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Triosephosphate isomerase deficiency: historical perspectives and molecular aspects

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In this chapter, the original descriptions and pre-molecular studies of triosephosphate isomerase (TPI) deficiency are summarized, and the molecular aspects of the disease presented. The gene is well characterized, and several mutations have been described. Structure–function studies have led to an increased understanding of impaired catalysis. All kindreds that have been studied with the predominant Glu104Asp mutation are linked by a common haplotype, indicating descent from a common ancestor. Variant upstream substitutions occur in high frequency in persons of African and East Asian lineage and in lower frequency in other groups, but the possible role, if any, of these variants in clinical TPI deficiency requires further investigation. The possible contribution of deviant lipid metabolism to the pathogenesis of the disorder has been extensively investigated, and an intriguing new area of inquiry is the apparent cell-to-cell transfer of enzyme in cell culture systems, raising the question of the feasibility of enzyme or gene replacement therapy.

Key words: triosephosphate isomerase; mutations; hereditary haemolytic anaemia; neuromuscular degeneration; membrane lipids; structure–function; haplotypes; enzyme replacement.

Triosephosphate isomerase (TPI) deficiency is a rare autosomal recessive multisystem disorder characterized by a markedly decreased activity of TPI, a housekeeping enzyme expressed in all tissues and encoded by a single gene located at chromosome 12p13. The clinical syndrome in homozygotes or compound heterozygotes is marked by profoundly decreased enzyme activity in all tissues that have been studied and is dominated by lifelong haemolytic anaemia and severe progressive neuromuscular degeneration, most often beginning about the seventh month of life. An increased propensity to infection is almost always noted, infectious episodes often being associated with increased anaemia and episodic hypotonia. Almost all cases have ended in death before the age of 5. There is no specific treatment, but aggressive supportive care, especially assisted respiration, has appeared to prolong life in some instances. Heterozygotes are clinically normal.

In spite of the rarity of affected subjects, a surprisingly extensive literature has accumulated since the original description of this disorder more than 35 years ago.[1,2] This review attempts to summarize the salient information that has become available, and to provide a historical perspective. The presentation is divided into three sections,

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generally coinciding with time periods but with some natural overlap: an initial section covering the period during which the first cases were described and the basic clinical and biochemical characteristics of the syndrome were defined; a second section, largely presented in summary form, covering an intervening period marked by reports from around the world describing new kindreds and clinically affected cases, as well as the addition of significant new insights into the clinical and biochemical features of the disease; and a third section detailing the time period that extends to the present, largely marked by an emphasis on the molecular aspects of TPI deficiency.

INITIAL DESCRIPTIONS: THE FRENCH-LOUISIANA GROUP

Case descriptions

The initial case descriptions are typical of those which followed. The first proposita[1,2] was a girl of mixed French and African-American lineage who had had a chronic haemolytic state that became evident at the age of 5 weeks. There had been a similarly affected sibling, a finding later observed to be common in affected families. Except for the anaemia, her development appeared to be normal until the age of 7 months, at which time a tightness of her Achilles tendons with an inability to dorsiflex her feet was noted. By the age of 13 months, generalized spasticity with increased deep tendon reflexes had become evident. At 14 months, seizure-like bouts of generalized stiffening and rigidity began. The patient died suddenly and unexpectedly at 20 months old, during convalescence from a respiratory infection. She had never been able to walk and had lost the ability to stand, sit or hold her head erect. The course of her illness had been interspersed with numerous severe infections, often associated with a marked increase in the degree of anaemia. Between bouts of infection, this girl had exhibited a chronic haemolytic state, her haemoglobin concentration varying from 7.5 to 9.0 g/dl.

Red cell morphological changes were nondescript, with a slight macrocytosis. A small number of target cells and scattered, small, densely contracted red cells with irregular margins were noted. Autohaemolysis[3] was pronounced after 48 hours but was averted by the addition of glucose, adenosine or ATP. Erythrocyte osmotic fragility was normal when fresh blood was studied, but there was a flattening of the curve after 24 hours' incubation. Routine haematological studies, including auto-haemolysis and osmotic fragility, were essentially normal in both parents and in two clinically unaffected brothers.

Although no direct evidence of consanguinity was established between the maternal and paternal sides of the patient's family, both were from a highly inbred, semi-isolated group of mixed French-Louisiana and African-American lineage, and both parents considered it almost certain that they were related in some way.

An assay of several enzymes of the glycolytic and pentose phosphate pathways and of glutathione reductase showed that these were generally elevated in the red cells of the patient, consistent with a young cell population. In marked contrast, the activity of erythrocyte TPI was reduced to approximately one-seventh that of normal, and there was a similar reduction in leukocyte enzyme activity. Both parents, as well as one of the two brothers and several other relatives, had a reduction in erythrocyte TPI activity to a level intermediate between the normal and that of the marked deficiency noted in the patient's erythrocytes. These findings, extended in the initial family and later repeated in many additional families, indicated that half-normal enzyme activity was characteristic of all clinically unaffected TPI parents as well as several close

relatives. TPI family members could be biochemically separated into three groups: a clearly definable, severely enzyme-deficient group consisting solely of affected patients, an intermediate group including all of the parents of the affected children as well as several other close relatives, and a third group indistinguishable from the normal population. These data supported the assumption that heterozygotes were biochemically defined by intermediate enzyme deficiency.

The second observed patient, of white French-Louisiana origin[2,4], was strikingly similar. Pallor had been apparent shortly after birth. At the age of 1 month, the haemoglobin concentration was 5.5 g/dl and the reticulocyte count varied from 10% to 15%. There had been several episodes of increasing anaemia during various febrile illnesses. Neurological abnormalities became evident at approximately 10 months of age, being at first characterized by generalized flaccidity. The patient survived until 12 years old, after a disease course characterized by muscular weakness and developmental and motor delay, followed by cerebellar dysfunction and finally spasticity and hyperreflexia. Haematological and enzymatic findings were similar to those of the first patient and her family. Again, a marked deficiency of erythrocyte TPI was noted in both the erythrocytes and the leukocytes, and again both parents exhibited intermediate enzyme deficiency consistent with the heterozygous state.

