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Inherited and de novo deletion of the tyrosine aminotransferase gene locus at 16q22.1 → q22.3 in a patient with tyrosinemia type II

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Summary. Tyrosinemia II is an autosomal-recessively inherited condition caused by deficiency in the liver-specific enzyme tyrosine aminotransferase (TAT; EC 2.6.1.5). We have restudied a patient with typical symptoms of tyrosinemia II who in addition suffers from multiple congenital anomalies including severe mental retardation. Southern blot analysis using a human TAT cDNA probe revealed a complete deletion of both TAT alleles in the patient. Molecular and cytogenetic analysis of the patient and his family showed one deletion to be maternally inherited, extending over at least 27 kb and including the complete TAT structural gene, whereas loss of the second TAT allele results from a small de novo interstitial deletion, del 16 (pter → q22.1::q22.3 → qter), in the paternally inherited chromosome 16. Three additional loci previously assigned to 16q22 were studied in our patient: haptoglobin (HP), lecithin: cholesterol acyltransferase (LCAT), and the metallothionein gene cluster MT1,MT2. Of these three markers, only the HP locus was found to be codeleted with the TAT locus on the del(16) chromosome.

Introduction

The transamination reaction converting tyrosine to *p*-hydroxyphenylpyruvate is the rate-limiting step in the catabolic pathway of this aromatic amino acid. This reaction is catalyzed by the hepatic cytosolic enzyme tyrosine aminotransferase (TAT; EC 2.6.1.5). Deficiency in TAT enzyme activity causes tyrosinemia II (Richner-Hanhart syndrome), a genetic disease following an autosomal recessive mode of inheritance. Characteristic symptoms of the disease are keratitis and clouding of the cornea, palmar and plantar hyperkeratosis, and sometimes mental retardation, accompanied by highly elevated serum and urine levels of tyrosine and its metabolites. The condition improves rapidly on a tyrosine- and phenylalanine-restricted diet (for reviews see Goldsmith 1983; Buist et al. 1985),

In 1967, a 2-year-old mentally retarded male child with keratitis, palmo-plantar hyperkeratosis, and tyrosinemia together with multiple congenital anomalies was reported briefly (Campbell et al. 1967). Enzyme studies performed on a liver biopsy demonstrated that this patient had virtually complete absence of TAT activity (Fellman et al. 1969) [Reduction or loss of hepatic TAT activity has subsequently been reported in a few other cases of tyrosinemia II (Goldsmith 1983; Buist et al. 1985)]. By all these criteria this patient could clearly be diagnosed as suffering from tyrosinemia II, and his epithelial lesions rapidly cleared after initiation of a tyrosine-restricted diet (Kennaway and Buist 1971). On the other hand, the multiple congenital anomalies presented by this patient usually are not part of the Richner-Hanhart syndrome. In addition, his severe degree of mental retardation contrasts with the milder forms frequently associated with tyrosinemia II (Goldsmith 1983; Buist et al. 1985). For these reasons, a chromosomal abnormality in this patient was suspected for many years. One could hypothesize that a chromosomal derangement, responsible for the congenital anomalies, could concomitantly have inactivated the TAT gene locus, which, when combined with a second mutant TAT allele, would lead to tyrosinemia II. However, several earlier cytogenetic analyses of the patient and his first-degree relatives had failed to reveal any karyotypic abnormality.

By somatic cell hybrid analysis and in situ hybridization using cDNA clones for TAT as probes, the human TAT gene locus has recently been assigned to chromosome 16q22 → q24 (Barton et al. 1986; Natt et al. 1986). With this knowledge, we carried out a cytogenetic reanalysis of our patient with high-resolution banding techniques and found a small interstitial deletion with breakpoints as 16q22.1 and 16q22.3 in one of his two homologues. This deletion must have occurred de novo, as the karyotypes of his parents and sibs are normal. Southern blot analysis of the patient and his family using a cloned human TAT cDNA as probe not only confirmed the expected TAT gene deletion due to the del(16) chromosome, but showed the patient to be completely nullisomic for TAT. Loss of the second TAT allele in the patient results from a maternally inherited TAT gene deletion, which has also been passed on to a brother. Consistent with TAT nullisomy, in situ hybridization of the patient's chromosomes with the same cloned TAT probe did not show above-background grain counts over 16q.

In addition to TAT, three other loci previously assigned to the 16q22 region were studied: haptoglobin (HP) at 16q22.1 (Magenis et al. 1970; Magenis and Chamberlin 1979; McGill et al. 1984), lecithin: cholesterol acyltransferase (LCAT) at16q22 (Azoulay et al. 1985), and the metallothionein gene cluster (MT1,MT2) at 16q22 (Le Beau et al. 1985). Of these three loci, only the HP locus could be assigned together with TAT to the segment missing on the del(16) chromosome of our patient.

