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Probable assignment of soluble isocitrate dehydrogenase (IDH_1) to 2q33.3

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Summary. Gene dosage effects for soluble isocitrate dehydrogenase (IDH_1) were investigated in four unrelated cases with abnormalities involving the long arm of chromosome 2. Case 1 was trisomic for 2q33.3→qter, Case 2 monosomic for 2q33.3→q35, Case 3 trisomic for 2q11.2→q24.2, and Case 4 monosomic for 2q23→q24.2. These abnormalities were de novo except in Case 1, where trisomy 2q resulted from a maternal translocation. The red cell IDH_1 levels were significantly reduced in Cases 1 (41.4% of normal value) and 2 (51.9%), while they were normal in Cases 3 and 4. The low IDH_1 level also in the father of Case 1 (43.6%), together with the common electrophoretic phenotype of IDH_1 in red cells as well as leukocytes, led us to suppose that Case 1 was really heterozygous for common and probable null alleles, and that the IDH_1 gene locus could be excluded from 2q33.3→qter. On the other hand, normal IDH_1 values in the parents of Case 2 were consistent with the hemizygosity for this locus in Case 2. The results suggested that the IDH_1 locus could be assigned to the 2q33.3 band, especially the proximal portion of it.

Introduction

NADP-dependent isocitrate dehydrogenase (E.C. 1.1.4.2) catalyzes the oxidative decarboxylation of L-isocitrate to α-ketoglutarate. The enzyme is known to exist in both soluble (IDH_1) and mitochondrial (IDH_2) forms. In humans, these two forms of IDH have been demonstrated to be under separate genetic control. Recently, somatic cell hybridization studies have assigned the IDH_1 locus to chromosome 2, specifically 2q32→qter (Hamerton et al. 1975; Weil et al. 1977). Since then, however, little advance has been achieved in regional mapping of this locus. Gene dosage studies in combination with high resolution banding analysis in a case with a certain chromosome alteration will make a more detailed localization of a specific gene marker possible. To our knowledge, there have been only two reports of cases with chromosome No. 2 abnormalities, where a gene dosage study of IDH_1 was performed (Jansen et al. 1982; Wyandt et al. 1982). This prompted us to study gene dosage effects for IDH_1 in four unrelated cases with partial trisomy or monosomy for the long arm of chromosome 2. The results suggested that the IDH_1 locus could be mapped to 2q33.3.

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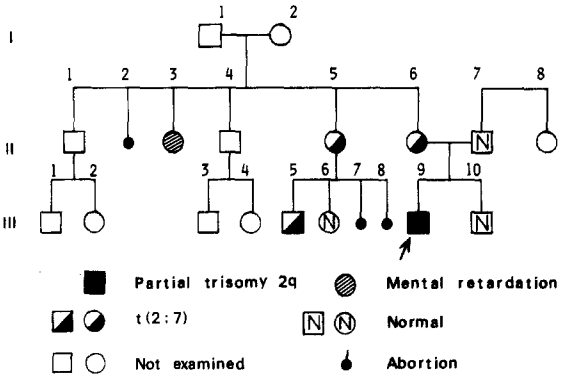

Fig. 1. Family pedigree of Case 1

Case reports

Case 1

This boy was first seen in our clinic at the age of 15 months because of dysmorphic features and psychomotor retardation. The family history revealed a maternal aunt with mental retardation (II-3 in Fig. 1) and another aunt with recurrent spontaneous abortions (II-5). His craniofacial dysmorphia, including square facies with prominent glabella, hypertelorism, antimongoloid palpebral fissures, broad nasal bridge and root with hypoplastic alae nasi, and thin upper lip, were in accordance with the partial trisomy 2q syndrome (Zankl et al. 1979).

Case 2

This girl was referred to us at the age of 4 years 5 months because of speech delay. She said several words with meaning at 2 years 6 months, but could not put words into sentences. Phenotypic abnormalities comprised only mongoloid palpebral fissures and internal strabismus. An IQ was estimated to be 70. At present, the patient is attending a normal school.

