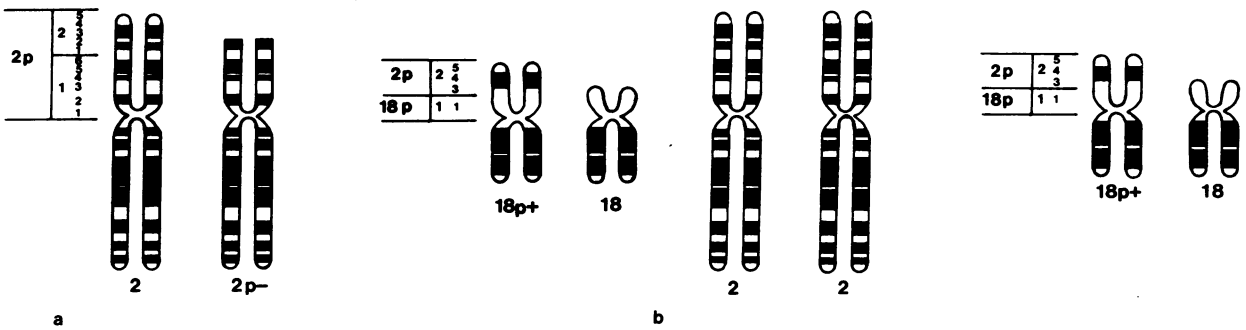

FIG. 3. Diagram modified from 1971 Paris Conference idiogram (38) of chromosomes 2 and 18 illustrating the bands involved in the translocation: (a) in the balanced carrier father, (b) in the trisomic proband.

Table 1. Quantitative measurement of erythrocyte acid phosphatase (units/g of hemoglobin ± SD)

Source	Phenotype					
	Observed			Theoretical		
	AA	BA	BB	P^A	P^B	BAB
Adult controls						
Spencer *et al.*(14)	122.4 ± 16.8	153.9 ± 17.3	188.3 ± 19.5	61	94	
Modiano *et al.* (15)	123.3 ± 20.7	142.6 ± 20.3	162.7 ± 20.0	62	81	
Our results	121.0 ± 5.0	145.2 ± 9.8	174.8 ± 15.2	61	82	225
Age matched controls	114.9	138.8 ± 10.9	160.9 ± 15.4	58	80	218
I-1 (balanced translocation)			178.2 ± 7.7			
I-2 (normal)		141.6 ± 1.4				
Proband (trisomic, 2p)						201.8 ± 10.4*

* Observed values for proband.

cording to this formulation, would have 218 units/g of hemoglobin. The proband's observed value of 201.8 ± 10.4 units/g of Hb lies much closer to that predicted for a BAB phenotype than that for a BA phenotype (138.8 ± 10.9 units/g of Hb). Both her father, who carries the balanced translocation, and her mother, who has normal cytogenetic findings, have acid phosphatase activities consistent with their genotypes at this locus.

Several erythrocyte enzymes, notably hexokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1), aspartate aminotransferase or glutamic oxalic transaminase (L-aspartate; 2-oxoglutarate aminotransferase, EC 2.6.1.1), and glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: $NADP^+$ 1-oxidoreductase, EC 1.1.1.49) are known to have higher activities in reticulocytes than in senescent erythrocytes (16–18). The proband has a normal reticulocyte count and normal erythrocyte indices. A further effort to exclude a younger mean cell age as the reason for the increase in erythrocyte acid phosphatase was made by measuring a group of erythrocyte enzymes (Table 2). None of these has increased activity.

All of the above results are consistent with the idea that the proband is trisomic at the acid phosphatase locus, and has the genotype $P^BP^AP^B$.

DISCUSSION

The causes and effects of additional loci for specific genes as compared to the wild type have long been of interest. Stern in 1929 (19) described the effect of multiple doses of the X-linked "bobbed" allele in *Drosophila melanogaster* and found them to be cumulative. Stewart and Merriam (20) described a 1.4-fold increase in isocitrate dehydrogenase ($NADP^+$) [*threo*-D_s-isocitrate: $NADP^+$ oxidoreductase (decarboxylating), EC 1.1.1.42] in *Drosophila melanogaster* trisomic for the left arm of chromosome 3. A dose effect of the autosomal locus for xanthine oxidase (xanthine: oxygen oxidoreductase, EC 1.2.3.2) has also been reported (21). Spofford (22) has presented a theoretical treatment of the evolutionary effect of gene duplications and in particular the outcomes expected if alleles involved are heterotic. She refers to a direct relationship between gene dose and gene product as *unregulated* protein production and points out that this property applies to some but not all loci in *Drosophila*.