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Materials and methods

Cell hybrids

Somatic cell hybrids were obtained by polyethylene-glycol-induced fusion of skin fibroblasts from patient TH with mouse A9 cells, which lack enzyme activities for both hypoxanthine phosphoribosyltransferase (HPRT) and adenine phosphoribosyltransferase (APRT). Mouse A9 cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (Seromed) supplemented with penicillin (100 units/ml) and streptomycin (100 µg/ml). Hybrid cells were selected either for $HPRT^+$ activity in HAT medium or for $APRT^+$ activity in medium containing 0.05 m*M* azaserine and 0.1 *M* adenine (AA medium) as described by Wigler et al. (1979). Presence of chromosome 16 of TH in the hybrids was scored by Southern blot analysis using the α-globin 3′ hypervariable region probe pα3′ HVR.64, which allowed for easy differentiation between the two parental chromosomes due to different-sized RFLPs.

Isolation of a TAT cosmid clone

About 2×10^6 recombinants of a human cosmid library in vector pcos2EMBL (Poustka et al. 1984), kindly provided by A. Frischauf and H. Lehrach, Heidelberg, were screened by colony hybridization with the human TAT cDNA clone phcTAT 2-16. A single positive clone was isolated and designated coshTAT-1.

DNA probes

The following probes were used: For TAT: phcTAT2-16, a 1.7-kb cDNA clone isolated from a human adult liver cDNA library (Chandra et al. 1983), extending from exon 2 to exon 12 of the TAT gene, and BS 0.9, a 0.9-kb BglII-SalI fragment derived from a genomic TAT phage clone (R. Rettenmeier and G. Scherer, unpublished).

For HP: phapto-6, a 1.5-kb human cDNA clone (Raugei et al. 1983), kindly provided by R. Cortese, Heidelberg, used for Southern blot analysis; and pULB 1741, a 1.2-kb human cDNA clone derived from haptoglobin $\alpha^{1S}\beta$ mRNA, received from A. Bollen, used for in situ hybridization.

For α-globin: pBRα-1.5, a 1.5-kb genomic PstI fragment from the α1 globin gene (Lauer et al. 1980), kindly provided by R. Gelinas, Seattle.

For the α-globin 3′ hypervarible region: p3′HVR.64, a 4.0-kb genomic Hinf I fragment (Jarman et al. 1986), kindly provided by D. R. Higgs, Oxford.

For MT: the 170-bp BamHI-PvuII fragment of phMT-II_3, corresponding to probe 1 in Karin et al. (1984). The clone was kindly provided by M. Karin, Los Angeles.

For LCAT: an 800-bp SstI fragment from the 1.4-kb human cDNA insert of clone LCAT 14A (Tata et al. 1987), kindly provided by F. Tata and S. Humphries, London.

Southern blot hybridization

Genomic DNA was prepared from peripheral blood according to Baas et al. (1984). DNA was extracted from mouse cells, human fibroblasts, and somatic cell hybrids by lysis in 10 m*M* Tris-HCl, pH 7.4, 10 m*M* EDTA, 0.5% SDS and 200 µg/ml proteinase K. After an overnight incubation at 37°C, DNA was purified by phenol extraction and isopropanol precipitation as above. 5 µg of DNA were digested to completion using a severalfold excess of the respective restriction enzyme purchased from Pharmacia or Boehringer-Mannheim. DNA fragments were separated by electrophoresis on 0.8% horizontal agarose gels and transferred to Gene Screen nylon membranes (NEN) as described by Reed and Mann (1985) with slight modifications. For all DNA probes used, inserts were first isolated and subsequently labeled with (α-^{32}P)dCTP (Amersham, Arlington Heights, IL) to a specific activity of 3×10^8–8×10^8 cpm/µg by the method of Feinberg and Vogelstein (1983). After prehybridization in 0.5 *M* sodium phosphate buffer, pH 7.2, 7% SDS, 1 m*M* EDTA for 30 min at 65°C, filters were hybridized for 16 h at 65°C in the same solution containing the heat-denatured probe (probe concentration at 5–10 ng/ml). Filters were washed subsequently in 120 m*M*, 80 m*M*, and 40 m*M* sodium phosphate buffer, pH 7.2, 1% SDS at 60°C for 30 min each time and exposed to Kodak XAR-5 film for 16–72 h at −70°C between two Dupont Cronex II intesifying screens.

Haptoglobin protein analysis

α-Haptoglobin phenotypes were determined by subjecting serum samples to starch gel electrophoresis (Poulik 1957) followed by staining for haptoglobin activity with peroxide benzedine. Quantification of serum haptoglobin levels was performed by Laurell electroimmunoassay (Laurell 1966).