Case 3

This male infant was found to have the following malformations immediately after birth: hypertelorism, flat nasal bridge, cleft lip and palate, macrostomia, micrognathia, low-set and malformed ears, heart murmurs, widely spaced nipples, cryptorchidism, and abnormal implantation of the second, fourth, and fifth toes. He died at 4 months, following cardiac catheterization which demonstrated atrial septal defect and pulmonary hypertension.

Case 4

This case, a 6-year-old girl with multiple congenital malformations, will be published elsewhere (Takahashi et al. to be published).

Materials and methods

Peripheral blood lymphocytes were cultured using the conventional technique. Cells in prometaphase or prophase were collected according to the method of Ikeuchi and Sasaki (1979). In brief, ethidium bromide (at a final concentration of 10 μg/ml of growth medium) and Colcemid (0.05 μg/ml) were added to the cultures 2 h prior to harvest. Chromosome analysis was performed using GTG bandings.

Hemolysates were prepared from heparinized blood as described by Beutler (1975). Leukocytes were separated by sedimentation in an ACD-dextran-dextrose solution. After lysis of contaminating erythrocytes by distilled water, leukocytes were centrifuged and the cell pellet was lysed by freezing and thawing in 0.9% saline.

Red cell IDH activity was measured according to the method of Bergmeyer and Bernt (1974) with modifications. The reaction mixture contained 80 m*M* triethanolamine/HCl buffer (pH 7.5), 4.4 m*M* DL-isocitrate, 50 m*M* NaCl, 0.32 m*M* NADP, and 3.9 m*M* $MnSO_4$. Fifty microliters of 1:20 stroma-free hemolysate was used for each milliliter of the reaction mixture. NADP or $MnSO_4$ was omitted from the blanks. After the blood sample and buffer/substrate solution were allowed to stand at 25°C for 10 min, the reaction was started by the addition of NADP/$MnSO_4$ solution. The generation of NADPH from NADP was measured: the change in the optical density (OD) was followed at 340 nm, using a Hitachi double wavelength double beam spectrophotometer model 557 with a full scale of 0.1 OD. The reaction was linear at least for 30 min, and there was a correlation between enzyme quantity and activity in 1:10 to 1:80 hemolysate ($r = 1.0$). The coefficient of variations in this assay was 4.5% ($n = 7$).

Erythrocyte malate dehydrogenase (MDH_1) was assayed by the method of Bergmeyer and Bernt (1974), and acid phosphatase (ACP_1) by the method of Hopkinson et al. (1964). Lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PD), and 6-phosphoglucuronate dehydrogenase (6PGD) activities were also measured in red cells (Beutler 1975). Starch gel electrophoresis and specific staining of IDH_1 and ACP_1 were carried out as described by Harris and Hopkinson (1976). Enzymatic activities were assayed on the same day as the blood samples were collected.

Results

Cytogenetic studies

Case 1. One of chromosomes 7 was found to have an elongated long arm (Fig. 2a). The mother's karyotype showed a balanced translocation between the long arms of chromosomes 2 and 7 (Fig. 2b). The breakpoints appeared to be at 2q33.3 and 7q36.3. The karyotype of the case was 46,XY,−7,+der(7), t(2;7)(q33.3q36.3)mat. Shortly after the case was seen in our clinic, the mother became pregnant. Prenatal diagnosis at 16 weeks' gestation disclosed a male fetus with a normal karyotype (III-10 in Fig. 1). A maternal aunt (II-5) and her son

