PDH E1 alpha missense mutations-supplement

The amino acid sequence of PDH E1a (isoform 1, shown in Figure 1, below) is presented at the following NCBI (National Center for Biotechnology Information) link:
http://www.ncbi.nlm.nih.gov/protein/4505685?report=genpept

BLAST analysis of this sequence at NCBI reveals conserved domains: the amino acids comprising the TPP binding site, the heterodimer interface, the tetramer interface and the phosphorylation loop region. These conserved domains are indicated by the underlined amino acids in figures 2 through 5. These conserved domains are identified at the following NCBI link:
http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?SEQUENCE=NP_000275.1&FULL

The three-dimensional structure of PDH E1a (Protein Data Base ID 3EXI) is presented at the NCBI at the following link:

http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?uid=68173

The E1a missense mutations are summarized in the context of E1a amino acid sequence (isoform 1) in Figure 1.

Figure 1 here

Legend for Figure 1. Reported missense mutations are listed below the corresponding mutated amino acid in the E1a sequence. Missense mutations are color coded to reflect the residual PDH enzyme activity reported for the particular mutation.

The missense mutations are scattered throughout the 390 amino acid sequence. One mutation, R10P, lies within the 29 amino acid mitochondrial targeting sequence, while all others lie within the mature protein. The PDH activity of 28% of normal reported for the R10P mutant may reflect a PDH insufficiency subsequent to the impaired mitochondrial targeting of the precursor protein. Discordant activities reported for similar mutations at the same site (Fig. 1) may reflect variation in the individual laboratory assays or, perhaps, different genetic backgrounds of the affected individuals.

Some of the reported missense mutations lie within, or close to amino acids comprising the thiamine pyrophosphate (TPP) binding site (Fig. 2)

Figure 2 here

Legend for Figure 2. Reported missense mutations are shown in relation to the amino acids (underlined) comprising the TPP binding site of E1a.

Mutations of amino acids within or flanking residues comprising the TPP binding site, such as G195A, A199T and Y227H likely reduce the TPP binding affinity directly, consistent with the severe PDH deficiency observed for these mutations (Figure 2). Individuals harboring such mutations may find that elevated levels of thiamine will compensate the reduced affinity, ameliorating the PDH deficiency. Elevated thiamine levels are also indicated for other mutations lying in the vicinity of the TPP binding site, as these may secondarily diminish TPP binding.

Several missense mutations lie within, or flanking regions known to comprise the heterodimer interface of Ela (Figure 3).

Figure 3 here

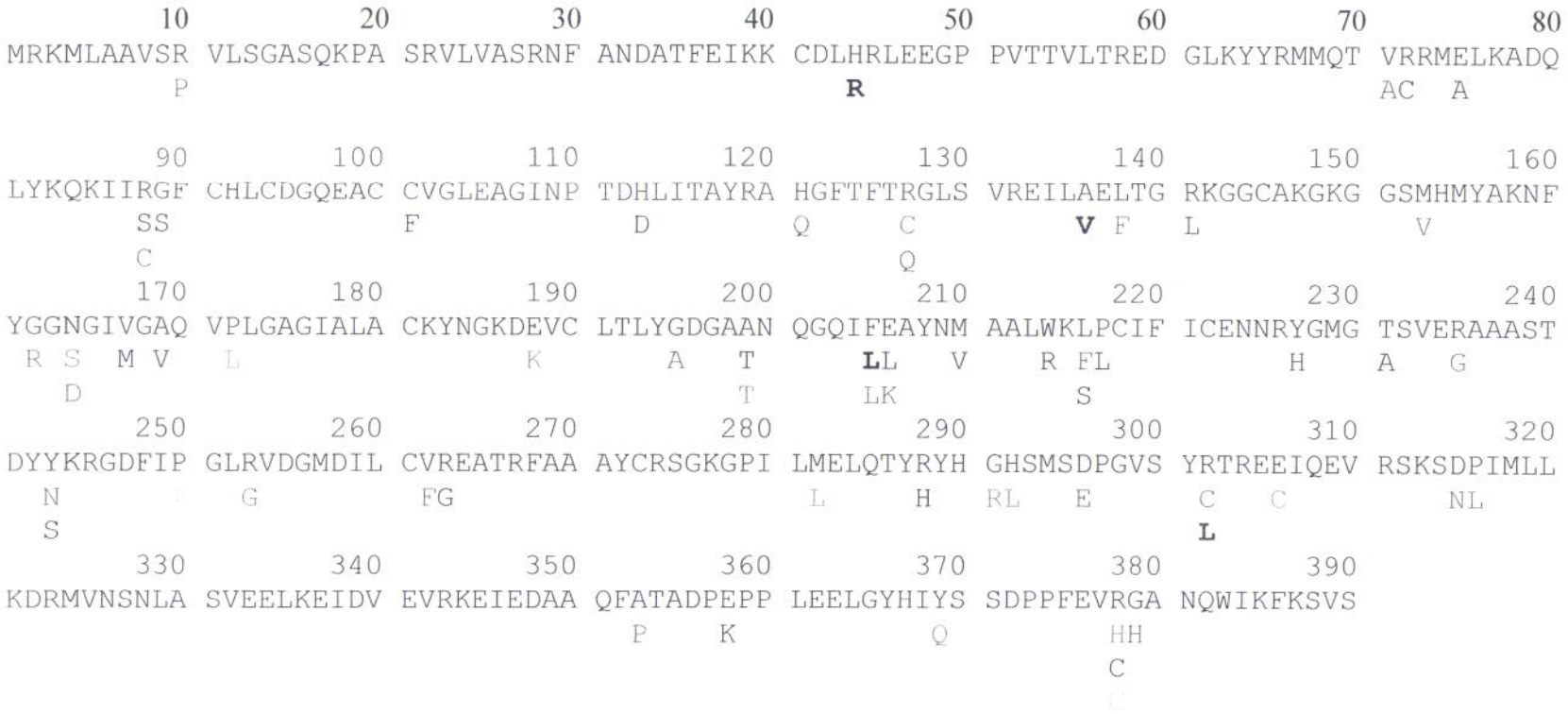
Legend for Figure 3. Reported missense mutations are shown in relation to the amino acids (underlined) comprising the heterodimer interface of E1a.

