International Journal of Pharma and Bio Sciences V1(2)2010

IN SILICO PHARMACOGENOMIC ANALYSIS OF ALCOHOL DEHYDROGENASE INVOLVED IN ALCOHOLISM

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ABSTRACT

Alcoholism is a disease characterized by lack of a person to metabolize the alcohol efficiently. Pharmacogenomic analysis deals with study how different individuals behave towards same drug or chemical. The present work examined *Insilco* sequence and structural differences in the isoenzymes of alcohol dehydrogenase involved in alcohol metabolism. **Methods:** Experiment, identified ADH1B, ADH1A, ADH1C and ADH4 as four different alcohol dehydrogenase isoenzymes, their sequences in the FASTA format was retrieved form uniprot knowledgedatabase. Sequence alignment, dendogram generation, motif identification, secondary structure prediction was carried out and examined with the help of different computational programs. **Results:** Multiple sequence alignment, Phylogenetic tree, amino acid composition, Percentage of different secondary structures reflect high degree of similarity in the results for ADH1B, ADH1A and ADH1C except there are some significant deviations in the results for ADH4. While analyzing the amino acids of active site region it was found that five out of nine amino acids are identical but four amino acid showed some variations. Ser at position 48 is replaced by Thr in ADH1B, ADH1A and ADH4. His at position 51, Ala at position 317 and Cys at position 146, is mutated with Thr in all three cases and all three mutations have occurred only in case of ADH4.*Insilco* analysis suggests that minimum mutations took place in the active site region of ADH1C, followed by ADH4, ADH1B and ADH1A.

It can be concluded that individuals may inclined towards alcoholism either due to the absence of normal form of isoenzyme or presence of mutated form of isoenzyme (Pharmacogenomics), which is unable to perform its normal function of metabolism.

KEYWORDS

Insilico, Pharmacogenomics, Alcoholism, Alcohol Dehydrogenase.

INTRODUCTION

Modern medical definitions describe alcoholism as a disease and addiction which results in a persistent use of alcohol despite negative consequences.

But in medical terms alcoholism can defines as a disease characterized by impaired regulation of alcohol consumption that, overtime, leads to impaired control overdrinking, tolerance, psychological dependence (craving) and physical dependence (withdrawal signs upon cessation). [1]

Pharmacogenomics is a science that examines the inherited variations in genes (variations/ in their DNA base content) that dictate drug response and explores

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the ways these variations can be used to predict whether an individual will have a good response to a drug, a bad response to a drug, or no response at all. When inherited variations in the genes translate, they give rise to different amino acid sequences, which finally leads to different protein products. The functional capacity of these proteins differs with each other. Some of them keep the original function of the genes while others may not be that much functionally active. An indivudual may show the Alcoholism due to the presence of variations the gene, that encodes for alcohol dehydrogenase. These variations in the gene sequences leads to the expression the different forms of alcohol dehydrogenase (ADH1B, ADH1A, ADH1C and ADH4). The body metabolizes alcohol extremely quickly. Once alcohol reaches the stomach, it begins to break down with the alcohol dehydrogenase [2]. The enzyme is contained in the lining of the stomach and in the liver. It catalyzes the oxidation of ethanol to acetaldehyde.

The above alcohol metabolizing enzyme alcohol dehydrogenase is found in the form of four isoenzymes ADH1B, ADH1A, ADH1C and ADH4 in the humans [8]. Furthermore the presence of any of these isoenzymes and alcohol metabolizing capacity of these isoenzymes in an individual varies significantly. It is reported that percentage of liver contribution of these enzymes ADH1B (21.8), ADH1A (8.1), ADH1C (41.5) and ADH4 (28.6) is different [3]. This means if a person has isoenzymes ADH1A the chances of alcoholism is maximum and presence of ADH1C reduces the possibility of alcoholism in the individual.The main objective of present work is to identify the isoenzymes involved in the alcohol metabolism for sequence and structural analysis by using various computational tools to get insight into the role of above isoenzymes in the alcoholism.

