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Duplication of Chromosome 10p: Confirmation of Regional Assignments of Platelet-Type Phosphofructokinase

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SUMMARY

A proband, clinically thought to have trisomy 10p, was found to have an inverted duplication of 10p [46,XY, inv dup(10)(qter→ p15.3::p15.3→p11.1:)]. The phenotypic findings and cytogenetic observations were supported by relevant biochemical studies. The activity of phosphofructokinase (platelet-type; *PFKP*), previously localized to 10p, and hexokinase-I (*HKI*), putatively on 10p, demonstrated 153% and 149% of control activity in the proband's fibroblasts. These gene-dosage effects confirmed the clinical and cytogenetic observations as well as the localization of *HKI* to 10p. Additionally, phosphofructokinase (PFK) and hexokinase (HK), which are control points in the glycolytic pathway, were shown to be syntenic.

INTRODUCTION

We describe here a newborn infant manifesting features suggestive of trisomy 10p, a discernible clinical syndrome [1, 2]. Because of the initial difficulties encountered in the cytogenetic identification of the de novo chromosomal abnormality in this patient, biochemical and immunochemical analyses of the isozymes of two key glycolytic enzymes assigned to chromosome 10 were performed [3–5] (i.e., platelet-type phosphofructokinase [E.C.2.7.1.11; *PFKP*], localized

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to 10p [6], and hexokinase-I [E.C.2.7.1.1.; *HKI*], whose regional assignment is still controversial [7, 8]).

Human PFK is under the control of three structural loci that encode for M (muscle-type), L (liver-type), and P (platelet-type) subunits, which are differentially expressed by various tissues; cultured fibroblasts express all three subunits with P and L types predominating [9, 10]. Random tetramerization of these subunits produces various homo- and heterotetrameric isozymes that are distinguishable from one another by ion-exchange chromatography and subunit-specific antibodies [9, 10].

Three distinct monomeric forms of HK (HK I-III) have been described in mammalian tissues that exhibit unique electrophoretic, chromatographic, and kinetic-regulatory properties [11]. The liver-specific, high K_m glucokinase has also been designated as *HKIV;* however, several lines of evidence indicate it to be a distinct isozyme (E.C.2.7.1.2) under separate genetic control. In contrast to mammalian PFK, the molecular and genetic basis for the multiplicity of mammalian HK is not known [11, 12].

Biochemical studies of the cultured fibroblasts from our patient revealed gene-dosage effects for both enzymes. Additional immunochemical studies of PFK isozymes strongly suggested that the increased enzymatic activity was secondary to a specific increase in the P subunit. The gene-dosage effects demonstrated by both *PFKP* and *HKI* activities suggested a duplication of 10p in this infant. Furthermore, these results also indicate that *HKI* is located on chromosome 10p, and, thus, these two control points of the glycolytic pathway [13] are syntenic.

CLINICAL REPORT

The proband, a Caucasian male of 31.5 weeks gestation, was delivered precipitously to unrelated parents (father's age 34; mother's age 30) because of occult cord prolapse and fetal distress. Birth length was 41 cm (25–50th percentile); birth weight was 1,620 g (50–75th percentile); occipitofrontal circumference was 30 cm (50–75th percentile). Examination revealed coarse facies, an 8–9-cm occipital cranial defect, a cephalohematoma, and low-set, posteriorly rotated ears. Limb anomalies included: bilateral congenital hip dislocation (the adductors were not tight with an acetabular index < 30 degrees), bilateral metatarsus adductus, hands held flexed at the wrist, and bilateral clinodactyly. There was chordee with hypospadius, bilateral cryptorchidism, bilateral inguinal hernias, and a deep sacral dimple. Neurological abnormalities noted in the neonatal period included: central hypotonia, peculiar jerking movements, spontaneous clonus of the upper limbs, motor dysfunction of the fourth and fifth fingers, and possible seizure activity. An EEG was abnormal, indicating bilateral involvement of the midtemporal areas. Hearing impairment was evident (50-decibel hearing loss in the left ear, 35-decibel loss in the right ear), as were bilateral optic atrophy and wandering eye movements. Also in the neonatal period, the proband demonstrated hypocalcemia, hyperbilirubinemia, hypotension, hypothyroidism, and lactose intolerance. A grade II/VI blowing systolic murmur and cardiomegaly were present; echocardiogram revealed the presence of a membrane within the left atrial cavity in addition to a moderately large atrial-septal defect.