Cell culture and chromosome studies

Peripheral blood lymphocytes were cultured using routine techniques and synchronized with amethopterin to increase the yield of early metaphase chromosomes (Yunis 1976; Francke and Oliver 1978). Chromosome analysis was accomplished using trypsin G-banding (Seabright 1971).

In situ hybridization

The TAT probe phcTAT2-16 and the HP probe pULB 1741 were radiolabeled by nick translation with (^{3}H)dTTP (65 Ci/mmol) and (^{3}H)dCTP (60 Ci/mmol; Amersham) to specific activities of 1.7×10^7 dpm/µg and 3.0×10^6 dpm/µg, respectively (Harper and Saunders 1981).

In situ hybridization was performed using the technique of Harper and Saunders (1981). The chromosomes were treated for 1 h with RNase at a concentration of 100 µg/ml in 2 × SSC at 37°C, denatured in 70% formamide (MC/B, Norwood, OH) 2 × SSC at 70°C for 2 min, then immersed in 70%, 80%, and 95% ethanol for 1 min each. Probes phcTAT2-16 and pULB 1741 were both diluted to a final concentration of 0.05 µg/ml in pH 7 hybridization buffer containing 50% formamide/2 × SSCP, 1% dextran sulfate, and 0.1 mg/ml sonicated denatured salmon sperm DNA. Chromosomes were hybridized for 12 h at 37°C; excess probe was then removed with three changes of 50% formamide/2 × SSC for 3 min each followed by five changes of 2 × SSC for 2 min each, all at 40°C. Chromosome preparations were again dehydrated with an alcohol series, and Kodak autoradiographic NTB-2 liquid emulsion was applied to the slide for a 10-day exposure. After the slides were developed, they were R-banded using a modification of the technique of Schweizer (1980).

Results

Monosomy for 16q22.1-q22.3 in patient TH

The patient TH studied in this report shows a complex clinical picture, where symptoms of tyrosinemia II are combined with

multiple congenital anomalies such as cleft lip and palate, clubbed feet, and absence of one kidney (Campbell et al. 1967; Fellman et al. 1969). In addition, TH is very severely mentally retarded and has been under permanent hospital care since the age of 5 years. Figure 1 shows recent photographs of TH illustrating his facial characteristics.

Earlier cytological studies of the patient's chromosomes had failed to reveal any abnormal features in karyotype. The recent assignment of the TAT gene locus to 16q22-q24 (Barton et al. 1986; Natt et al. 1986) stimulated us to a cytogenetic reanalysis of this case by high-resolution banding techniques. As shown in Fig. 2, a small interstitial deletion with breakpoints at 16q22.1 and 16q22.3 is found in one chromosome 16 homologue of patient TH. This deletion must have occurred de novo as the karyotypes of his parents and sibs are all normal.

Complete deletion of both TAT gene copies

As our tyrosinemia II patient carried a cytogenetically visible deletion on the long arm of one chromosome 16, we looked for the expected TAT gene deletion by hybridizing a human TAT cDNA probe, phcTAT2-16, to a Southern blot prepared from Eco RI digested genomic DNA of the patient and his family. The probe used detects a 5.8-kb fragment, which actually is a doublet (see Fig. 5). Surprisingly, the TAT signal is completely missing in the patient's DNA (Fig. 3, lane II-1). Furthermore, the signal intensity is reduced by half in the lanes of his mother and his brother (Fig. 3, lanes I-2 and II-3) compared with his father and his sister (Fig. 3, lanes I-1 and II-2). To confirm that about equal amounts of DNA were loaded per lane, the TAT probe was removed and the same filter was subsequently hybridized to an α-globin probe (Fig. 3, lower part) which detects a 22.5-kb Eco RI fragment from the α-globin gene cluster on the short arm of chromosome 16.

In situ hybridization of TH's chromosomes using the ^{3}H-labeled TAT probe phc TAT2-16 showed no grains on 16q out of 178 grains observed in 100 metaphases. In contrast, of 218 grains observed in 100 metaphases from a control, 42 (19.3%) were found associated with mid 16q. These results corroborate loss of the TAT genes from both the deleted and cytologically normal chromosomes 16 of TH.

These data confirm the expected deletion of one TAT allele in patient TH as the result of the de novo interstitial deletion at 16q22.1-q22.3. The Southern blot in Fig. 3 shows in addition that absence of the second TAT allele in the patient results from inheriting a TAT gene deletion from his mother, which has also been passed on to his brother but not his sister. As heterozygotes for this deletion, the mother and brother are both asymptomatic with respect to tyrosinemia type II. The existence of a maternally inherited TAT gene deletion is in turn evidence that the de novo deletion occurred on a paternal chromosome. This is confirmed by the haptoglobin data presented below.