An investigation of the extended families of the first two patients revealed three additional affected subjects, several heterozygotes and a possible link between the two families. One child had been hospitalized for haemolytic anaemia at the age of 10 months and had died aged 1 year of respiratory disease. A sibling had died several hours after birth of what had been thought to be haemolytic disease of the newborn. A third child of the same marriage died suddenly at 5 years old after a progressive illness characterized by chronic haemolytic anaemia, recurrent infection, slow motor development, and weakness progressing from spasticity to generalized flaccidity.

The French-Louisiana group numbered six affected subjects in all[5], all but two dying before the age of 5. Some of the patients appeared to have increased susceptibility to infection; all had severe generalized disease. Recently, a new French-Louisiana case has been reported, but the relationship to the original group has not yet been determined.[6,7]

Metabolic studies

In order to evaluate the metabolic effect of TPI deficiency, leukocyte-free TPI-deficient erythrocytes were suspended in native plasma with added glucose, and the concentration of several compounds of interest was determined initially and at intervals throughout 150 minutes of incubation at 37°C.[8,9] Erythrocytes are unable to metabolize pyruvate further via the tricarboxylic acid cycle and depend entirely upon anaerobic glycolysis for the production of high-energy phosphate compounds. In addition, the TPI step is the only known means whereby dihydroxyacetone phosphate (DHAP) can be further metabolized in the red cells. If a complete block were present, the expected findings might well include a decrease in the glycolytic rate, a decline in the rate of ATP synthesis, an accumulation of DHAP and perhaps other intermediate compounds above the level of the block, and a marked reduction by almost half in the amount of three carbon compounds that are eventually converted to lactate.

Except for the expected accumulation of DHAP, the findings were in fact quite different. Alterations in glucose utilization, ATP and lactate production, and glycolytic intermediates were unimpressive. Thus, major compensatory mechanisms must have been at play, and it was suggested that such compensation occurred by way of the

pentose phosphate pathway. Further increases in glucose utilization in incubations with added methylene blue were considerably less marked than in the controls. This was thought to indicate that the shunt was already functioning at a near-maximal level and that this was the probable explanation for the increased glucose utilization and the almost proportionate production of lactate. The most striking deviation from normal was a marked increase in the initial concentration of DHAP to values more than 20-fold higher than the control values and almost 10-fold greater than in high-reticulocyte controls. The paucity of other metabolic abnormalities stood in rather marked contrast to the striking clinical abnormalities in the affected subjects, and it was suggested[5,9] that the accumulation of DHAP itself or of some derivative product might be injurious to cells. Alternatively, even though mature erythrocytes lack any known mechanism for the metabolism of DHAP other than conversion to glyceraldehyde-3-phosphate catalysed by TPI, this is not true of most other tissues. It was suggested that, in tissues such as the brain, diversion toward the synthesis of α-glycerophosphate might lead to lipid abnormalities of pathogenetic significance.[9]

Early studies of the erythrocyte enzyme

As a preliminary step towards further purification, DEAE-Sephadex A-25 chromatography with K_2HPO_4/KCl linear gradient elution was performed on stroma-free haemolysate.[9,10] TPI activity was found to occur in two distinct peaks, Michaelis constants for glyceraldehyde-3-phosphate for the two peaks being the same. Except for a marked reduction in total enzyme activity, the findings from a homozygous TPI-deficient subject were essentially identical to those from normal controls. These studies were interpreted as suggesting that the enzyme might exist in more than one molecular form, even though further experiments were consistent with the two fractions representing a single peptide in a monomer–multimer equilibrium.

Using the disappearance of NADH fluorescence under long-wave ultra-violet light as a marker in an α-glycerophosphate dehydrogenase/NADH linked system, Jean Claude Kaplan, working in the laboratory of Ernest Beutler, found three major bands of TPI enzyme activity when normal haemolysates were subjected to starch gel electrophoresis.[11] A moderate decrease in activity of the slowest moving band was observed in a homozygous TPI-deficient subject, with a virtual disappearance of the fastest moving band. It was initially suggested that the finding of three bands might be the result of a dimerization of two separate subunits, and that in TPI deficiency the disappearance of a band represented a failure of synthesis of one of the subunits. It was also considered possible that the three-banded pattern represented different configurational forms of the same enzyme.

THE INTERVENING PERIOD

The next stage of development was marked by the description of new clinical cases and a further definition of the clinical syndrome. The many significant events of this 'pre-molecular' period, overlapping with both the initial period and the period that followed, are summarized in a 'vertical time-line' fashion in Table 1.

The notable highlights of this period are many. Enzyme deficiency was demonstrated in a number of other tissues in addition to red cells and leukocytes. These included skeletal muscle, serum and cerebrospinal fluid[13], skin fibroblasts[20], platelets[21], lymphocytes[30], amniotic cells[33,34] and fetal red cells.[36] The diagnostic importance of

Table I. Investigation of triosephosphate isomerase (TPI) deficiency: the 'pre-molecular' period.