MATERIALS AND METHODS

The sequence and structural data of the identified isoenzymes, ADH1B, ADH1A, ADH1C and ADH4 involved in the metabolism of alcoholism was retried in the FASTA format from uniprot knowledgedatabase available at the expert protein analysis server (ExPASy) server. This was used for the *in-silico* analysis. Several Web-accessible sites offer useful tools for sequence analysis via sequence similarity searches, motif searches, and structural comparisons. The Multiple sequence alignment of the amino acid sequences of ADH1B, ADH1A, ADH1C and ADH4 in FASTA format was carried out by using clustalW server (align.genome.jp/) to identify the identical, similar and different regions in the sequences and to generate dendogram to establish phylogenetic relationship among the selected isoenzymes. The motif was identified in amino acid sequences of ADH1B, ADH1A, ADH1C and ADH4 by using motif search server (motif.genome.jp). Identification of motif gives the insight into the pattern of conserve amino acids in the sequences. The amino acid composition in the amino acid sequences of ADH1B, ADH1A, ADH1C and ADH4 was determined by using protparam tool available at the ExPASy server (http://www.expasy.ch/tools/protparam.html). The different secondary structures and their corresponding amount of ADH1B, ADH1A, ADH1C and ADH4 was predicted by using GOR IV secondary structure prediction method. In this way pattern of secondary structure of selected enzymes was analyzed.

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RESULTS

Four metabolic isoenzymes, which plays active role in metabolism of alcohol, were used for the study. These isoenzymes were known to facilitate the interconversion between alcohols and aldehydes or ketones.

Table 1.1
Metabolic isoenzymes identified

Entry results — Hide | Top

	Accession	Entry name	Protein names	Organism	Gene
☐	P00326	ADH1G_HUMAN	**Alcohol dehydrogenase 1C** (EC 1.1.1.1) (Alcohol dehydrogenase subunit gamma)		ADH1C (ADH3)
☐	P00325	ADH1B_HUMAN	**Alcohol dehydrogenase 1B** (EC 1.1.1.1) (Alcohol dehydrogenase subunit beta)		ADH1B (ADH2)
☐	P07327	ADH1A_HUMAN	**Alcohol dehydrogenase 1A** (EC 1.1.1.1) (Alcohol dehydrogenase subunit alpha)		ADH1A (ADH1)
☐	P08319	ADH4_HUMAN	**Alcohol dehydrogenase 4** (EC 1.1.1.1) (Alcohol dehydrogenase class II pi chain)		ADH4