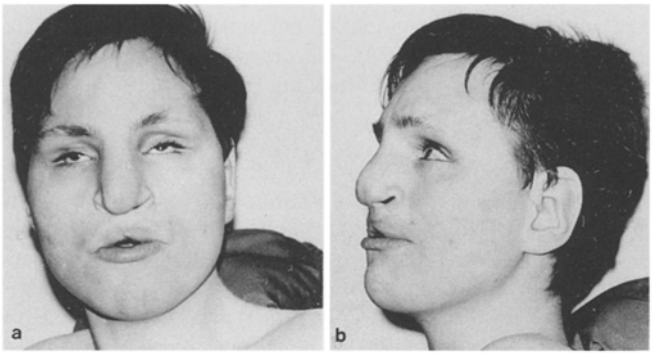

Fig. 1a, b. Patient TH at age 21. **a** Frontal view showing repaired cleft lip, prominent nose, mildly upslanted palpebral fissures, deep-set eyes, ptosis, and narrow forehead. **b** Side view. Note simple ears, deep-set eyes and prominent nose

Characterization of the maternal TAT deletion

Using phcTAT2-16 as probe, we isolated a cosmid clone from a human cosmid library in pcos2EMBL (Poustka et al. 1984). This clone, designated coshTAT-1, carries a human insert of 33 kb with the complete human TAT gene, which consists of 12 exons and extends over 11 kb (R. Rettenmeier, E. Westphal, H. Zentgraf and G. Scherer, unpublished; see Fig. 5).

Subclones derived from the extreme ends of the genomic insert in coshTAT-1 were used in Southern blot experiments to determine the extent of the maternally inherited TAT deletion. The results (not shown) placed one deletion breakpoint outside the region covered by the cosmid and the second deletion breakpoint near the right end of the cosmid insert as drawn in Fig. 5. This breakpoint was finally identified more precisely by using a restriction fragment from the 3′ end of the TAT gene, BS 0.9, to probe an Eco RI blot of patient TH and

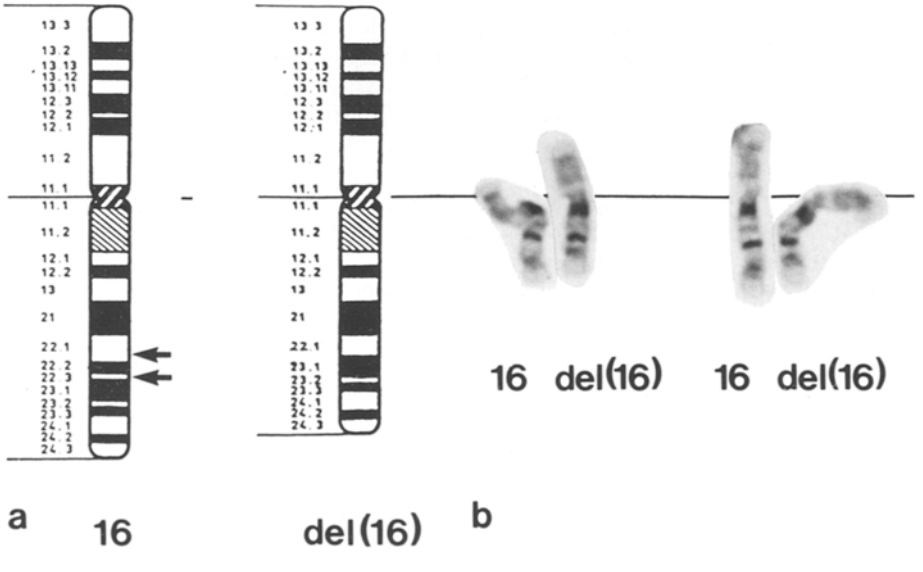

Fig. 2. a Idiogram of normal and abnormal chromosomes 16 at 850-band stage (ISCN 1985) illustrating breakpoints in bands q22.1 and q22.3. **b** G-banded normal and deleted chromosomes 16 at 850-band stage from patient TH