Year	No. of cases	Observations of special note	Reference
1964, 1965	2	First reported cases: mixed French-Louisiana/ African-American lineage	1, 2
1965	1	Second reported case: white French-Louisiana lineage	2, 4
1965		Dihydroxyacetone phosphate elevation as evidence of metabolic block	8, 9
1966	3	Extended studies of French-Louisiana/African-American family. Three additional cases described	5
1965–1968		Metabolic studies. Increased DHAP is the most striking finding. No abnormality in TPI DEAE Sephadex chromatography	8, 9, 12
1968	1	Enzyme deficiency in red blood cells, white blood cells, skeletal muscle, serum and cerebrospinal fluid. Additional case of English ancestry Three electrophoretic bands with loss of the most rapidly moving band	13 11
1970	1	English-Irish origin. Survival into adult life. Emphasized the importance of increased DHAP to confirm the metabolic block	14
1970	1	New case. Sudden unexplained death emphasized	15
1974, 1975		Localization of TPI gene to chromosome 12	16, 17
1975	4	Non-consanguineous French family	18
1977		Further localization of the TPI gene locus to between 12pter and 12p12.2	19
1977	3	Three new cases, one from Spain. Enzyme deficiency in fibroblasts. First description of thermolability	20
1978		Large consanguineous Spanish family of previously reported Spanish case. Decreased TPI in platelets. DHAP markedly elevated. Evidence for single gene origin of three electrophoretic bands	21
1979		Population screening for TPI heterozygotes in Germany, initial report. Follow-up in 1984 (see below)	22
1981		Population screening for TPI heterozygotes in USA	23
1982		TPI Manchester, catalytically normal but thermolabile electrophoretic variant	24
1982		First description of neuropathological findings. Subject was one of original group of TPI cases	25

Table I continues over the page

Table I. Continued.

Year	No. of cases	Observations of special note	Reference
1982		High-frequency TPI heterozygosity in African-Americans, presumably due to embryolethal null allele	26
1983	2	Two siblings in non-consanguineous Spanish family. Neuropathological findings reported	27
1983		Population study in Japan	28
1984		Definitive German population study	29
1985	2	Two new cases in two non-consanguineous unrelated Italian families. TPI decreased in lymphocytes. Very high DHAP level	30
1985	7	Seven homozygotes in 5 unrelated families: 2 from France, 1 from Algeria, 2 siblings from Yugoslavia, 2 siblings from Morocco. DHAP increased. Thermolability	31
1985		Neuropathological aspects of TPI deficiency. Describes findings in two French cases and reviews literature	32
1985–1986	1	Australian family. Successful prenatal diagnosis in 16th week, based on enzyme assay and thermolability in amniotic cells. Importance of thermolability stressed	33, 34
1986		Platelet function defect described in a previously described Italian case	35
1986		Prenatal diagnosis in 18th week by enzyme assay and thermolability of fetal red cells obtained by cordocentesis	36
1986		Large French family. Prenatal diagnosis performed on mother of proposita previously reported by Rosa et al (see above)	37, 38
1987		Prenatal diagnosis in first trimester by chorionic villus sampling and at 16th week by amniocentesis. Utilized enzyme activity, thermolability and DHAP concentration. Predicted that first-trimester chorionic villus sampling and DNA analysis would soon be method of choice	39
1988	1	New US case with stable clinical course after several years of aggressive supportive care. Note: This case was the first Glu104Asp homozygote reported in early molecular studies from the Maquat group (see text)	40
1988	1	New case from a non-consanguineous Spanish family	41

Table I continues.

Table 1. Continued.

Year	No. of cases	Observations of special note	Reference
1989–1990	1	First case from the UK. In subsequent pregnancy at risk, prenatal diagnosis performed in 19th week on fetal red cells obtained by cordocentesis. Utilized enzyme assay, thermolability and DHAP concentration. Emphasized importance of increased DHAP level in prenatal diagnosis. Predicted that DNA diagnosis with chorionic villus sampling would soon be available	42, 43
1990–1993	2	Two Hungarian brothers: one with haemolytic anaemia and neurological changes, the other with haemolytic anaemia but without neurological changes. Note: Both brothers were later found to have an identical genotype: Glu145Stop/Phe240Leu (see text)	44, 45
1990–1991	1	Case from Germany. Atypical clinical picture with changes similar to those of mitochondrial myopathy. Note: Later shown to be homozygous Val231Met (see text)	46, 47

DHAP elevation as an indicator of metabolic block was emphasized by several authors.[14,21,30,31,39,42,43] Marked thermolability of the mutant enzyme was first reported in 1977 from the Beutler laboratory[20], and this, along with other evidence, strongly suggested that the enzyme deficiency was caused by molecular instability of the mutant enzyme. The question concerning multiple isoenzymic forms of TPI was resolved when it was demonstrated that these resulted from post-translational modifications[20,48] and that the enzyme protein was encoded by a single gene.[49] The gene was localized to chromosome 12 between 12pter and 12p12.2.[16,17]

Population screening for heterozygotes was carried out in a number of populations.[22,23,26,28,29,50] A surprisingly high incidence of TPI heterozygosity was reported in African-Americans, and it was suggested that the rarity of homozygotes was the result of the embryolethality of a null allele.[26] Successful 'pre-molecular' prenatal diagnosis was performed in several laboratories.[33,36,39,42,43,51,52] Thirty-four clinically affected cases were reported during this period, including the original cases and additional subjects from the USA, as well as cases from France, Spain, Italy, Germany, Australia, Hungary and the UK.

THE 'MOLECULAR PERIOD': GENE CHARACTERIZATION, DESCRIPTION OF MUTATIONS AND PRENATAL DIAGNOSIS. FURTHER INVESTIGATIONS CONTINUING TO THE PRESENT

In 1975, the amino acid sequence of rabbit muscle TPI was deduced by the characterization of overlapping tryptic peptides.[53] The enzyme was found to have two identical subunits, each consisting of 248 amino acid residues. A comparison with coelacanth[54] and chicken enzymes[55] revealed a high degree of homology. By sequencing randomly

selected clones from a rabbit muscle cDNA library and comparing the nucleotide sequences with the known amino acid sequence of TPI, Putney et al were in 1983 able to isolate an 84 base pair TPI cDNA fragment corresponding to amino acid residues 56–83.[56]

Characterization of the gene and description of the first mutations

With the stage thus set, Lynne Maquat and her co-workers, in a series of landmark papers, determined the nucleotide sequence of human TPI cDNA[57]; cloned, sequenced and characterized the gene[49,58]; described three intronless processed pseudogenes[49]; and for the first time delineated specific mutations involved in TPI deficiency.[59–61] Using the TPI cDNA probe from rabbit muscle[56] and a plasmid adult human liver cDNA library, they were able to isolate and sequence an almost full-length human TPI cDNA that included the last two nucleotides of the methionine initiation codon and the entire 744 base pair coding region, as well as the entire 448 base pair 3′ untranslated region.[57] This represented the first demonstration of the human coding sequence, although a human TPI sequence that differed by 16 residues had previously been determined by amino acid sequencing.[62]