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P00326   MSTAGKVIKCKAAVLWELKKPFSIEEVEVAPPKAHEVRIKMVAAGICRSDEHVVSGNLV-  59   ADH1G_HUMAN
P00325   MSTAGKVIKCKAAVLWEVKKPFSIEDVEVAPPKAYEVRIKMVAVGICRTDDHVVSGNLV-  59   ADH1B_HUMAN
P07327   MSTAGKVIKCKAAVLWELKKPFSIEEVEVAPPKAHEVRIKMVAVGICGTDDHVVSGTMV-  59   ADH1A_HUMAN
P08319   MGTKGKVIKCKAAIAWEAGKPLCIEEVEVAPPKAHEVRIQIIATSLCHTDATVIDSKFEG  60   ADH4_HUMAN
         *.* *********: **  **:.**:********:****:::*...:* :*  *:...:
P00326   TPLPVILGHEAAGIVESVGEGVTTVKPGDKVIPLFTPQCGKCRICKNPESNYCLKN-DLG  118  ADH1G_HUMAN
P00325   TPLPVILGHEAAGIVESVGEGVTTVKPGDKVIPLFTPQCGKCRVCKNPESNYCLKN-DLG  118  ADH1B_HUMAN
P07327   TPLPVILGHEAAGIVESVGEGVTTVKPGDKVIPLAIPQCGKCRICKNPESNYCLKN-DVS  118  ADH1A_HUMAN
P08319   LAFPVIVGHEAAGIVESIGPGVTNVKPGDKVIPLYAPLCRKCKFCLSPLTNLCGKISNLK  120  ADH4_HUMAN
          .:***:**********:* ***.**********  * * **:.* .* :* * *  ::
P00326   NPRG---TLQDGTRRFTCSGKPIHHFVGVSTFSQYTVVDENAVAKIDAASPLEKVCLIGC  175  ADH1G_HUMAN
P00325   NPRG---TLQDGTRRFTCRGKPIHHFLGTSTFSQYTVVDENAVAKIDAASPLEKVCLIGC  175  ADH1B_HUMAN
P07327   NPQG---TLQDGTSRFTCRRKPIHHFLGISTFSQYTVVDENAVAKIDAASPLEKVCLIGC  175  ADH1A_HUMAN
P08319   SPASDQQLMEDKTSRFTCKGKPVYHFPGTSTFSQYTVVSDINLAKIDDDANLERVCLLGC  180  ADH4_HUMAN
         .* .    ::* * ****  **::**.* *********.:  :****  : **:***:**
P00326   GFSTGYGSAVKVAKVTPGSTCAVFGLGGVGLSVVMGCKAAGAARIIAVDINKDKFAKAKE  235  ADH1G_HUMAN
P00325   GFSTGYGSAVNVAKVTPGSTCAVFGLGGVGLSAVMGCKAAGAARIIAVDINKDKFAKAKE  235  ADH1B_HUMAN
P07327   GFSTGYGSAVNVAKVTPGSTCAVFGLGGVGLSAIMGCKAAGAARIIAVDINKDKFAKAKE  235  ADH1A_HUMAN
P08319   GFSTGYGAAINNAKVTPGSTCAVFGLGGVGLSAVMGCKAAGASRIIGIDINSEKFVKAKA  240  ADH4_HUMAN
         *******:*:: ********************.:********:***.:***.:**.***
P00326   LGATECINPQDYKKPIQEVLKEMTDGGVDFSFEVIGRLDTMMASLLCCHEACGTSVIVGV  295  ADH1G_HUMAN
P00325   LGATECINPQDYKKPIQEVLKEMTDGGVDFSFEVIGRLDTMMASLLCCHEACGTSVIVGV  295  ADH1B_HUMAN
P07327   LGATECINPQDYKKPIQEVLKEMTDGGVDFSFEVIGRLDTMMASLLCCHEACGTSVIVGV  295  ADH1A_HUMAN
P08319   LGATDCLNPRDLHKPIQEVIIELTKGGVDFALDCAGGSETMKAALDCTTAGWGSCTFIGV  300  ADH4_HUMAN
         ****:*:**:* :******: *:*.*****:::  *  :** *:* *   . *:..::**
P00326   PPDSQNLSINPMLLLTGRTWKGAIFGGFKSKESVPKLVADFMAKKFSLDALITNILPFEK  355  ADH1G_HUMAN
P00325   PPASQNLSINPMLLLTGRTWKGAVYGGFKSKEGIPKLVADFMAKKFSLDALITHVLPFEK  355  ADH1B_HUMAN
P07327   PPDSQNLSMNPMLLLTGRTWKGAILGGFKSKECVPKLVADFMAKKFSLDALITHVLPFEK  355  ADH1A_HUMAN
P08319   AAGSKGLTVFPEELIIGRTINGTFFGGWKSVDSIPKLVTDYKNKKFNLDALVTHTLPFDK  360  ADH4_HUMAN
         .. *:.*:: *  *: *** :*:. **:** : :****:*:  ***.****:*: ***:*
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Fig 1.1 ***Sequence alignment of selected enzymes***

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Figure 4.1 represents the sequence alignment of ADH1B, ADH1A, ADH1C and ADH4. It can be seen that most of the amino acid are identical in all four enzymes at the corresponding positions, which is represented by * below the alignment. But some amino acids are identical in two enzymes, some are identical in three enzymes and some are different in all enzymes. Position 60,117,125,126,127 is filled with different amino acids in ADH4. The positions are missing in ADH1B, ADH1A, and ADH1C.

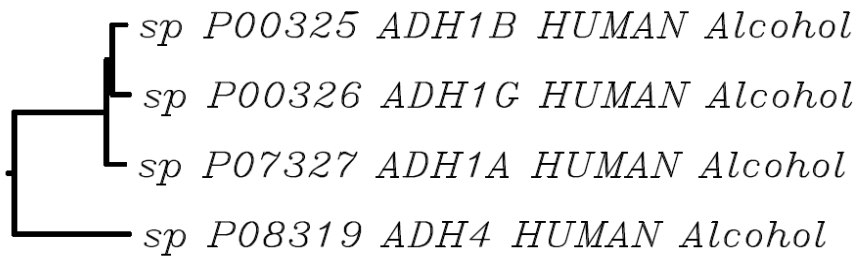

Fig 1.2 ***Phylogenetic tree generated for selected enzymes***

Figure 1.2 Phylogenetic tree represents the Phylogenetic relationship of ADH1B, ADH1A, ADH1C and ADH4. The branch length represent degree of Phylogenetic distance among the enzymes. Closely related enzymes are connected with same branch in single group. It can be seen that ADH1B and ADH1C are highly identical as far as their ancestral relationship is concerned. ADH1A is also connected with the same branch.
But ADH4 is connected with a separate long branch, suggesting that it is the most distinct enzyme among all four.