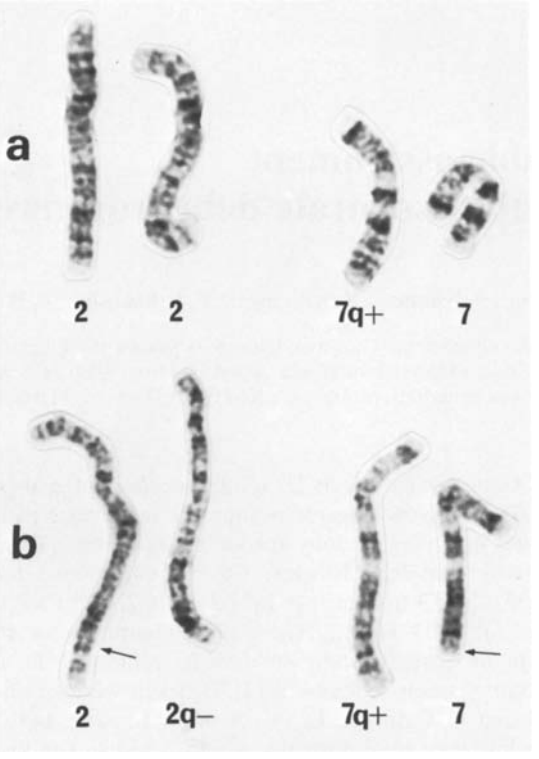

Fig. 2a, b. Partial G-banded karyotypes of Case 1 (**a**) and the mother (**b**). *Arrows* indicate the breakpoints

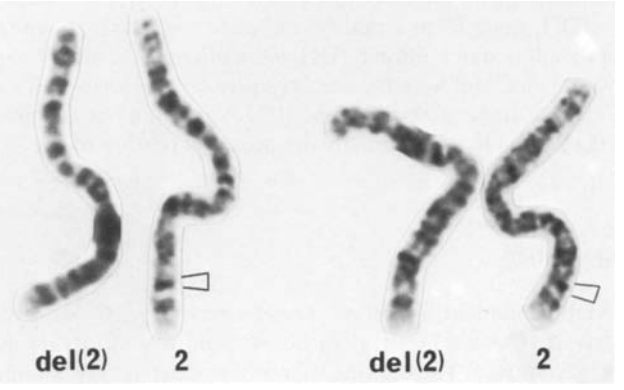

Fig. 3. Partial G-banded karyotypes of Case 2. *Brackets* indicate a segment deleted

(III-5) were also found to be a translocation carrier. Cytogenetic studies of other family members were not available.

Case 2. An interstitial deletion of the long arm of chromosome 2 was detected in all cells examined (Fig. 3). The breakpoints appeared to be at 2q33.3 and 2q35, and the karyotype was 46,XX,del(2)(q33.3q35). The parents had normal chromosomes.

Case 3. One of chromosomes 2 was found to have an extra amount of chromosome material in the proximal long arm (Fig. 4). From the banding patterns, the rearrangement was interpreted to be a direct tandem duplication of the segment 2q11.2→q24.2. The karyotype of the case was 46,XY,dir dup(2)(pter→q11.2::q11.2→q24.2::q11.2→qter). The parents' karyotypes were normal.

Case 4. The karyotype was 46,XX,del(2)(q23q24.2) de novo.

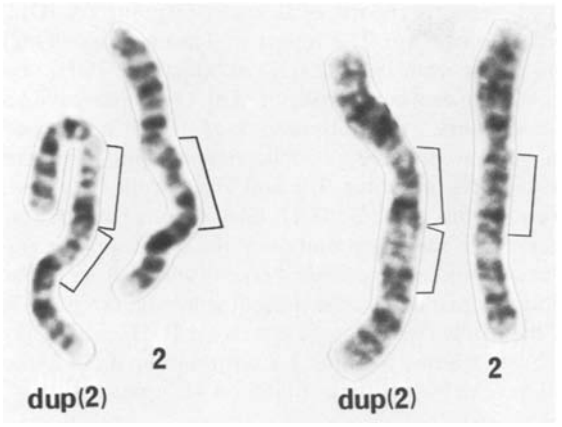

Fig. 4. Partial G-banded karyotypes of Case 3. *Brackets* indicate a segment duplicated

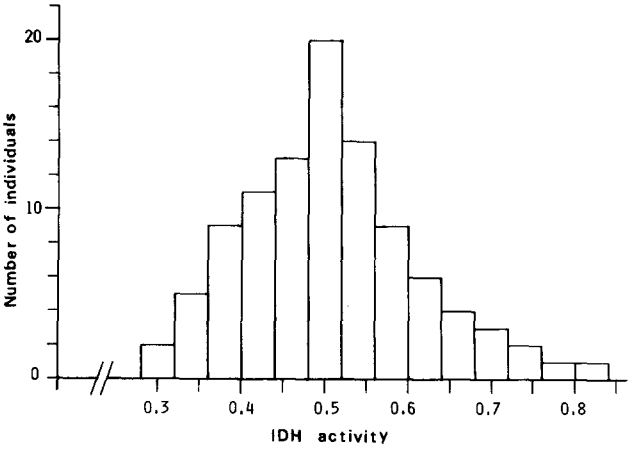

Fig. 5. IDH activities of red cells from 100 normal individuals. IDH activities were expressed in μmol/min/g Hb on the abscissa