In cultured fibroblast cell lines from two unrelated subjects with TPI deficiency, TPI activity was markedly reduced, and an analysis of TPI steady-state mRNA in cultured fibroblasts revealed a normal level in one of the two subjects and a reduction to about 40% of normal in the other. This was interpreted as indicating heterogeneity in the genetic basis of TPI deficiency. It was further suggested that, in the subject with reduced mRNA, the defect must have resulted from an alteration affecting TPI gene expression, such as gene transcription, transcript processing, transport from the nucleus to the cytoplasm, or stability of the mature message.[57]

In continuing studies from the same group[49], a human genomic DNA library was screened with the previously isolated human cDNA probe. Four screened λ Charon 4A plaques contained different TPI sequences, these sequences being identified as the functional TPI gene and three intronless afunctional pseudogenes. Sequence analysis revealed that the functional TPI gene spanned 3.5 kbp and consisted of seven exons and six introns, the exonic coding sequence predicting an amino acid sequence identical to that previously predicted from the isolated cDNA. It was concluded that although there was a family of human TPI genes, there was only a single functional gene. Upstream sequences from the transcription start site contain an atypical TATATAA box located 27–21 base pairs upstream and a novel cap proximal element at −18 to −6.[58]

The Glu104Asp and Arg189Stop mutations

Continuing with their work, the Maquat group[59] reported further studies on cultured fibroblasts from the two previously studied TPI-deficient subjects. A full-length expressed gene cloned into phage EMBL3B was isolated from each cell line and sequenced in its entirety. Both subjects were found to have the same mutation, 104 (GAG : Glu → GAC : Asp). It was further noted that the mutant protein was thermolabile in the cultured fibroblasts as well as in stably transformed Chinese hamster ovary cells. Computer modelling indicated that the loss of the side chain methylene group was sufficient to counterbalance charges that normally exist within a hydrophobic pocket of the native enzyme.

One of the two TPI-deficient subjects was found to have the same mutation in both alleles. Initially, only a single allele was characterized in the second subject. In a subsequent report[60], however, the remaining allele was shown to harbour a nonsense mutation, 189 (CGA : Arg → TGA : Stop), generating a truncated protein without demonstrable enzyme activity. It was of considerable interest that the resultant mRNA was shown to have a reduced cytoplasmic half-life. This observation and similar observations with the beta-thalassaemias[63,64] were the stimulus for a continuing series of investigations of the mechanisms by which nonsense and frameshift mutations give rise to unstable mRNAs.[65]

Additional reports of Glu104Asp

The Glu104Asp mutation was again reported, this time from France, in two siblings of Bulgarian origin studied by Raymonde Rosa, Jean Rosa and their co-workers in Créteil.[66] To avoid interference from the afunctional pseudogenes, they extracted expressed RNA from lymphoblastoid cell lines from each of their two subjects, converted the mRNAs to the corresponding cDNAs, and amplified the resulting DNA sequences using the polymerase chain reaction (PCR). The mutation was demonstrated by a dot-blot technique with allele-specific oligonucleotide hybridization and discriminant temperatures, and was confirmed by electroelution and sequencing. The authors concluded that both subjects were homozygous for the 104 mutation.

Notably, in 1991 and 1992, molecular prenatal diagnosis was performed by chorionic villus sampling on two fetal siblings of an affected subject from Greece, both being shown to be Glu104Asp homozygotes. (The affected subject is discussed more fully in a later section.) To distinguish between amplification of the functional gene and of the unprocessed pseudogenes, PCR primers were located at the beginning of exon 3 and the middle of exon 4, yielding a 136 base pair fragment for the pseudogenes and a 210 base pair fragment for the gene. After electrophoresis, the longest DNA fragment was electroeluted, blotted and hybridized with labelled normal and mutant oligonucleotides (Raymonde Rosa et al, unpublished observations, communicated by M. Cohen-Solal, 1999).

In 1992, another subject with the Glu104Asp mutation was reported from Germany in a child of Turkish origin.[67,68] Prenatal diagnosis was performed on a fetal blood sample obtained by cordocentesis during the 19th week of gestation. DNA was amplified by PCR using exonic primers. Direct DNA sequencing as well as allele-specific oligonucleotide hybridization revealed that the unborn sibling of the affected subject did not carry the 104 mutation, and the pregnancy was continued.

Additional kindreds were reported in 1995[6] (see below for details), and more recently, two additional instances of the 104 mutation were reported by Luciano Baronciani and his colleagues in Milan.[69] The mutation was found in both sets of parents of the two unrelated Italian cases that had been previously reported.[30]

The Val231Met mutation

A novel TPI deficiency variant reported by Bardosi et al[46] was clinically characterized by degenerative myopathy, an increase in intracellular glycogen level and mitochondrial changes similar to those seen in mitochondrial myopathies. The authors suggested a possible failure of the 'α-glycerin phosphate shuttle' wherein α-glycerophosphate crosses the mitochondrial membrane, where it serves as substrate for α-glycerophosphate dehydrogenase. This is the mechanism by which DHAP

re-crosses the mitochondrial membrane back to the cytosol, thus facilitating the transfer of the electron change represented by NAD → NADH. The same TPI-deficient subject was more fully characterized by Stefan Eber and his co-workers the following year[47], and shortly thereafter the mutation was characterized as being 231 (GTG : Val → ATG : Met).[67] It was noted that the affected amino acid residue is entirely conserved from bacteria to man and is known to be involved in substrate binding.