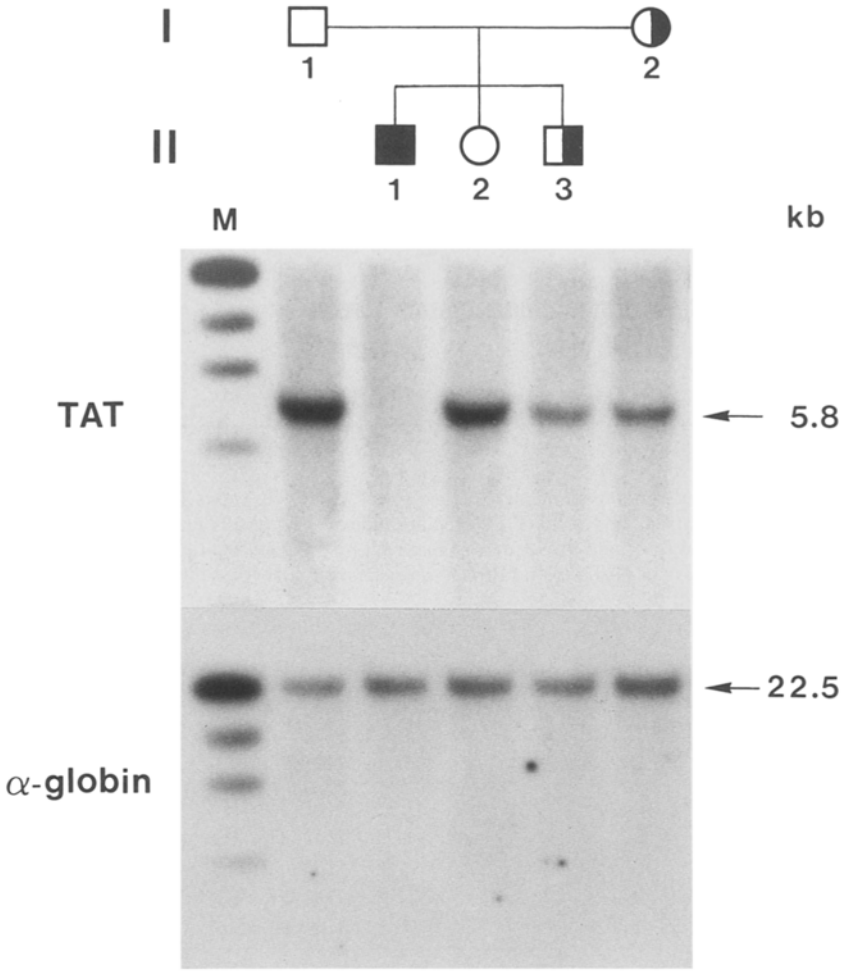

Fig. 3. Homozygous and heterozygous tyrosine aminotransferase (TAT) gene deletion in a tyrosinemia II family. DNA from patient TH *(II-1)*, his parents, and sibs was digested with Eco RI and subjected to Southern blot analysis. The same filter was first hybridized with the TAT cDNA probe phcTAT2-16 (*upper*, see also Fig. 5), and subsequently with a genomic α-globin probe (*lower*). *M* phage lambda Hind III fragments as size markers (23.6, 9.6, 6.6, and 4.3 kb). Genomic fragment sizes are given in kb on the right

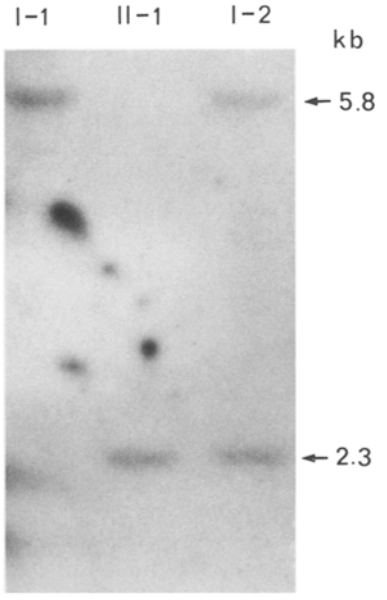

Fig. 4. Detection of one maternal TAT deletion breakpoint. Probe BS 0.9 (see Fig. 5) was hybridized to Eco RI-digested DNA from patient TH *(II-1)*, his father *(I-1)* and his mother *(I-2)*. The probe reveals the normal 5.8-kb fragment (Fig. 5) and a new fragment of 2.3 kb

his parents. As can be seen from Fig. 4, lane I-1, the probe detects a 5.8-kb Eco RI fragment in DNA prepared from the father of TH, as expected from the restriction map shown in Fig. 5. This fragment is absent in the patient's DNA and replaced by a new fragment of 2.3 kb (Fig. 4, lane II-1), while both the 5.8-kb and the 2.3-kb fragments are present in his mother's DNA (Fig. 4, lane I-2).

The fact that the sequences detected by the cDNA probe phcTAT2-16 fall completely within the maternal TAT deletion (Fig. 3), whereas those sequences detected by probe BS 0.9 are, at least in part, outside the deletion allows us to locate one deletion breakpoint to an interval of about 1 kb at the 3′ end of the TAT gene (see Fig. 5). This breakpoint could still be within the last, twelfth exon of the TAT gene, the exact size of which has not yet been determined. The maternal deletion, therefore, has removed (almost) the whole 11-kb TAT gene and at least 16 kb of 5′ flanking sequences.

Codeletion of the HP gene locus on the del(16) chromosome

The human haptoglobin (HP) gene has been localized to a region near the fragile site at 16q22.1 (Magenis et al. 1970; Magenis and Chamberlin 1979; McGill et al. 1984). We therefore examined whether, in addition to TAT, the HP locus has also been deleted on the del(16) (q22.1q22.3) chromosome.