Table 1. Erythrocyte IDH activity[a] and phenotype in the cases and their relatives

	IDH_1 activity	IDH_1 phenotype
Case 1	0.209[b]	1
Father	0.220	1
Mother	0.540	1
Sibling (III-10)	0.501	1
Aunt (II-5)	0.461	1
Cousin (III-5)	0.561	1
Cousin (III-6)	0.475	1
Case 2	0.262[b]	1
Father	0.492	1
Mother	0.537	1
Case 3	0.510	1
Case 4	0.479	1
Normal controls	0.505 ± 0.103	1

[a] Expressed as μmol/min/g Hb
[b] Mean of three separate measurements

Biochemical studies

The mean IDH activity of red cells from 100 unrelated individuals with an electrophoretically confirmed phenotype of IDH_1 1 was 0.505 ± 0.103 μmol/min/g Hb. The control values, ranging from 0.309 to 0.810 μmol/min/g Hb, scattered in a near normal distribution (Fig. 5). The results of IDH_1 assays in the cases and their relatives are shown in Table 1. The IDH_1 levels in Cases 3 and 4 were within normal ranges, while those in Cases 1 and 2 were significantly reduced: 0.209 μmol/min/g Hb (41.4% of normal value) in Case 1 and 0.262 μmol/min/g Hb (51.9% of normal) in Case 2. Since both parents of Case 2 had normal IDH_1 activity, the result in Case 2 could be compatible with the hemizygosity for the IDH_1 locus. On the other hand, the reduced IDH_1 level in Case 1 was difficult to explain. The father of Case 1 also had a low IDH_1 level (43.6% of normal), suggesting that the decrease in Case 1 was a consequence of inheriting a catalytically very low IDH_1 allele from the father.

Starch gel electrophoresis of red cell IDH showed that all cases had a phenotype of IDH_1 1. The intensity of the IDH band by specific staining in each case was proportional to the respective activity of IDH_1 assayed in red cells. In both Case 1 and the father, electrophoretic mobility of IDH_1 in leukocyte lysate was identical to that in red cells. Activities of other red cell enzymes, including MDH_1 and ACP_1 of which gene loci have been assigned to 2p, were found to be normal in all cases (Table 2).

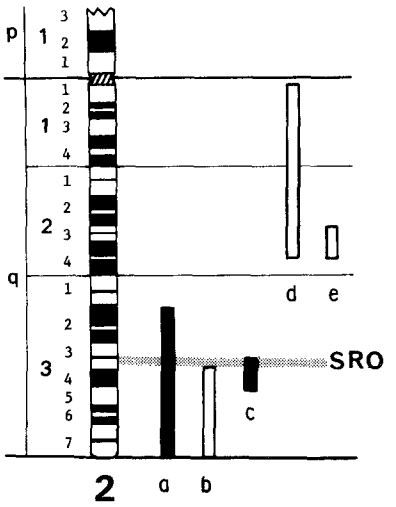

Fig. 6. Diagram of regional mapping of IDH_1. *Solid bars* represent the segment to which the IDH_1 locus was assigned, and *open bars* the segment from which the locus was excluded. SRO denotes the shortest region of overlap. *a* Weil et al. (1977), *b* Case 1, *c* Case 2, *d* Case 3, and *e* Case 4

Table 2. Activities[a] of erythrocyte enzymes other than IDH_1 in the cases

	Case 1	Case 2	Case 3	Case 4	Normal controls ($n = 20$)
ACP_1	147.0	167.8	203.8	145.1	186.7 ± 19.4 (B)
	(BA)[b]	(BA)	(B)	(BA)	157.3 ± 15.9 (BA)
MDH_1	77.7	92.1	84.0	81.6	82.1 ± 12.5
LDH	228.5	181.1	212.6	212.8	189.1 ± 24.2
G6PD	7.44	10.08	7.82	10.77	9.14 ± 1.77
6PGD	8.44	7.97	9.22	10.21	8.56 ± 1.54