He was hospitalized for bronchiolitis at age 5 months and developed pneumonia. At this time, signs of developmental delay were also apparent. Three months later he died of congestive heart failure.

Family history is positive only for mild metatarsus adductus on both sides; the proband's parents and half-sister were normal.

MATERIALS AND METHODS

Cytogenetics

Chromosomal preparations were obtained from both phytohemagglutinin-stimulated lymphocytes and cultured skin fibroblasts of the proband and from lymphocytes of both parents. Prometaphase chromosomes were prepared from synchronized fibroblast cultures using a modified amethopterin technique [14, 15]. Banding was achieved by a modified GTG procedure [16] and C-banding of fibroblast chromosomes by using a modified $Ba(OH)_2$ procedure [17].

Fibroblast Cultures

Four normal human diploid fibroblast strains were established from skin biopsies of controls. The proband's skin fibroblasts were designated FB KB, while FB 88 was a fibroblast cell strain from an individual known to possess an inherited duplication of 10p [(46,XX, −14, + der(10),t(10;14)(q11.23;q11.2)] [6]. Cells were harvested when cultures became approximately 80% confluent and were counted by the standard hemocytometer method.

Preparation of Cell Extracts

The harvested cells were washed three times in chilled phosphate-buffered saline, pH 7.4, and pelleted. Approximately 200 μl of chilled cell lysis buffer [50 mM potassium phosphate buffer, pH 8.0, containing 5 mM EDTA, 5 mM $(NH_4)_2SO_4$, 0.6 mM AMP, 3 mM dithiothreitol, 0.6 mM fructose 6-phosphate, and 10 mM KF] was added. The cells were disrupted by sonication (three 10-second bursts) using a Branson sonifier (model 185) at 30 W, followed by centrifugation at 10,000 *g* for 10 min. Supernatants were used for the enzymatic assays and the immunoprecipitation and chromatographic studies to determine PFK isozymic profiles and for protein determination.

PFK and HK Activity Assays

PFK activity assays were performed with a Gilford model 260 spectrophotometer at 26°C as described [9]. One unit of PFK was defined as the amount of enzyme needed to convert 1 μmol of fructose 6-phosphate to fructose-1,6-diphosphate in 1 min. HK assays were performed in a final volume of 1.0 ml of 50 mM Tricine buffer, pH 8.1, containing 5 mM ATP, 12.5 mM $MgCl_2$, 2 mM glucose, 0.5 mM NADP, 1 mM dithiothreitol, and 0.2 U glucose-6-phosphate dehydrogenase; from the blanks, either glucose or HK was omitted. The reduction of NADP was monitored for 15–20 min at 340 nm. All assays of fibroblasts were performed in duplicate on extracts freshly made as described above. The proband's fibroblasts were always investigated concurrently with two of the four normal human diploid fibroblast strains from the pool serving as controls. In addition, FB 88, exhibiting both trisomy 10p as well as a gene-dosage effect for PFK activity, was also concurrently studied to provide a positive control. The enzyme activity of fibroblasts was expressed as $U/10^{10}$ cells as well as mU/mg protein.

Subunit-Specific Antibodies to the PFK Subunits

Production and characterization of mouse monoclonal antibodies against the M and L subunits, and a mouse antiserum against the P subunit of PFK have been described in detail [6, 17].

Enzyme Immunoprecipitation of PFK Isozymes

Enzyme precipitation studies using all three types of antibodies were performed by the immunoprecipitation assay technique described [18]. Briefly, the diluted (1:200) monoclonal antibody-bearing ascites (anti-M or anti-L) or anti-P antiserum (1:64) was mixed with

diluted cell extract (0.06 U/ml); the control tubes contained diluted nonimmume mouse serum. The mixtures were incubated, and the soluble antigen-antibody complexes were precipitated by staphylococci-bearing protein A. The supernatants were assayed for residual enzyme activity: only precipitation values greater than 7% of the concurrent controls were considered significant [6]. Each fibroblast extract was tested in duplicate on at least two separate occasions.