The severity of these mutations (V167M, A169V and W214R) reflects the perturbed interaction of the E1a and E1b proteins in constituting the PDH holoenzyme. Additional mutations reported for amino acids comprising the PDH tetramer interface (Figure 4) have equally severe effects on PDH activity, likely interfering with the active tetrameric form of the enzyme. It is noted that several mutations within or flanking the phosphorylation loop region of E1a (Figure 5) also result in severely diminished PDH activity.

Legend for Figure 4. Reported missense mutations are shown in relation to the amino acids (underlined) comprising the tetramer interface of E1a.

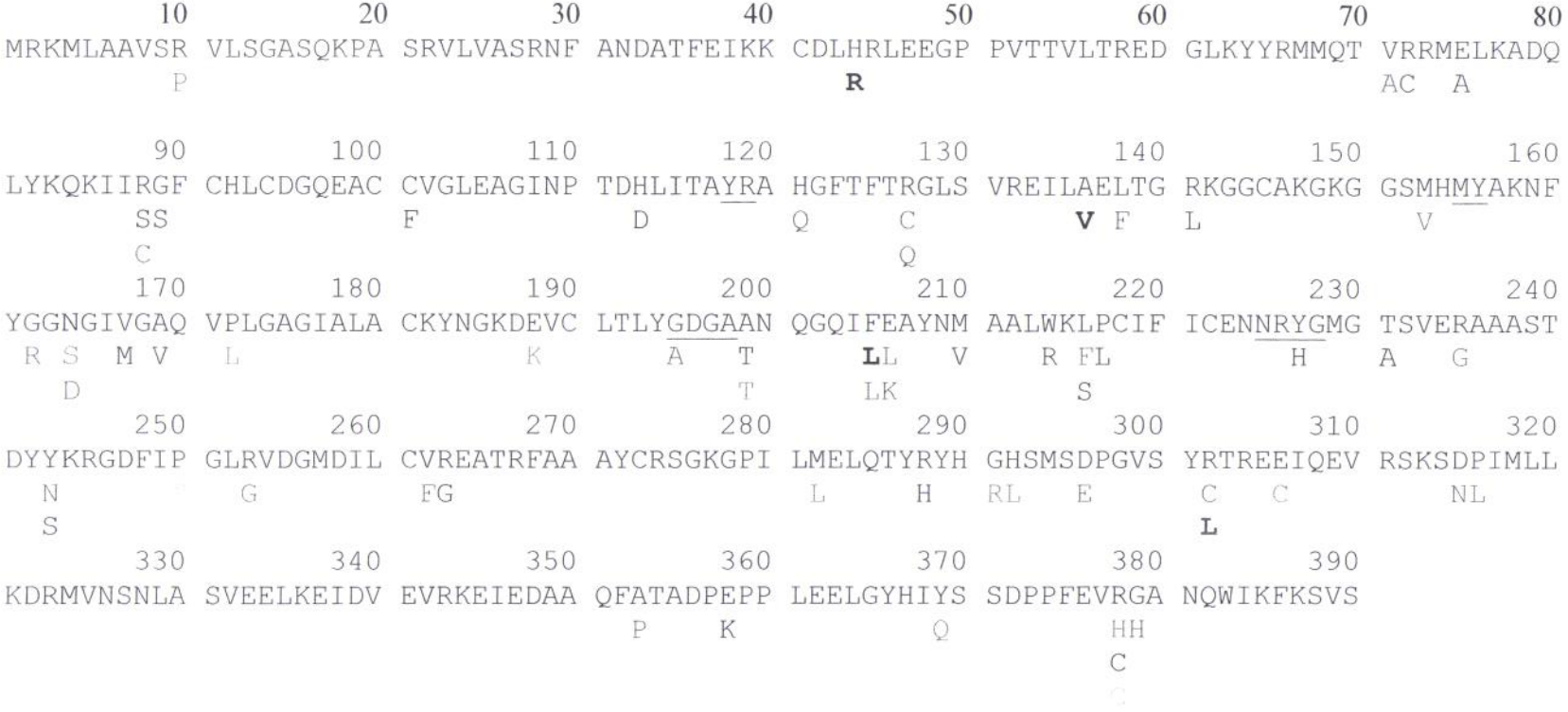
Legend for Figure 5. Reported missense mutations are shown in relation to the amino acids (underlined) comprising the phosphorylation loop region of E1a.