[a] U/g Hb. In ACP_1, however, one unit was expressed as 1 μmol/30 min
[b] ACP_1 phenotype was given in parenthesis

Discussion

Our method of IDH_1 assay was simple and reproducible. The IDH_1 activity in normal individuals was slightly higher in the present study than in the previous ones: 0.505 ± 0.103 μmol/min/g Hb versus 0.35 ± 0.12 (Turner et al. 1974) or 0.31 ± 0.09 μmol/min/g Hb (Jansen et al. 1982). This is probably due to the differences in the method of assay, pH of buffer, or concentration of substrates used. In any case, the considerable variation in IDH_1 activity among different subjects indicates the necessity in gene dosage studies of IDH_1 to examine not only the patients but also their parents.

It was unexpected that in Case 1 despite trisomy for the distal long arm of chromosome 2, the IDH_1 level was below half of the normal value. In view of the fact that the father also had a low IDH_1 level, the most pertinent interpretation is as follows: the structural gene for IDH_1 was in fact not present on the segment 2q33.3→qter translocated onto the long arm of chromosome 7, while the normal chromosome 2 of paternal origin contained an allele resulting in very low enzyme activity, and the homologue of maternal origin a common IDH_1 allele.

What kind of a variant allele was involved in Case 1 and the father? Genetic variations of IDH_1 are relatively rare, and a total of five phenotypic variants are known at present: IDH_1 2-1, 3-1, 4-1, 5-1, and 6-1 (Chen et al. 1972; Turner et al. 1974). Of these variants, IDH_1 2-1, 3-1, or 4-1 have additional components which migrate more cathodally or anodally than does an isoenzyme of IDH_1 1. In contrast, IDH_1 5-1 and 6-1 are unique in that the products of alleles 5 and 6 are not detectable in red cells but present in other tissues including leukocytes, placenta, and fibroblasts. It was believed that the polypeptides from these variant alleles are less stable in vivo than the usual type, and accordingly that IDH activities of red cells from heterozygotes for these variants are much lower as compared with those from individuals with IDH_1 1 (Turner et al. 1974). Electrophoretic study of Case 1 and the father showed that IDH_1 in leukocytes lysate had the same electrophoretic mobility as that in red cells. This suggests that the allele in question may be a new allele or probable null allele rather than a variant allele 5 or 6. The incidence of rare variant heterozygotes in Caucasians was estimated to be 1 in 205 (Turner et al. 1974). The present study of red cell IDH from 100 unrelated Japanese revealed none with a rare variant. The findings suggested that the incidence of rare variant heterozygotes other than IDH_1 5-1 or 6-1 is also low in Japanese.

The normal IDH_1 levels in Cases 3 (trisomy for 2q11.2→q24.2) and 4 (monosomy for 2q23→q24.2) were in good agreement with the previous report of the assignment of the IDH_1 locus to 2q32→qter. The hemizygous deficiency of IDH_1 in Case 2 (monosomy for 2q33.3→q35), together with merely duplex gene dosage effects for IDH_1 (genotype 1-0?) in Case 1 (trisomy for 2q33.3→qter), argues strongly that the shortest overlapping region for the IDH_1 locus could be pinpointed to the 2q33.3 band, especially the proximal portion of it (Fig. 6).

The two previous reports of deletion mapping on IDH_1 have been inconclusive. The report of Jansen et al. (1982) concerned a case with r(2)(p25q37) in which the IDH_1 was normal. On the other hand, Wyandt et al. (1982) described a peculiar case where a ring chromosome r(2)(p25.2q33.2) and a rearrangement involving duplication of the segment 2q33.3→qter were present in 30% and 70% of cells examined, respectively. In this case, the IDH_1 level was also within normal ranges. Since the interpretation of the result was complicated by the mosaicism, the finding is not contradictory to our postulation. Unfortunately, the present study did not provide evidence that triplex gene dosage effects for IDH_1 exist in any individual with trisomy for 2q33.3. Confirmation of our hypothesis will have to await future studies on such patients.

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