DNA from all family members was digested with Eco RI and Pst I, and the resulting Southern blots were hybridized with an HP probe which detects, in addition to the two haptoglobin alleles Hp1 and Hp2, restriction fragments derived from the haptoglobin-related (Hpr) gene that directly flanks both Hp1 and Hp2 (Bensi et al. 1985; Oliviero et al. 1985).

As lane I-1 in Fig. 6 shows, the father carries an Hp1 and an Hp2 allele, which are characterized, respectively, by a 10.1-kb and 11.8 kb Eco-RI fragment (Fig. 6A) and a 4.2-kb and 5.9-kb Pst I fragment (Fig. 6B). In addition, the 8.3-kb Eco RI and 4.4-kb Pst I fragments corresponding to the Hpr gene are

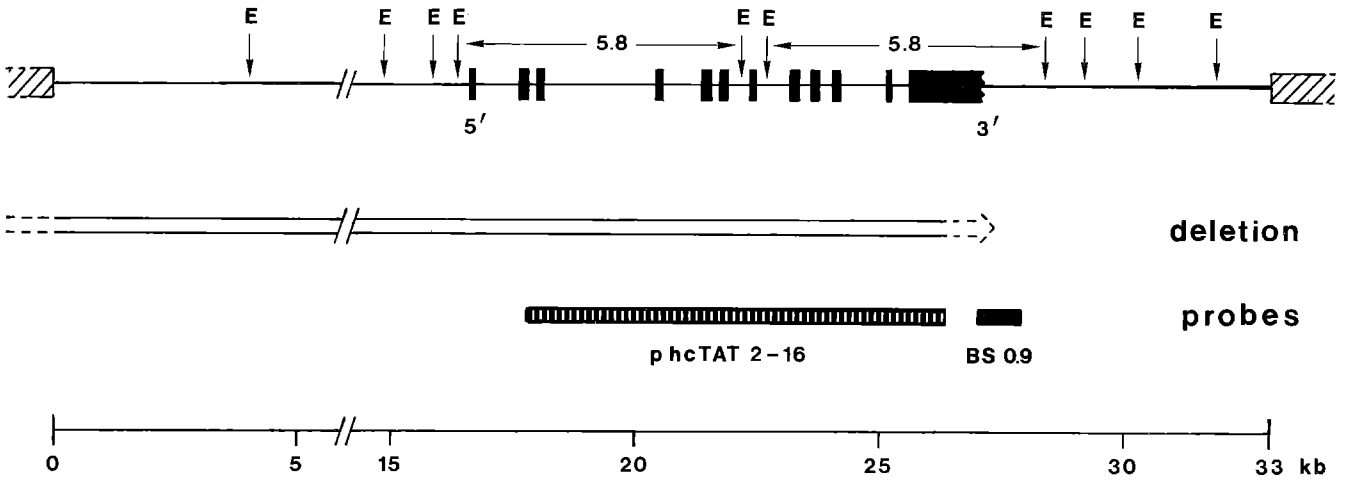

Fig. 5. Extent of maternal TAT deletion. *Top line* shows Eco RI *(E)* restriction map of part of clone coshTAT-1, with vector sequences indicated by *hatched boxes*. Sizes are given only for the relevant 5.8-kb fragments. Exons of the TAT gene are indicated by *black bars*, with exact size of the last exon still undetermined. Direction of transcription is indicated by 5′ and 3′. Sequences removed by the maternal deletion are indicated by the *double line*. One breakpoint maps to the interval between probes phcTAT2-16 and BS 0.9 *(dashed arrow)*, whereas the second breakpoint lies beyond the cloned sequences *(dotted lines)*. Scale in kb at bottom of figure

present. The mother's DNA (lane I-2 in Fig. 6A) shows the 11.8-kb fragment diagnostic for Hp2, whereas the 10.1-kb Eco RI fragment corresponds not to Hp1 but to a polymorphic Hp2 allele, $Hp2^V$ in the nomenclature of Oliviero et al. (1985). This is evident from the Pst I pattern (Fig. 6B) where only the 5.9-kb band corresponding to Hp2 and $Hp2^V$ (which is not polymorphic with Pst I) is visible, but no band at 4.2 kb corresponding to Hp1. The Pst I pattern also reveals that she has the common Hpr allele with a 4.4-kb band and a variant allele, Hpr^V, characterized by the two bands of 2.9 kb and 1.5 kb. With Eco RI, both Hpr and Hpr^V produce the same fragment of 8.3 kb (Fig. 6A).

The results obtained from Fig. 6 are summarized in Table 1 for all family members in the form of Hp-Hpr haplotypes taking into account that $Hp2^V$ and Hpr^V are in complete linkage disequilibrium (Oliviero et al. 1985). Clearly, patient TH has inherited only the $Hp2^V$-Hpr^V alleles from his mother, but none of the Hp or Hpr alleles from his father. Therefore, the de novo deletion must have occurred on a paternally inherited chromosome, codeleting the HP locus with TAT.