Chromatographic Separation of PFK Isozymes

PFK isozymes from the FB KB fibroblasts were resolved chromatographically on a DEAE-Sephadex A-25 column as described [9, 10].

Protein Determinations

These were performed according to Lowry et al. [19] using bovine serum albumin as a standard.

RESULTS

Cytogenetics

In the proband, the metaphases studied (both lymphocytes [25 counts] and fibroblasts [50 counts]) demonstrated a modal number of 46 chromosomes with one chromosome 10 having an elongated short arm (10p+). Interpretation of the G-banded karyotype was compatible with an inverted tandem duplication of almost the entire 10p (fig. 1), and was designated as [46,XY,inv dup(10)(qter→p15.3::p15.3→p11.1:)]. C-banding indicated but a single positive region in this abnormal chromosome. Both parents were found to have normal chromosomes.

Enzyme Activities in Fibroblasts

PFK activity of the proband's fibroblasts was 669.38 U/10^{10} cells or 85.0 mU/mg protein, compared with 379.65 U/10^{10} cells or 55.3 mU/mg protein in control fibroblasts (table 1). In relative terms, PFK activity was 176% and 153% of the control fibroblasts when expressed on a per cell basis and on a per mg protein basis, respectively. This increase in PFK activity was comparable to that observed in concurrently tested known trisomy 10p cells (FB 88) [6]. Similarly, the HK activity levels were 135% and 149% of the control values when expressed on a per cell basis and on a per mg protein basis, respectively. Fibroblasts from the proband's parents were not available for study.

Subunit-specificity of Anti-PFK Antibodies

Each of the three antibodies exhibited strict subunit-specificity (i.e., anti-M, anti-L, or anti-P). Since a given antibody reacts with its respective subunit, whether contained within a homo- or heterotetramer, it precipitates all the tetrameric isozymes (intraspecific and interspecific) that contained at least one of the respective human subunits. Thus, anti-P antiserum precipitates not only P_4 isozyme, but also P_3L, P_2LM human isozymes, etc.

Enzyme Immunoprecipitation Studies

Table 2 lists the precipitation values of PFK from normal and FB KB fibroblasts with all three types of antibodies. The anti-P and anti-L antibodies precipitated