TPI Manchester

Another mutation was found in a previously described thermolabile electrophoretic variant with cathodal migration and normal activity and kinetics, which had been designated TPI Manchester.[24] This variant was found to have a G → A transition coding for the substitution of a glycine by an arginine at position 122.[70]

The Phe240Leu and Glu145Stop mutations

In an intriguing set of investigations extending over the past decade[44,45,61,71–79], Susan Hollán and her co-workers described two Hungarian brothers, both with lifelong haemolytic anaemia, markedly reduced red cell TPI activity with no residual activity after 10 minutes incubation at 55°C, slow electrophoretic migration of the enzyme, and increased red cell DHAP. The TPI activity in both clinically unaffected parents was approximately one-half that of normal. The younger brother presented with hyperkinetic extrapyramidal signs and symptoms beginning at the age of 11, remaining stable after the age of 13. The other brother, an amateur wrestler, has remained free of neurological manifestations. Molecular analysis revealed that both brothers had identical genotypes and were compound heterozygotes for two previously unknown mutations. One of the mutations was 240 (TTC : Phe → CTC : Leu)[61], creating a thermolabile protein. The other reduced the abundance of TPI mRNA 10–20-fold[61] and, not surprisingly, was later shown by Cohen-Solal and Hollán[74,76,80,81] to be a nonsense mutation, 145 (GAG : Glu → TAG : Stop).

It had been noted earlier that the glycolytic consequences of TPI deficiency were surprisingly few[8,9], and it had been suggested that TPI deficiency might lead to lipid abnormalities.[9,45,71,72] DHAP, which is greatly elevated in TPI deficiency, is a precursor of acyl-DHAP, which is in turn an obligatory precursor of glycerol ether lipids (alkyl glycerol ethers and plasmalogens) as well as being converted to non-ether glycerolipids.[82]

In the search for the pathogenesis of the differing phenotypes in the two compound heterozygous brothers with identical genotypes, an ongoing search for lipid abnormalities in TPI deficiency has led to a number of interesting findings, including increased fatty acid chain mobility in the external lipid layer in red cells, ghosts and inside-out vesicles as measured by fluorescence anisotropy with fluorophores[45,71,72,76], changes in the composition of major phospholipid subclasses, and a decrease in membrane alkenylacyl-phosphatidylethanolamines (phosphoethanolamine plasmalogens) in the cells that have been studied, and presumably in neural tissue as well.[75,78] Other changes include increased activity of erythrocyte acetylcholinesterase and Ca^{2+}-ATPase[73] and increased binding of TPI to CNS tubulin.[83] It has been suggested that the altered steric properties of TPI might have an effect on protein–protein interactions as well as on the activity of enzymes and membrane transport functions.

The foregoing changes, particularly the decreased plasmalogen level, were more marked in the neurologically affected Hungarian subject than in his non-neurologically affected sibling. A decrease in plasmalogen level was also noted[79] in a subject with TPI deficiency caused by the Glu104Asp mutation.[84,85] It is noteworthy that decreased plasmalogens have been described in a variety of neurodegenerative disorders, including Alzheimer's disease and adrenoleukodystrophy, as well as Zellweger's syndrome, a neurodegenerative disorder in which the phenotype is caused by mutations in any of several different genes involved in the biogenesis of peroxisomes. Both the vinyl ether bond of phosphatidylethanolamine plasmalogen and polyunsaturated fatty acids are major targets in oxidative stress; thus, these lipid modifications suggest that free radicals may be involved in the pathogenesis of the neurological manifestations of TPI deficiency, as in fact appears to be the case in Alzheimer's disease.[86] It has also been suggested that a clinical trial with docosahexaenoic acid in TPI deficiency might be warranted[79], since such therapy has been found to increase the plasmalogen concentration in adrenoleukodystrophy.

The repeated occurrence of the 104 mutation in multiple apparently unrelated families

Two new kindreds with TPI deficiency, one from Greece and the other from the USA (Alabama), were reported by our group in 1994[87], a third kindred from the USA (Louisiana) being added the following year[6] and later reported separately by others.[7] In all three instances, genomic DNA directly sequenced after amplification by PCR exhibited the Glu104Asp mutation.

At this point, a total of nine families throughout the world, all apparently unrelated to one another, had been reported with the Glu104Asp mutation.[6,59–61,66–68,87] In addition, we became aware of four other affected families from France and others from the UK. This stood in sharp contrast to the other known mutations affecting amino acids 122[70], 189[60], 232[67] and 240[61], each of which had been reported only in single families. The demonstration that Glu104Asp was the most frequent mutation had immediate practical significance since questions concerning prenatal diagnosis are often paramount among the concerns of affected families. Prenatal diagnosis depends upon a precise knowledge of the nature of the mutation in each parent, a reasonable initial approach to this problem being first to examine affected subjects and their parents for the Glu104Asp mutation. This can be easily accomplished by a simple restriction digest using *Dde*I, which targets a restriction site created by the mutation.[6,88,94] Thus, once the diagnosis of TPI deficiency has been made in a previously uncharacterized family, the presence or absence of the Glu104Asp mutation can be established by this assay, setting the stage for subsequent prenatal diagnosis if required.

The provocative frequent recurrence of the Glu104Asp mutation in multiple unrelated families was initially investigated by studying five polymorphic short tandem repeat and microsatellite markers that were known to lie within a 1.77 megabase region that included the TPI gene. Initially, seven families were available for study[89], including the three previously reported by our group (two from the USA and one from Greece)[6], three new families from France, and an additional US family, previously reported by others.[60] Four of the five markers were linked, but in apparent equilibrium. In contrast, a polymorphic 162 base pair short tandem repeat sequence in the *CD4* gene, which lies telomeric to the TPI gene, was in complete linkage disequilibrium with the TPI 104 mutation. We pointed out that these findings support

the hypothesis that TPI Glu104Asp arose as a single mutation in a common ancestor of the affected families.[89]