The Hp phenotypes obtained by Southern blot analysis are in complete agreement with α-haptoglobin phenotypes of TH, his parents and his brother obtained by starch gel electrophoresis. Quantification of serum haptoglobin levels by Laurell electroimmunoassay showed approximately half normal levels for TH consistent with gene dosage (data not shown). Furthermore, in situ hybridization using the Hp probe pULB 1741 resulted in 16 grains of a total of 159 grains (10.1%) located over bands q22 → q23 of chromosome 16 in 100 metaphases from a normal control. In 100 metaphases from TH, only 9 of 147 grains (6.1%) were associated with 16q, approximately half the control value. These results are also consistent with loss of one of the Hp genes in TH.

MT1,MT2, and LCAT are not deleted

Two additional loci previously mapped to 16q22 were included in our investigation. The metallothionein gene cluster (MT1,MT2) and lecithin : cholesterol acyltransferase (LCAT). Southern blots were prepared using DNA from patient TH, his parents, two somatic cell hybrids containing only the paternal or maternal chromosome 16 of TH, respectively (see Materials and methods), and mouse A9 fibroblasts. The blots were hybridized with cDNA probes for MT and LCAT, using an MT probe that detects both the MT1 and MT2 gene cluster (Karin et al. 1984). The hybridization signals obtained were of the same intensity in the patient's DNA compared with his parents, and the corresponding bands were present in both cell hybrids (data not shown). Therefore, both MT1,MT2 and LCAT must be located outside the segment deleted on the paternal chromosome.

Table 1. Haptoglobin (Hp-Hpr) haplotypes derived from the data shown in Fig. 6 of patient TH (II-1), his parents (I-1, 1-2) and sibs (II-2, II-3)

Individual	Haplotypes
I-1	Hp1-Hpr/Hp2-Hpr
I-2	Hp2-Hpr/$Hp2^V$-Hpr^V
II-1	$Hp2^V$-Hpr^V
II-2	Hp2-Hpr/Hp2-Hpr
II-3	Hp1-Hpr/$Hp2^V$-Hpr^V

Discussion

This report describes the cytogenetic and molecular analysis of a patient with unusual clinical features where clear symptoms of tyrosinemia II are combined with multiple congenital anomalies not related to this syndrome. Our results allow for a satisfactory explanation of this complex clinical picture.

Using high-resolution banding techniques, a small interstitial deletion on the long arm of one of the two chromosome 16 homologues could be identified in the karyotype of the patient, but not in the karyotypes of his parents or sibs. The breakpoints of this de novo interstitial deletion are in bands 16q22.1 and 16q22.3. We conclude that monosomy for this chromosomal segment is the cause for the various congenital anomalies observed in the patient. Some of the symptoms such as psychomotor retardation, palpebral fissures slant, cleft lip and palate, small hands, and clubbed feet have been described in other cases of de novo interstitial deletions on 16q