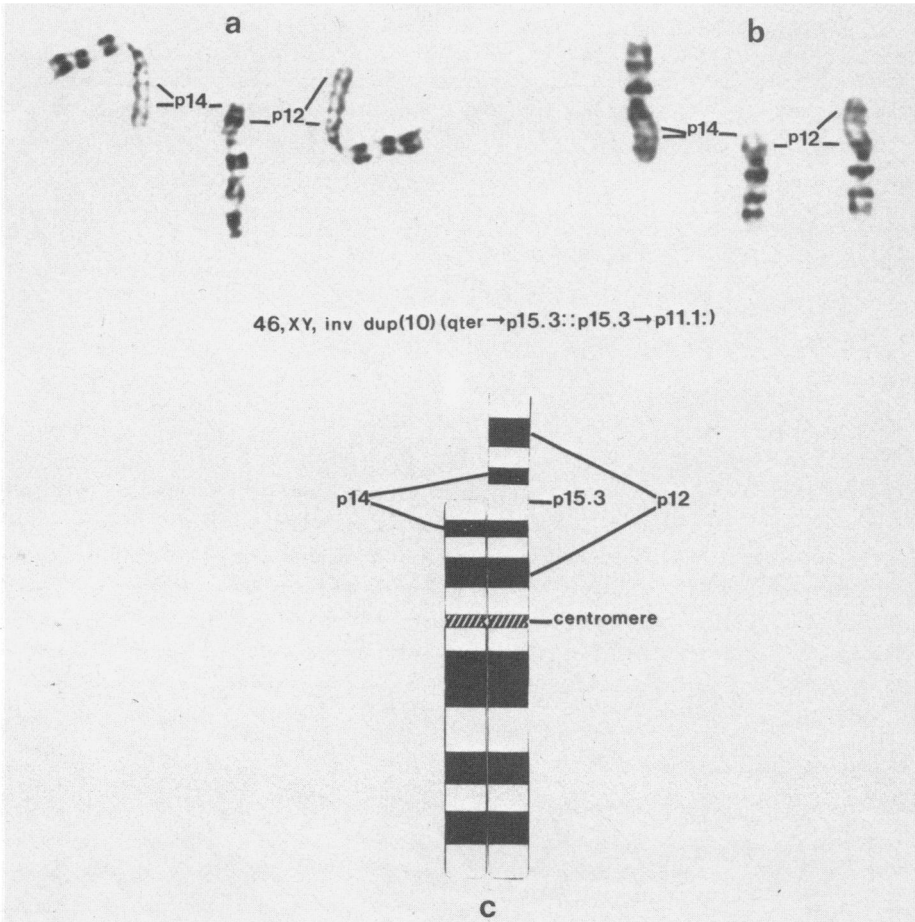

FIG. 1.—*a*, Partial GTG-banded prometaphase karyotype of a normal no. 10 (*center*) and two duplicated no. 10s; bands p12 and p14 are illustrated on both the normal homolog and the inverted tandem 10p duplication. *b*, Partial GTG-banded metaphase demonstrating the normal no. 10 (*center*) and additional short-arm material (10p+) indicating the duplication. *c*, Diagrammatic representation of the normal no. 10 (*left*) and the inverted tandem 10p duplication.

comparable amounts of PFK from both control and FB KB (patient's) fibroblasts. However, anti-M antibody, which usually precipitates a signficant amount of PFK from normal fibroblasts, consistently failed to precipitate PFK from FB KB, indicating marked reduction or absence of expression of the M subunit in the proband's cells. The FB 88 cells demonstrated a very low level of expression of *PFKM* (approximately 5% precipitation) as reported [6] (data not shown).

Chromatographic Studies

The isozymic composition of FB KB was not significantly different from that of normal fibroblasts (data not shown).

TABLE 1

PFK AND HK ACTIVITY VALUES OF THE PROBAND'S FIBROBLASTS

CELL STRAIN	PFK ACTIVITY		HK ACTIVITY	
	U/10^{10} cells	mU/mg protein	U/10^{10} Cells	mU/mg protein
Proband*	669.38 ± 50.25	85.0 ± 16.0	118.1 ± 9.81	15.6 ± 4.3
Controls†	379.65 ± 81.33	55.3 ± 18.0	87.0 ± 12.95	10.5 ± 2.2

NOTE: Data are mean ± SD. U = units.
* Five independent investigations on the proband's fibroblasts.
† Ten independent observations on four different control fibroblast cell lines.

DISCUSSION

Including the present proband, 40 cases of 10p duplication have been reported (for review, see [1, 2]). Although some phenotypic variation is apparent, these cases demonstrate a relatively specific MCA/MR syndrome. The major clinical findings include: severe mental, developmental, and growth retardation; a characteristic facies; central nervous system, ocular, renal, and genital abnormalities; and congenital heart disease. Skeletal anomalies found in this syndrome include: congenital hip dysplasia, flexion deformities of the hands and fingers, and clubfoot. Although our patient did not manifest all the typical facial features of trisomy 10p at birth, he did have a number of other malformations associated with the syndrome.

Of the 39 previously reported cases of trisomy 10p [1, 2], 33 resulted from malsegregation of familial translocations; two were due to familial pericentric inversions [20, 21]; two resulted from de novo translocations [22, 23]; one was due to a de novo partial duplication of 10p [46,XX,dup(10p)(pter→p12::p12::p12→qter)] [24]; and one was due to a supernumerary chromosome composed of 10p material [25]. Our proband represents only the second case with a de novo duplication, and the first with an inverted tandem duplication of the entire short arm of chromosome 10 [46,XY,inv dup(10)(qter→p15.3::p15.3→p11.1:)]. However, due to the inherent difficulties encountered in the identification of de novo chromosomal rearrangements, confirmation of the 10p duplication was attempted utilizing biochemical methods.