Fig. 1. PDH E1alpha iso 1 missense mutations



* PDH activity 0-25%, **26-49%**, 50-74%, **75-100%**

Fig. 2. PDH E1alpha iso 1 missense mutations TPP Site



* PDH activity 0-25%, **26-49%**, 50-74%, **75-100%**

Fig. 3. PDH E1alpha iso 1 missense mutations Heterodimer Interface

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        10         20         30         40         50         60         70         80
MRKMLAAVSR VLSGASQKPA SRVLVASRNF ANDATFEIKK CDLHRLEEGP PVTTVLTRED GLKYYRMMQT VRRMELKADQ
         P                                      R                             AC  A

        90        100        110        120        130        140        150        160
LYKQKIIRGF CHLCDGQEAC CVGLEAGINP TDHLITAYRA HGFTFTRGLS VREILAELTG RKGGCAKGKG GSMHMYAKNF
       SS              F            D        Q    C         V F   L            V
        C                                         Q
       170        180        190        200        210        220        230        240
YGGNGIVGAQ VPLGAGIALA CKYNGKDEVC LTLYGDGAAN QGQIFEAYNM AALWKLPCIF ICENNRYGMG TSVERAAAST
  R S  M V   L                K       A   T     LL    V    R FL          H    A   G
    D                                     T     LK           S
       250        260        270        280        290        300        310        320
DYYKRGDFIP GLRVDGMDIL CVREATRFAA AYCRSGKGPI LMELQTYRYH GHSMSDPGVS YRTREEIQEV RSKSDPIMLL
   N          G         FG                    L     H   RL   E      C   C         NL
   S                                                                L
       330        340        350        360        370        380        390
KDRMVNSNLA SVEELKEIDV EVRKEIEDAA QFATADPEPP LEELGYHIYS SDPPFEVRGA NQWIKFKSVS
                                    P    K           Q         HH
                                                               C
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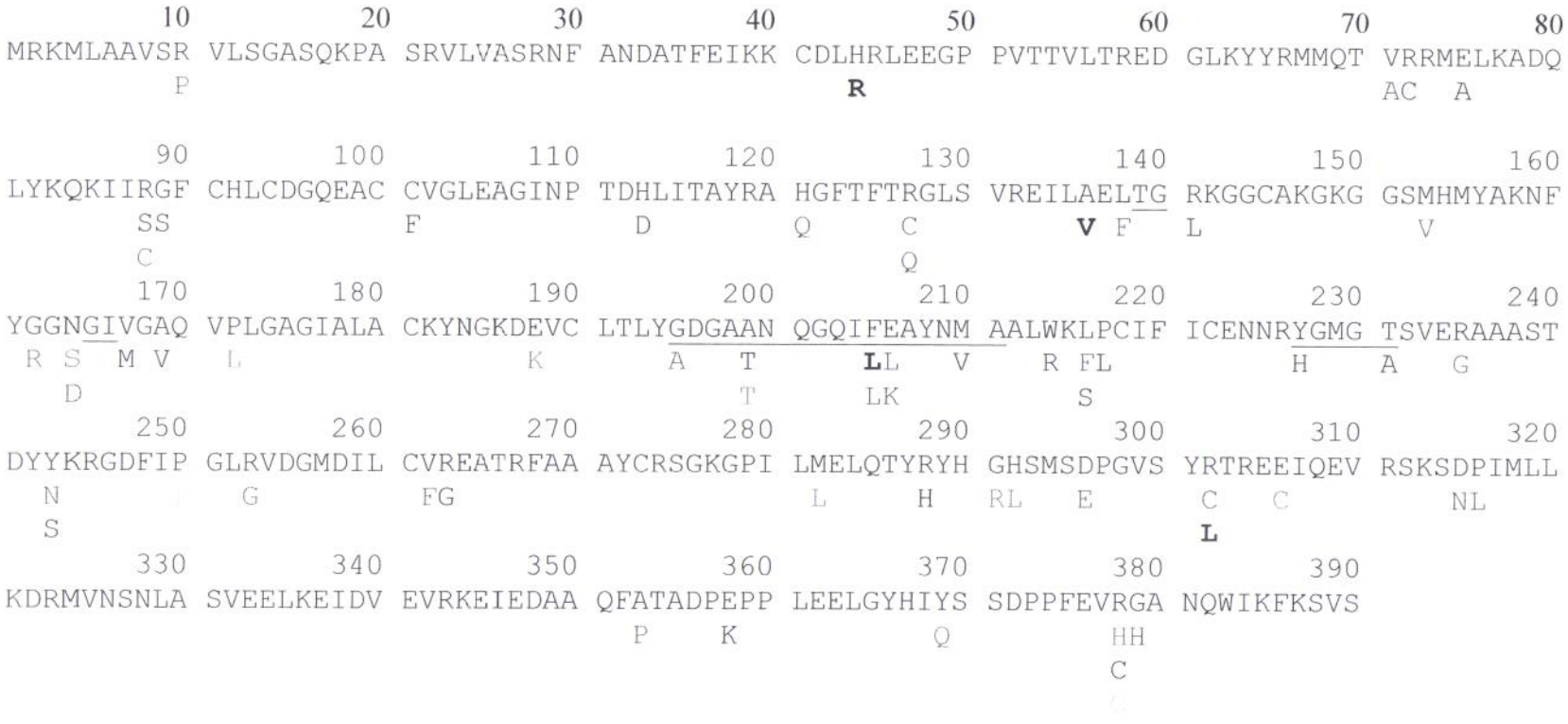