At the time this study was first presented by our group, we were informed by Roopen Arya of the British group at King's College in London that he and his colleagues had made similar observations, unpublished at that time, in a group of subjects with the Glu104Asp mutation, using an intragenic polymorphism that they had discovered within intron 5 of the TPI gene. In a published report, they proposed that the predominance of a single mutant allele reflected a founder effect, and they also reported successful first-trimester molecular diagnosis by chorionic villus sampling in two families with the Glu104Asp mutation.[90]

The studies by our group were expanded to include all of the known Glu104Asp kindreds: five families from the USA, three families from France, one family of Turkish origin from Germany, one family from Greece and two families from Australia.[91,92] The *CD*4 pentameric 162 base pair repeat was again found to be in complete linkage disequilibrium with the TPI 104 mutation. We also evaluated the intragenic polymorphism previously discovered by the British group, an A or G at genomic position 2895. The intronic polymorphism, in the A form, was also in complete linkage disequilibrium with the 104 mutation. All chromosomes with Glu104Asp carried the CD4-162/TPI-A haplotype, this haplotype being found in only 2 out of 38 chromosomes without the Glu104Asp mutation.

These findings were amplified by the formal reports from the British group, which included all the known UK and Australian families diagnosed since 1970: five families from the UK and two from Australia. All carried the TPI 104 mutation, and as we had previously been told, there was a strong linkage of TPI Glu104Asp with the A form of the C/A polymorphism at nucleotide 2892.[52,85]

It is noteworthy that the two Australian families were studied by both our group and the British group, providing a common link between all of the TPI 104 families. Thus, when both sets of findings were combined, all the families in the world that were known at that time to carry Glu104Asp were found to have an identical linkage. We interpreted the data as showing that, despite the disparate geographical distribution of the affected families, TPI Glu104Asp originated in a common ancestor who carried the CD4-162/TPI-A haplotype. It was further suggested that the original mutation occurred well in excess of 1000 years ago, probably in an ancestor who dwelt in England or France.[92]

The Cys41Tyr and Ile170Val mutations

In 1995, Arya and his co-workers first reported their detailed findings on the aforementioned seven kindreds from the UK and Australia, also communicating two new mutations.[90] All of their subjects carried the TPI 104 mutation. In four of the families, the propositus was a TPI 104 homozygote, and in the remaining three families, compound heterozygosity was demonstrated. In two of the families, the second allele was shown to be 41 (TGT:Cys → TAT:Tyr), and in the remaining family the second mutation was 170 (ATT:Ile → GTT:Val). Interestingly, the propositus in the family with the Ile170Val mutation was free of neurological disease, and it was suggested that an altered enzyme structure rather than abnormal catalysis might play an important role in the generalized clinical manifestations of TPI deficiency. These findings were reported in further detail in subsequent publications.[52,85]

The Gly72Ala and Val154Met mutations. The −5, −8 and −24 upstream variants

In 1982, Mohrenweiser and Fielek reported the heterozygote incidence of TPI deficiency as determined by enzyme activity in a population survey.[26] The heterozygote frequency was 0.024 in US whites, similar in magnitude to the frequencies reported from Germany[22,29] and Japan.[28] Of considerable interest was the 10-fold higher heterozygote frequency of 0.24 in African-Americans.[26] In a 1996 paper of considerable interest, Watanabe et al[93] reported the re-investigation of the blood samples that had been stored for many years from the earlier Mohrenweiser and Fielek study in order to define the molecular basis of the reduced enzyme activity findings. In the white group, Watanabe et al reported two new mutations: 72 (GGG : Gly → GCG : Ala) and 154 (GTG : Val → ATG : Met), as well as a single subject with the previously described Glu104Asp mutation. In the African-American group, each of the seven heterozygous subjects had two substitutions −5 and −8 base pairs upstream of the transcription start site. These changes were −5 A → G and −8 G → A. Additionally, two of the seven subjects had yet another substitution: −24 T → G. The −8 substitution lies within the cap proximal element and the −24 substitution within the TATATAA box, known regulatory elements within the TPI gene.[58,94]

Tabulation of all known TPI mutations

A complete listing of all known TPI mutations was first presented in 1996[80] and is shown in a modified form in Table 2. Three previously unpublished mutations were included that had been characterized by Michel Cohen-Solal and his co-workers in Créteil. These included a nonsense mutation 145 (GAG : Glu → TAG : Stop); the second allele from the two Hungarian brothers with the previously described Phe240Leu mutation[61]; a mutation involving the initiation codon, ATG : Met → AAG : Lys, later designated TPI Paris[95]; and a frameshift mutation, 28 (CTG : Leu → C del 2nt : frameshift), later designated TPI Alfortville.[95] The genotypes of all reported instances were also tabulated. Homozygosity had been reported only for the Glu104Asp and Val231Met mutations. Several compound heterozygotes had been identified, all but two in combination with Glu104Asp. One of these two displayed the aformentioned Phe240Leu and Glu145Stop, the other involving the newly reported initiation codon defect along with the −5 and −8 upstream substitutions. One subject was a compound heterozygote for Glu104Asp in combination with the −5, −8 and −24 upstream substitutions.

Assignment of the known mutations to functional domains of the enzyme protein: structure–function relationships

The delineation of a significant number of mutations prompted our group to investigate the structure–function relationships of the known mutations in TPI deficiency, our preliminary studies being reported in 1996.[96,97] Similar studies were reported (essentially simultaneously) by Arya and his colleagues from the King's College group.[98] Our approach was to map the amino acid residues as well as the first- and second-degree contacts of all of the residues comprising each of three functional domains of the TPI enzyme protein: the substrate binding, flexible loop and dimer interface domains; evaluate the limitations in 'permissible' substitutions of each

Table 2. Triosephosphate isomerase (TPI) mutations and variant substitutions: observed genotypes.