TABLE 2

IMMUNOPRECIPITATION VALUES OF PFK FROM THE PROBAND'S FIBROBLASTS

Cell strain	Anti-M	Anti-L	Anti-P
Proband*	0	59.09 ± 4.95	85.29 ± 2.55
Controls†	18.97 ± 10.44	69.09 ± 12.49	81.3 ± 13.43

NOTE: Data are mean ± SD. Values given are the percent of enzyme activity precipitated as compared to the control; each value represents the average of duplicate determinations on three separate occasions.
* Mean value from three independent investigations on the proband's fibroblast cell lines.
† Mean value from three independent observations on the three control fibroblast cell lines.

Gene-dosage effects for those enzymes definitively or presumptively localized to 10p were studied. Vora et al. [6] recently assigned the locus for the platelet-type isozyme of PFK to the short arm of chromosome 10 using both somatic cell hybrids and gene-dosage studies. In a cell line (FB 88) bearing a duplication of chromosome 10p (pter→cen), these authors demonstrated PFK activity values 180% of normal. Since normal human fibroblasts express all three PFK subunits with the platelet-type predominating, the gene-dosage effect in FB 88 was considered to result from a specific increase in the platelet-type PFK.

In contrast to *PFKP*, the regional assignment of human *HKI*, the only isozyme expressed in fibroblasts [3] and the major form (~90%) expressed in red cells [26], is still controversial [7]. Initial studies, using somatic cell hybrids, assigned *HKI* to chromosome 10 [3] with further regional assignment to 10pter→10q24 [4]. Since then, a number of investigators have reported a gene-dosage effect for HK in either red cells or fibroblasts of patients exhibiting various aberrations of chromosome 10 (see table 3). The results of these studies have been conflicting at best. In general, most suggest a long chromosomal region (10pter→10q24) as the likely site of *HKI* [4, 25, 27]. However, Dallapicolla's studies [22, 28] localize this gene to a very short segment of the short arm of chromosome 10. This assignment of *HKI* is contradicted by two studies: Rochon et al. [29] reported the locus to be in 10p11→10q24, and Gitelman and Simpson [7] reported it to be in 10p11→10q23. Junien et al. [30] localized *HKI* to 10pter→10q23 but believed that the gene may actually lie in band 10q23. We reasoned, therefore, that the demonstration of a gene-dosage effect for *HKI* in our patient might help resolve this controversy. In addition, such studies may also establish linkage between *PFKP* and *HKI*.

Enzymatic study of the proband's fibroblasts (FB KB) demonstrated approximately 50% increments in the activities of both PFK and HK (table 1). The immunochemical studies strongly suggest that the increment in PFK activity resulted from a specific increase in the P subunit. As previously demonstrated with another trisomy 10p cell strain (FB 88) [6], fibroblasts of the proband showed a nearly total lack of *PFKM* expression (table 2) and a normal isozymic profile.

TABLE 3

PUBLISHED ASSIGNMENTS FOR *HKI* TO HUMAN CHROMOSOME 10

Author/Reference	Method	Cell type	Chromosome/Region
Shows (1974) [3]	SCH	FB	Chromosome 10
Chern (1976) [4]	SCH	FB	10(pter→q24)
Sparkes et al. (1978) [27]	GDE	RBC	10(pter→q23)
Turleau et al. (1979) [25]	GDE	RBC	10(pter→q24)
Rochon et al. (1979) [29]	GDE	RBC	10(p11→q24)
Junien et al. (1979) [30]	GDE	RBC	10(pter→q23);10q23?
Dallapicolla et al. (1979) [22]	GDE	RBC	10(pter→p12)
Dallapicolla et al. (1981) [28]	GDE	RBC	10(pter→p13)
Gitelman and Simpson (1982) [7]	GDE	FB	10(p11→q23)
Present study (1983)	GDE	FB	10(pter→cen)

NOTE: SCH, somatic cell hybrid; FB, fibroblasts; GDE, gene-dosage effect; RBC, red blood cell.