Table 1.2
Motif and there position in ADH1B, ADH1A, ADH1C and ADH4

S.No.	Enzyme	Position of Motif	Motif Found
1	ADH1B	67..81	GHEAAGIVESVGEGV
2	ADH1A	67..81	GHEAAGIVESVGEGV
3	ADH1C	67..81	GHEAAGIVESVGEGV
4	ADH4	68..82	GHEAAGIVESIGPGV

Table 1.2 Composition of amino acid in ADH1B, ADH1A, ADH1C and ADH4 In the above table and sequence motifs, it can be seen that single motif is present in all four enzymes. The position of motifs is same (67...81) in ADH1B, ADH1A, ADH1C but the position of motif in ADH4 is different (68...82). Significant point is that the motif is identical at most of the positions; characters in motif in all sequences are same except two positions in ADH4 at 78 and 80. The motif can be said to be conserved.

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Table 1.3
Composition of amino acids present in ADH1B, ADH1A, ADH1C and ADH4

S.No.	Amino Acid	ADH1B Composition (%)	ADH1A Composition (%)	ADH1C Composition (%)	ADH4 Composition (%)
1	Ala	8.3	8.3	8.0	9.2
2	Arg	2.9	2.4	2.9	2.1
3	Asn	3.2	2.9	3.2	3.4
4	Asp	4.5	4.5	4.3	5.3
5	Cys	4.0	4.3	4.0	4.2
6	Gln	1.6	1.9	1.6	1.6
7	Glu	5.1	5.3	5.6	4.5
8	Gly	10.1	9.6	9.9	10.3
9	His	1.9	2.1	1.6	1.6
10	Ile	5.9	6.9	6.4	8.2
11	Leu	7.7	7.7	7.7	7.9
12	Lys	8.5	8.5	8.8	8.7
13	Met	2.1	2.9	2.1	1.3
14	Phe	4.3	4.0	4.5	5.0
15	Pro	5.3	5.3	5.3	4.5
16	Ser	5.6	6.1	6.4	6.1
17	Thr	6.4	5.9	5.9	7.1
18	Trp	0.5	0.5	0.5	0.8
19	Tyr	1.6	1.1	1.1	1.3
20	Val	10.4	9.6	10.1	7.1

The amino acid composition in ADH1B, ADH1A, ADH1C and ADH4 is similar with few exceptions in the composition of ADH4. Amino acid number 10 (Ile), 13(Met), 17(Thr), 20(Val) of ADH4 (Table 1.3) has significant variation in their compositions.

Table 1.4
***Composition of secondary structures of ADH1B, ADH1A, ADH1C and* ADH4**

S.No.	Enzyme	Alpha helix (%)	Extended Strand (%)	Random coil (%)
1	ADH1B	24.00	24.27	51.53
2	ADH1A	27.73	21.87	50.40
3	ADH1C	28.80	22.67	48.53
4	ADH4	23.42	21.84	54.74

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Table 1.4, the secondary structure of ADH1B, ADH1A, ADH1C and ADH4 were compared. It has been observed that there are some variations in the percentage of different secondary structures like Alpha helix, Extended Strand and Random coil among ADH1B, ADH1A, ADH1C and ADH4. Percentage of Alpha helix is maximum in ADH1C and minimum in ADH4. Percentage of extended strand is maximum in ADH1B and similar in rest of the enzymes. Percentage of random coil is maximum in ADH4 and minimum in ADH1C. There is also some change in pattern of folding of amino acid into secondary structure.

Table 1.5

Amino acid composition of active site of alcohol dehydrogenase

S.No.	Amino acid	Position in Amino acid sequence
1	His	67
2	Cys	174
3	Cys	46
4	Ser	48
5	His	51
6	Ile	269
7	Val	292
8	Ala	317
9	Phe	319

Table 1.5 showed that active site of enzyme is responsible for its catalytic activity. Substrate has to bind with the amino acids of active site. If there is some mutation or deletion of amino acids in the active site region, substrate is no longer able to bind with the enzyme; hence enzyme fails to catalyze the reaction.

While comparing the amino acid of active site with the amino acid sequence of ADH1B, ADH1A, ADH1C and ADH4 it is found that five out of nine amino acids are identical but four amino acid show some variations. Ser at position 48 is replaced by Thr in ADH1B, ADH1A and ADH4. His at position 51, Ala at position 317 and Cys at position 146, is mutated with Thr in all three cases and all three mutations have occurred only in case of ADH4. The mutation of Ser by Thr at position 48 is the most significant one as, Ser-48 plays it role in the deprotonation of the alcohol, which makes it an aldehydes.