* PDH activity 0-25%, **26-49%**, 50-74%, **75-100%**

Fig. 4. PDH E1alpha iso 1 missense mutations Tetramer Interface



* PDH activity 0-25%, **26-49%**, 50-74%, **75-100%**

Fig. 5. PDH E1alpha iso 1 missense mutations Phosphorylation Loop Region

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        10         20         30         40         50         60         70         80
MRKMLAAVSR VLSGASQKPA SRVLVASRNF ANDATFEIKK CDLHRLEEGP PVTTVLTRED GLKYYRMMQT VRRMELKADQ
         P                                     R                             AC  A

        90        100        110        120        130        140        150        160
LYKQKIIRGF CHLCDGQEAC CVGLEAGINP TDHLITAYRA HGFTFTRGLS VREILAELTG RKGGCAKGKG GSMHMYAKNF
       SS              F            D        Q     C          V F   L             V
       C                                           Q
       170        180        190        200        210        220        230        240
YGGNGIVGAQ VPLGAGIALA CKYNGKDEVC LTLYGDGAAN QGQIFEAYNM AALWKLPCIF ICENNRYGMG TSVERAAAST
 R S  M V    L                K       A   T      LL   V    R FL          H    A   G
   D                                      T      LK          S
       250        260        270        280        290        300        310        320
DYYKRGDFIP GLRVDGMDIL CVREATRFAA AYCRSGKGPI LMELQTYRYH GHSMSDPGVS YRTREEIQEV RSKSDPIMLL
  N           G         FG                     L     H   RL   E      C   C         NL
  S                                                                 L
       330        340        350        360        370        380        390
KDRMVNSNLA SVEELKEIDV EVRKEIEDAA QFATADPEPP LEELGYHIYS SDPPFEVRGA NQWIKFKSVS
                                   P    K            Q          HH
                                                                C
```

* PDH activity 0-25%, **26-49%**, 50-74%, **75-100%**