The apparent lack of a specific increase in the P subunit results from both the inherent limitation of immunoprecipitation technique, which does not detect the absolute amount of the P subunit but only the P-containing isozymes, as well as from the natural predominance of the P subunit in cultured fibroblasts [10]. The latter fact results in a lack of observable increases in the P and P-containing isozymes in the patient's fibroblasts, despite a quantitative increase in total PFK activity. Since our proband has a duplication of almost the entire short arm of chromosome 10 (i.e., pter→p11.1), the dosage effect for HK (in FB KB) corroborates the studies of Dallapicolla et al. [22, 28], suggesting that the most likely assignment of *HKI* appears to be in 10pter→10p13 [22].

Thus, our data indicate that the loci for both *PFKP* and *HKI* are located on the short arm of chromosome 10. Since PFK and HK represent the primary and secondary control points of the glycolytic pathway [13], their synteny is of special significance. It is conceivable that their physical proximity on 10p may be involved in their coregulation in a number of pathophysiological states. For instance, in the experimental rat carcinomas, a specific expression of the isozyme pattern of HK, PFK, and pyruvate kinase (PK) is seen which is considered to be conducive to accelerated glycolysis [31], a characteristic of neoplasia [32]. A similar hypothesis has been suggested with respect to both orotate phosphoribosyltransferase and oritidine-5′-phosphase, which catalyze the conversion of orotic acid to uridine-5′-phosphase. *Both* enzymes demonstrate reduced activities in the hereditary orotic aciduria type I [33]. The studies performed on this patient clearly illustrate the successful integration of biochemical and cytogenetic methodologies to elucidate unresolved basic and clinical questions.

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REFERENCES

1. SLINDE S, HANSTEEN IL: Two chromosomal syndromes in the same family: monosomy and trisomy for part of the short arm of chromosome 10. *Eur J Pediatr* 139:153–157, 1982
2. GONZALEZ GH, BILLERBECK AEC, TAKAYAMA LC, WAJNTAL A: Duplication 10p in a girl due to a maternal translocation t(10;14)(p11;q12). *Am J Med Genet* 14:159–167, 1983
3. SHOWS TB: Synteny of human genes for glutamic oxaloacetic transaminase and hexokinase in somatic cell hybrids. *Birth Defects: Orig Art Ser* X(3):143–145, 1974
4. CHERN CJ: Localization of the structural genes for hexokinase-1 and inorganic pyrophosphatase on region (pter→q24) of human chromosome 10. *Cytogenet Cell Genet* 17:338–342, 1976
5. WEIL D, COTTREAU D, NGUYEN VAN CONG, ET AL.: Assignment of the gene for F-type phosphofructokinase to human chromosome 10 by somatic cell hybridization and specific immunoprecipitation. *Ann Hum Genet* 44:11–16, 1980
6. VORA S, MIRANDA AF, HERNANDEZ E, FRANCKE U: Regional assignment of the human gene for platelet-type phosphofructokinase (*PFKP*) to chromosome 10p: novel use of polyspecific rodent antisera to localize human enzyme genes. *Hum Genet* 63:374–379, 1983
7. GITELMAN BJ, SIMPSON NE: Regional mapping of the locus for hexokinase-1 (HKI) to 10p11→q23 by gene dosage in human fibroblasts. *Hum Genet* 60:227–229, 1982