DISCUSSION

Four metabolic isoenzymes, which plays active role in metabolism of alcohol, were used for the study. These alcohol dehydrogenase isoenzymes were known to facilitate the interconversion between alcohols and aldehydes or ketones. These aldehydes or ketones will further oxidized into acetyl CoA by aldehydes dehydrogenase, acetyl CoA will enters into citric acid cycle generating carbon- di -Oxide, water and ATP molecules. Hence the alcohol get metabolized and converted to hydrophilic compounds so that it can leave the body with minimum toxitcity**.**

The sequence alignment of ADH1B, ADH1A, ADH1C and ADH4 (Figure 4.1) represents, that most of the amino acid are identical in all four enzymes. Amino acid position 60,117,125,126,127 is filled with different amino acids in ADH4. The positions are missing in ADH1B, ADH1A, and ADH1C.

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The Phylogenetic tree represents the Phylogenetic relationship of ADH1B, ADH1A, ADH1C and ADH4.The branch length represent degree of Phylogenetic distance among the enzymes. Closely related enzymes are connected with same branch in single group. It can be seen that ADH1B and ADH1C are highly identical as far as their ancestral relationship is concerned. ADH1A is also connected with the same branch. But ADH4 is connected with a separate long branch, suggesting that it is the most distinct enzyme among all four. This appears important because these insertions of the amino acids in ADH4 may be important for the relatively high catalytic activity in comparison with ADH1B and ADH1A.Therefore due to these insertions ADH4 is phylogenitically most distinct isoenzymes among selected isoenzymes.

Motifs were also predicted in the four enzymes, it could be seen that single motif is present in all four enzymes. The position of motifs is same (67...81) in ADH1B, ADH1A, ADH1C but the position of motif in ADH4 is different (68...82). Significant point is that the motif is identical at most of the positions; characters in motif in all sequences are same except two positions in ADH4 at 78 and 80. So it can be said that all four isoenzymes shares almost similar pattern of motif except ADH4.

The amino acid composition in ADH1B, ADH1A, ADH1C and ADH4 is similar with few exceptions in the composition of ADH4. Amino acid number 10 (Ile), 13(Met), 17(Thr), 20(Val) of ADH4 has significant variation in their compositions.

The secondary structure of ADH1B, ADH1A, ADH1C and ADH (Table 4.4) were compared, there are some variations in the percentage of different secondary structures like Alpha helix, Extended Strand and Random coil among ADH1B, ADH1A, ADH1C and ADH4. Percentage of Alpha helix is maximum in ADH1C and minimum in ADH4. Percentage of Extended Strand is maximum in ADH1B and similar in rest of the enzymes. Percentage of Random coil is maximum in ADH4 and minimum in ADH1C.

Active site of enzyme is responsible for its catalytic activity. Substrate has to bind with the amino acids of active site. If there is some mutation or deletion of amino acids in the active site region, substrate is no longer able to bind with the enzyme; hence enzyme fails to catalyze the reaction. While comparing the amino acid of active site (Table 4.5) with the amino acid sequence of ADH1B, ADH1A, ADH1C and ADH4 it is found that five out of nine amino acids are identical but four amino acid show some variations. Ser at position 48 is replaced by Thr in ADH1B, ADH1A and ADH4. His at position 51, Ala at position 317 and Cys at position 146, is mutated with Thr in all three cases and all three mutations have occurred only in case of ADH4. The mutation of Ser by Thr at position 48 is the most significant one as, Ser-48 plays it role in the deprotonation of the alcohol, which makes it an aldehydes. Ser is present in ADH1C and thus it can be explained that why ADH1C is able to metabolize 48% of alcohol.

CONCLUSION

On the bases of above finding it can be concluded that the contribution of alcohol metabolism by isoenzymes is affected by the occurrence of mutations in their amino acid sequences. ADH1B, ADH1A and ADH4 are the derived from ADH1C. ADH4 is most phylogenitically distinct isoenzyme as it deviates from rest of the selected isoenzymes.

The least mutated isoenzyme ADH1C was reported to contribute maximum in the alcohol metabolism. ADH4 has undergone mutations with retaining its metabolic activity in comparison with the ADH1B and ADH1A.

If a person shows the symptoms of alcoholism under normal conditions, it means that the metabolism of alcohol is not taking place properly. Therefore it can

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be concluded that Pharmacogenomic of the Individuals is responsible for different pattern of alcohol metabolism, alcoholism may take place due to the expression of that form of isoenzymes which is least responsible for alcohol metabolism in the individual.

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