Mutation/variant	Comments	Observed genotypes
−5 A → G, −8 G → A [93]	Upstream variant. Found in population survey	Normal/−5 −8 [93] −5 −8/MetInitLys [80]
−5 A → G, −8 G → A, −24 T → G [93]	Upstream variant. Found in population survey	Normal/−5 −8 −24 [93] −5 −8 −24/Glu104Asp [80]
Init (ATG:Met → AAG:Lys) [80]	TPI Paris	−5 −8/MetInitLys [80]
28 (CTG:Leu → C del 2nt:frameshift) [80]	TPI Alfortville	Leu28 del 2nt/Glu104Asp [80]
41 (TGT:Cys → TAT:Tyr) [90]		Cys41Tyr/Glu104Asp [90]
72 (GGG:Gly → GCG:Ala) [93]	Found in population survey	Normal/Gly72Ala [93]
104 (GAG:Glu → GAC:Asp) [59]	Most frequently occurring mutation. Common haplotype in all affected families	−5 −8 −24/Glu104Asp [80] Cys41Tyr/Glu104Asp [90] Glu104Asp/Glu104Asp [59] Ile170Val/Glu104Asp [90] Glu104Asp/Arg189Stop [60]
122 (GGA:Gly → AGA:Arg) [70]	TPI Manchester: thermolabile electrophoretic variant	Normal/Gly122Arg [70]
145 (GAG:Glu → TAG:Stop) [80]		Glu145Stop/Phe240Leu [80]
154 (GTG:Val → ATG:Met) [93]	Found in population survey	Normal/Val154Met [93]
170 (ATT:Ile → GTT:Val) [90]		Ile170Val/Glu104Asp [90]
189 (CGA:Arg → TGA:Stop) [60]		Glu104Asp/Arg189Stop [60]
231 (GTG:Val → ATG:Met) [67]	Mitochondrial-type myopathy	Val231Met/Val231Met [67]
240 (TTC:Phe → CTC:Leu) [61]	Two siblings with identical genotype but differing phenotypes	Glu145Stop/Phe240Leu [80]

Init = initiation codon.
References indicated in square brackets.

Table 3. Amino acids in each of the defined functional domains of the TPI enzyme protein.

Substrate binding	Flexible loop	Dimer interface
11Asn, 13Lys, 95His, 165Glu, 169Ala, 170Ile, 171Gly, 210Gly, 211Ser, 230Leu, 231Val, 232Gly, 233Gly	165Glu, 166Pro, 167Val, 168Trp, 169Ala, 170Ile, 171Gly, 172Thr, 173Gly, 174Lys	11Asn, 13Lys, 14Met, 15Asn, 16Gly, 17Arg, 18Lys, 44Pro, 45Thr, 46Ala, 47Tyr, 49Asp, 64Gln, 67Tyr, 69Val, 70Thr, 71Asn, 72Gly, 73Ala, 74Phe, 75Thr, 76Gly, 77Glu, 78Ile, 79Ser, 81Gly, 82Met, 85Asp, 86Cys, 95His, 97Glu, 98Arg, 101Val, 102Phe

Protein Data Bank files 1HTI.PDB (human) and 1TPH.PDB (chick) were analysed as described in the text with the Look program (Molecular Applications Group, Palo Alto, California). The major contribution of Dr Clarke Halfman in the derivation of these data and those in Table 4 is gratefully acknowledged.

determined residue by its evolutionary conservation; and then evaluate the mutation sites in relation to the characterized domains (Table 3).

Human and chicken PDB files were analysed. Each file was derived from crystallized TPI protein with a substrate analogue bound in the active site of one of the two subunits. Substrate-binding residues were determined as contact sites (defined as within 4 Å) of the bound substrate analogue. Flexible loop residues were determined as those comprising the portion of the random coil separating the beta-sheet and the alpha-helix in loop 6 that were non-superimposed in the 'closed' (substrate-bound) configuration compared with the 'open' (no substrate) configuration. Dimer interface residues were determined to be residues in one subunit that had contact sites within the other subunit. Evolutionary conservation was taken as an indicator of the validity of the approach to the selection of the amino acid residues comprising each of the three functional domains. All of the determined substrate contact sites, as well as all of the determined flexible loop residue sites, were found to be conserved across the entire phylogenetic spectrum from *Escherichia coli* to man. A similar conservation was found for 11 out of the 34 determined dimer interface residues. A high degree of conservation was also found for near neighbours, amino acid residues determined by their proximity within 4 Å of the previously defined residues in the three functional domains. Similarly determined second-degree (neighbours of neighbours) and third-degree neighbours were also highly conserved.

The mutation sites were then evaluated in relation to the defined functional domains. We found that the enzyme deficiency mutations were not random but fell in one or more of the three domains or among the first- or second-degree contacts of the domain residues (Table 4). It was predicted that mutation sites in or interacting with the substrate-binding site or flexible loop would be expected to manifest catalytic abnormalities. Mutation sites in or interacting with the dimer interface would be expected to exhibit molecular instability manifest as thermolability. Assignment to one domain did not preclude assignment to or interaction with other domains.

In the main, the predictions based on domain assignment were in accord with phenotypic observations. Our group predicted catalytic defects for the mutations in or interacting with the substrate-binding site – Cys41Tyr, Gly72Ala, Ile170Val, Val231Met and Phe240Leu – and with the flexible loop: Ile170Val. We also predicted molecular instability for the mutations in or reacting with the dimer interface: Cys41Tyr, Gly72Ala, Glu104Asp, Ile170Val and Val231Met. These predictions were in

Table 4. Assignment of triosephosphate isomerase missense mutations to defined functional domains of the enzyme protein.