In murine species, rat fibroblast cell lines that are tetraploid have been shown to synthesize collagen at twice the rate of diploid cell lines (23). Similarly, heteroploid mouse cell lines, trisomic for chromosome 7, were found to have increased amounts of glucosephosphate isomerase (D-glucose-6-phosphate ketol-isomerase EC 5.3.1.9), and lines tetrasomic for the centromere proximal region of chromosome 1 had increased amounts of isocitrate dehydrogenase (EC 1.1.1.42) in proportion to the number of alleles for these two autosomal loci (24).

In man, carrier detection for autosomal recessive diseases

Table 2. Erythrocyte enzymes (international units/10^{10} erythrocytes)*

Enzyme	EC no.	Proband	Control (95% range)
Glucose 6-phosphate dehydrogenase	1.1.1.49	2.18	1.35—2.64
Phosphogluconate dehydrogenase	1.1.1.44	1.85	1.53—3.05
Gluthione reductase [NAD(P)H]	1.6.4.2	1.38	0.67—1.74
Hexokinase	2.7.1.1	0.14	0.11—0.31
Glucosephosphate isomerase	5.3.1.9	9.90	6.46—14.32
Phosphofructokinase	2.7.1.11	1.46	1.37—3.57
Triosephosphate isomerase	5.3.1.1	176	104—276
Glyceraldehydephosphate dehydrogenase	1.2.1.12	26.5	13.4—31.5
Phosphoglycerate kinase	2.7.2.3	30.9	25.2—59.5
Enolase	4.2.1.11	1.74	1.87—3.47
Pyruvate kinase	2.7.1.40	2.85	1.77—3.77
Gluthione peroxidase	1.11.1.9	4.48	2.31—5.27
Glutamate oxalate transaminase or aspartate aminotransferase	2.6.1.1	0.14	0.15—0.39

* Enzyme assays were carried out by means of previously described methods (37).

is based on the assumption of approximately half normal activities or amounts for the gene products assayed. Only a few human autosomal loci have been shown to have a dosage effect in individuals who lack or are trisomic for the locus. Lactate dehydrogenase (L-lactate: NAD^+ oxidoreductase, EC 1.1.1.27) on the short arm of chromosome 12 (25), erythrocyte acid phosphatase (EC 3.1.3.2) as reported by Ferguson-Smith *et al.* (1), and the α-chain of hemoglobin (26–28) which has not been placed on a specific chromosome, are examples of deletion. Superoxide dismutase (superoxide: superoxide oxidoreductase, EC 1.15.1.1) (29) and possibly 6-phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) (30, 31) are examples of dosage effect in individuals trisomic for chromosome 21. In addition, cell lines in culture show a dosage effect for antiviral protein on chromosome 21 (32). The results reported here confirm the placement for erythrocyte acid phosphatase on the distal short arm of chromosome 2, and its inclusion among loci which are *unregulated*.

Extensive data describing gene frequencies for the P^A, P^B, and P^C alleles at the acid phosphatase locus have been collected (33), but the structure and function of this enzyme are still unknown. Sensabaugh (34) has studied several kinetic properties and the tissue distribution of "red cell" acid phosphatase, based on these properties. Of the natural substrates he examined, flavin mononucleotide dephosphorylation has a low K_m. Several compounds with structural similarities to riboflavin act as inhibitors, and the enzyme is activated by adenine and adenine analogs in the absence of organic phosphate. He also was able to demonstrate its occurrence in other human tissues including kidney, liver, placenta, and brain at levels higher than can be accounted for by contamination with blood.

The increased activity of erythrocyte acid phosphatase in our proband is comparable to that of individuals homozygous for the P^C allele (35). Because P^C homozygotes do not share the widespread developmental anomalies that characterize our patient and two others (36) trisomic for the distal short arm of chromosome 2, it is unlikely that this locus can account for their phenotype. Other genetic loci carried on this chromosomal region need to be identified to explain the disturbed morphogenesis which they share.

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