8. MAGNANI M, DALLAPICOLLA B: Regional mapping of the locus for hexokinase-1 (HKI). *Hum Genet* 62:181, 1982
9. VORA S, SEAMAN C, DURHAM S, PIOMELLI S: Isozymes of human phosphofructokinase: identification and subunit structural characterization of a new system. *Proc Natl Acad Sci USA* 77:62–66, 1980
10. VORA S: Isozymes of human phosphofructokinase in blood cells and cultured cell lines: molecular and genetic evidence for a trigenic system. *Blood* 57:724–732, 1981
11. PURICH DL, FROMM HJ, RUDOLPH FB: The hexokinases: kinetic, physical, and regulatory properties. *Adv Enzymol* 39:249–326, 1973
12. COLOWICK SP: The hexokinases, in *The Enzymes,* vol IX, *Group Transfer,* part B, edited by BOYER FD, New York, Academic Press, 1973, pp 1–48
13. MINAKAMI S, YOSHIKAWA H: Studies on erythrocyte glycolysis. II. Free energy changes and rate limiting steps in erythrocyte glycolysis. *J Biochem* 59:139–144, 1966
14. VOGEL W, SCHEMPP W, SIGWARTH I: Comparison of thymidine, fluorideoxyuridine, hydroxyurea, and methotrexate blocking at the G1/S phase transition of the cell cycle, studied by replication patterns. *Hum Genet* 45:193–198, 1978
15. SCHEMPP W, SIGWARTH I, VOGEL W: Demonstration of replication patterns corresponding to G- and R-type banding of chromosomes after partial synchronization of cell cultures with BrdU or dT surplus. *Hum Genet* 45:199–202, 1978
16. DE GROUCHY J, TURLEAU C: *Clinical Atlas of Human Chromosomes.* New York, John Wiley, 1977
17. SUMNER AT: A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res* 75:304–306, 1972
18. VORA S, WIMS LA, DURHAM S, MORRISON SL: Production and characterization of monoclonal antibodies to the subunits of human phosphofructokinase: new tools for the immunochemical and genetic analyses of isozymes. *Blood* 58:823–829, 1981
19. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265–275, 1951
20. SEKHON GS, SLY WS: Trisomy 10p due to familial pericentric inversion. *Birth Defects: Orig Art Ser* XIV(6c):422–423, 1978
21. LANSKY-SHAFER SC, DANIEL WL, RUIZ L: Trisomy 10p produced by recombination involving maternal inversion inv(10)(p11q26). *J Med Genet* 18:59–61, 1981
22. DALLAPICOLLA B, CHERSSA L. VIGNETTI P, FERRANTE E, GANDINI E: Increased HKI activity levels in the red cells of a patient with a de novo trisomy 10p: t(Y;10)(p11;p12). *Hum Genet* 50:45–49, 1979
23. ALLER V, ABRISQUETA J, PEREZ-CASTILLOA A, DEL MAZO J, MARTIN-LUCAS MA, DETORESS ML: Trisomy 10p due to a de novo t(10p;13p). *Hum Genet* 46:129–134, 1979
24. FRYNS JP, DEROOVER J, HAEGEMANN J, VAN DEN BERGHE H: Partial duplication of the short arm of chromosome 10. *Hum Genet* 47:217–220, 1979
25. TURLEAU C, RETHORÉ M-O, JUNIEN C, LEJEUNE J, DE GROUCHY J: Mosaïque 46,XX/46,XX,10p−/47,XX,+r/47,XX,10p−,+r et phénotype de la trisomie partielle 10p. *Ann Genet (Paris)* 22:178–181, 1979
26. STOCCHI V, MAGNANI M, CANESTRARI F, DACHA M, FORNAIN G: Multiple forms of human red blood cell hexokinase, preparation, characterization, and age dependence. *J Biol Chem* 257:2357–2364, 1982
27. SPARKES RS, BASS HN, SPARKES MC: 10q(q23→qter) duplication: GOTs, HK1, and other gene markers. *Hum Genet* 42:267–270, 1978
28. DALLAPICOLLA B, SERENA-LUNGAROTTI M, MAGNANI M, DACHA M: Evidence of gene dosage effect for HK I in the red cells of a patient with trisomy 10pter→p13. *Ann Genet (Paris)* 24:45–47, 1981
29. ROCHON M, POWELL J, BLANCHARD R, PARE C, LEMIEUX B: La trisomie 10p: étude clinique et biochimique. *Union Med Can* 105:190–193, 1979

30. JUNIEN C, TURLEAU C, BUGNON C, BRESSON JL, ROCHE P, DE GROUCHY J: Localisation de TGOs en 10q24q262 et suggestion de localisation de HK-I en 10q23. *Ann Genet (Paris)* 22:50–52, 1979
31. TANAKA T, IMAMURA K, ANN T, TANIUCHI K: Multimolecular forms of pyruvate kinase and phosphofructokinase in normal and cancer tissues, in *Gann Monograph on Cancer Research,* vol 13, 1972, pp 219–234
32. WEBER G: Enzymology of cancer cells. *N Engl J Med* 296:541–557, 1977
33. HOWELL RR, KLINENBERG JR, KROOTH RS: Enzyme studies on diploid cell strains developed from patients with hereditary orotic aciduria. *Johns Hopkins Med J* 120:81–88, 1967