Mutation	Relation to defined domains	Conservation	Expected properties
Cys41Tyr	Near neighbour of 10Gly and 12Trp, which in turn are near neighbours of 11Asn and 13Lys, both of which are substrate-binding and dimer interface residues	Six out of 18 species	Catalytic abnormalities and molecular instability
Gly72Ala	A substrate-binding residue and a near neighbour of 13Lys (in the other monomer), which is a substrate-binding and dimer interface residue; and of 71Asn, 73Ala and 74Phe, which are dimer interface residues	All species studied	Catalytic abnormalities and molecular instability
Glu104Asp	Near neighbour of 98Arg and 103Phe, which are dimer interface residues	All species studied	Molecular instability
Gly122Arg	No relation to defined functional domains	Six out of 18 species	No abnormalities
Val154Met	No relation to defined functional domains	Ten out of 18 species. Others substitute a Leu for a Val	No abnormalities
Ile170Val	A substrate-binding and flexible loop residue and a near neighbour of 167Val and 168Trp, both flexible loop residues; to 169Ala and 171Gly, both substrate-binding and flexible loop residues; and to 173Thr, a flexible loop residue	All species studied	Catalytic abnormalities and molecular instability
Val231Met	A substrate-binding residue and near neighbour of 11Asn, a substrate-binding and dimer interface residue; and to 230Leu and 232Gly, both substrate-binding residues	All species studied	Catalytic abnormalities and molecular instability
Phe240Leu	Near neighbour of 231Val, a substrate-binding residue and itself a mutation site	Fourteen out of 18 species	Catalytic abnormalities

concert with phenotypic observations.[59,61,67,84,93] The mutation Gly122Arg had no demonstrable interaction with a functional domain, which was in accord with the unimpaired catalytic activity of the mutation at this site. In addition, Val154Met had no interaction with a functional domain, and the reported catalytic deficiency of this mutation remains unexplained.

Putative high incidence of TPI heterozygosity in African-Americans

Our group next turned to the investigation of the putative high incidence of TPI deficiency heterozygosity in the African-American population and its reported relationship to the −5, −8 and −24 substitutions.[93] We performed TPI enzyme assay and determined haplotypes for the three substitutions in 424 African-American subjects and 75 white subjects.[99,100] Overall TPI enzyme activity was somewhat less in the African-American group than in the white group, but in contrast to the earlier findings of Mohrenweiser and Fielek[26], we were unable to identify deficient heterozygotes with any certainty. The variant haplotypes were surprisingly frequent, 41% of the African-American subjects carrying the upstream substitutions. Four haplotypes were identified in the African-Americans: no substitutions, −5 only, −5 with −8, and −5, −8 and −24 together. Three subjects were homozygous for the −5, −8 haplotype, a finding inconsistent with the earlier suggestion that the haplotype was associated with a TPI null allele, incompatible with life in homozygotes.[93] Also, although the −5, −8 haplotype was associated with a somewhat reduced TPI enzyme activity, accounting for the generally decreased activity in the African-American group, there was no clear-cut relationship with a reduction in enzyme activity sufficient to identify TPI deficient heterozygotes.

These findings were augmented by the recent studies by Humphries and her colleagues from London[101], who in a study of 378 subjects identified yet another infrequent (1/378) haplotype – that of −8 alone – and, significantly, pointed out that the upstream polymorphisms were more widely distributed than had been realised. Although the frequency was highest in African and Caribbean populations, small numbers were also found in Northern European (3/57), Middle Eastern (3/42) and Asian Indian (2/46) populations. In East Asian populations, a surprisingly high frequency (26/47) of the −5 haplotype was noted. The −5, −5 with −8, and −5, −8 and −24 together haplotypes were each found to be in linkage disequilibrium with the previously described[52] intragenic polymorphism in intron 5. This evidence, along with the relative frequencies of the haplotypes, suggested that the polymorphisms arose from a common −5 ancestral haplotype.

These workers also concluded that the −5 substitution had no effect on TPI enzyme activity, in contrast to the −8 and −24 substitutions, which were associated with a progressive diminution in enzyme activity. Such reductions in activity were minor, however, and these authors considered it unlikely that these substitutions might contribute to compound heterozygosity and clinical TPI deficiency. This remains an open question, however, since it remains possible that decreased activity, such as that observed at the lower end of the frequency distribution of enzyme activities in subjects with the −5 with −8, and −5, −8 and −24 haplotypes, might sometimes contribute to clinical TPI deficiency.[100] This issue will hopefully be clarified by the study of additional clinically affected subjects, such as the one observed by our group[80,99,100] and the one from France[95], each with the co-inheritance of a TPI deficiency allele in compound heterozygosity with one of the variant promoter haplotypes.

TPI enzyme transfer: can deficient enzyme activity be restored?

In an interesting group of reports[102–106], Ationu and his colleagues in London have recently suggested that it might be possible to reverse the metabolic block in TPI deficiency. Initially, in vitro stimulation by plasma of TPI mRNA expression in TPI-deficient cells was demonstrated, as was a direct stimulation of TPI gene expression by butyrate in a human endothelial cell line.

Following these observations, the possibility of enzyme secretion from exogenous sources and recapture by TPI-deficient cells was investigated in a number of ways. A transient increase in serum and lymphocyte TPI activity and a decreased lymphocyte DHAP concentration after the transfusion of normal red cells were demonstrated in a TPI-deficient patient. An in vitro system was devised in which TPI-deficient lymphoblastoid cells or primary skeletal myoblasts were cultured with exogenous rabbit muscle enzyme or co-cultured with human erythroleukaemia cells as a renewable source of enzyme. The two types of cell were separated by a semi-permeable membrane that prevented cell-to-cell contact or mixing, but allowed the diffusion of proteins and smaller molecules. Extraordinarily, a restoration of intracellular enzyme activity and a reduction of DHAP to normal levels was achieved in both types of deficient cell.

Apparent enzyme transfer was also observed with non-deficient human lymphoblastoid, muscle and astrocytoma cell lines. In addition, an increased intracellular TPI activity and a decreased DHAP level were observed when TPI-deficient cells were cultured in the presence of fresh frozen plasma as a source of exogenous enzyme. It was concluded that a transport mechanism must exist that permits the transfer of functional enzyme across the cell membrane, and that enzyme replacement therapy in TPI deficiency might thus be possible. It was suggested that enzyme might be continuously provided by haemopoietic stem-cell transplantation and might be delivered to deficient neural cells by bone marrow-derived microglial cells.

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