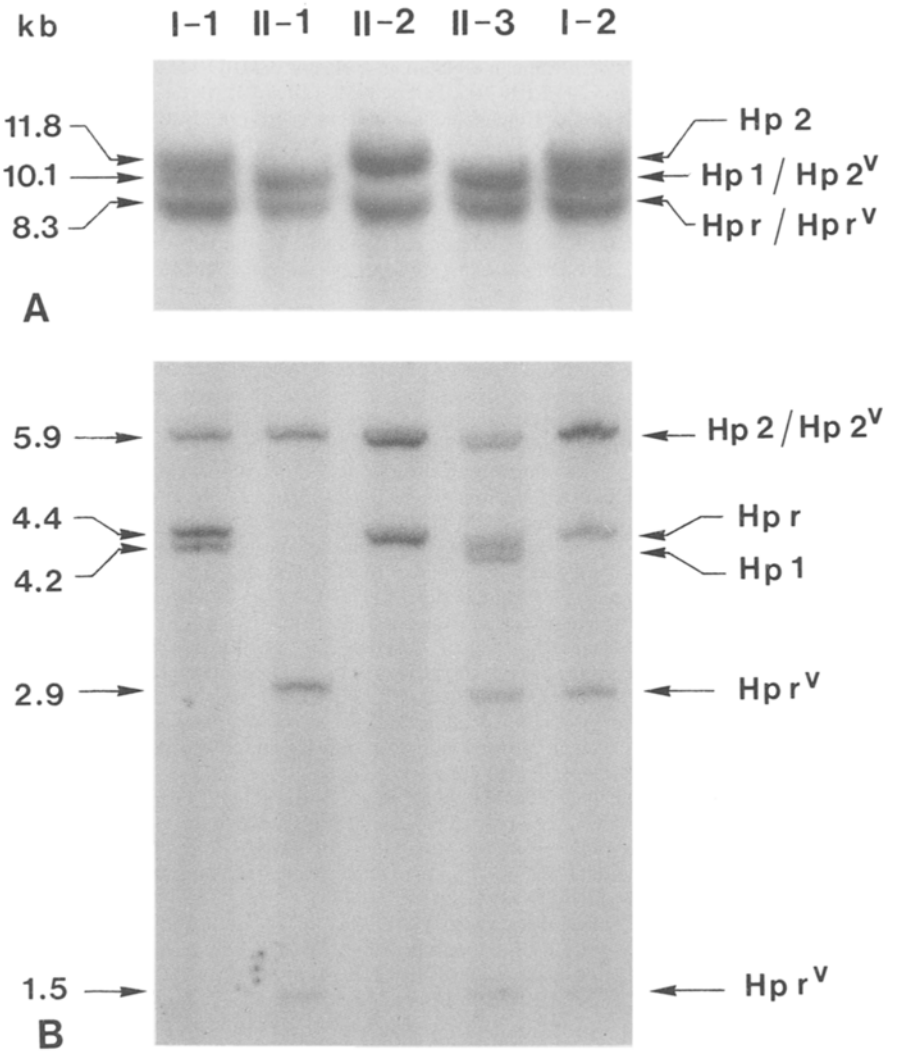

Fig. 6A, B. Southern blot analysis of haptoglobin *(Hp)* phenotypes. DNA from TH *(II-1)*, his parents *(I-1, I-2)* and sibs *(II-2, II-3)* (see Fig. 3) was digested with Eco RI (**A**) or Pst I (**B**) and hybridized with the Hp probe phapto-6. Fragment sizes are given in kb on the *left* and the corresponding Hp and Hpr phenotypes on the *right*, using the nomenclature of Oliviero et al. (1985). The results are summarized in Table 1

(Lin et al. 1983). While milder forms of mental retardation belong to the classical symptoms defining the Richner-Hanhart syndrome, the very severe degree observed in patient TH is regarded as atypical and is attributed to his 16q monosomy, not to his TAT deficiency.

Southern blot analysis of patient TH and his family with cloned probes for TAT and HP revealed (Figs. 3, 6 and Table 1) that the de novo deletion had occurred on a paternal chromosome 16, codeleting the loci for both TAT and HP. This assigns the locus for TAT to the interval 16q22.1–16q22.3, in accordance with and refining the previous assignment of TAT to the region 16q22 → q24 (Barton et al. 1986).

The results obtained with the TAT probe showed that patient TH is completely nullisomic for TAT. Loss of the second TAT allele results from a maternally inherited TAT gene deletion (Fig. 3). This deletion extends over at least 27 kb and completely removes 11 of the 12 exons of the TAT gene and part or all of the last, 12th exon as well (Fig. 5). As we have not yet characterized the exact size of this 3′ exon, the maternal deletion could still spare a small part of this exon. Nullisomy for the TAT gene in the patient explains his tyrosinemia II phenotype and confirms the previous observation of complete cytosolic TAT enzyme deficiency in cell extracts prepared from fresh liver tissue (Fellman et al. 1969). Patient TH, who was the first case of tyrosinemia II on which enzyme studies were performed, now is the first case fully characterized at the molecular level.

The Southern blot data obtained for TAT and HP showed close physical proximity for both loci, as both are absent from the del(16) chromosome. In addition, the segregation patterns of the maternal TAT and Hp-Hpr alleles in the pedigree are suggestive of genetic linkage between these loci: the TAT gene deletion segregates with the $Hp2^V$-Hpr^V haplotype in the patient and his brother, while the sister has inherited the normal TAT gene together with the maternal Hp2-Hpr haplotype (Figs. 3, 6; Table 1). With the help of recently identified RFLPs at the TAT gene locus we can in fact demonstrate genetic linkage between TAT and HP (Westphal and Scherer, unpublished).

Norum's disease, which results from LCAT deficiency, has been shown to be closely linked to HP, with a maximum lod score of 3.41 at $\hat{\theta} = 0.00$ (Teisberg et al. 1975). However, we have shown that LCAT is not included in the deleted region 16q22.1 → q22.3, whereas HP, whose most likely location is at 16q22.1 (Magenis et al. 1970; Magenis and Chamberlin 1979; McGill et al. 1984), falls within the deleted segment. This result may indicate that LCAT and HP are not very closely linked.

Results by Le Beau et al. (1985) place the metallothionein gene cluster in the proximal region of band 16q22. We have

shown that MT1,MT2, like LCAT, lie outside the deleted segment 16q22.1 → q22.3. From this information and from the other data reported here, we propose the following gene order on 16q: cen – MT1,MT2; LCAT (at q22.1) – HP; TAT (at q22.1 → q22.3) – ter.

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