Identification and in silico analysis of cattle DExH/D box RNA helicases

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Abstract
The helicases are motor proteins participating in a range of nucleic acid metabolisms. RNA helicase families are characterized by the presence of conserved motifs. This article reports a comprehensive in silico analysis of Bos taurus DExH/D helicase members. Bovine helicases were identified using the helicase domain sequences including 38 DDX (DEAD box) and 16 DHX (DEAH box) members. Signature motifs were used for the validation of these proteins. Putative sub cellular localization and phylogenetic relationship for these RNA helicases were established. Comparative analysis of these proteins with human DDX and DHX members was carried out. These bovine helicase have been assigned putative physiological functions. Present study of cattle DExH/D helicase will provides an invaluable source for the detailed biochemical and physiological research on these members.

Keywords: RNA helicases, DEAD box, Bioinformatics, Bovine, Bos taurus

Background
A fundamental cellular action of RNA helicases is to unwind nucleic acid duplexes and thus, they are required for different cellular processes involving RNA. Among these helicases several members perform their functions in pre-mRNA processing and ribosome biogenesis (Linder 2006). The DEAD and DEAH are the subgroups of the DExH/D family (Staley and Guthrie 1998). The DDX code is used for DEAD box and DHX is used for DEAH box. The basis of nomenclature of these DExH/D helicases is the composition of conserved amino acids in their motif II. DEAD-box and DEAH-box, helicases have D-E-A-D (Asp, Glu, Ala and Asp) and D-E-A-H (Asp, Glu, Ala and His) amino acids respectively at this motif. These proteins have role in RNA metabolism viz. transcription, translation, RNA editing and folding, nuclear transport, RNA degradation and RNA-ribosomal complex formations (Linder and Daugeron 2000; Patel and Donmez 2008). Alignments of the protein sequences obtained from various organisms have revealed nine highly conserved motifs in DEAD-box proteins (Q, I, Ia, Ib, and II–VI) and eight in DEAH-box proteins I, Ia, Ib, II, III, IV, V and VI (Tuteja and Tuteja 2004a, 2004b; Tanner et al. 2003). Among these motifs, motif II (or Walker B motif) along with motif I (or Walker A motif) and Q-motif are necessary for ATP binding and hydrolysis (Tanner et al. 2003) whereas, motifs Ia, Ib, II, IV and V may be involved in RNA binding (Svitkin et al. 2001).

Genome sequencing of variety of organisms have revealed the presence of different numbers of DExH/D helicases. In a genome-wide comparative study 161, 149, 136 and 213 different RNA helicase genes have been identified in Arabidopsis thaliana, Oryza sativa, Zea mays and Glycine max respectively (Xu et al. 2013). Also, ...
31 DEAD and 14 DEAH putative RNA helicases have been reported from human beings (Umate et al. 2011).

Recently, Steimer and Klostermeier summarised involvement of RNA helicases in infection and diseases (Steimer and Klostermeier 2012). For example dysregulation of these helicases has been linked to a wide variety of cancers. In addition, these proteins have a role in the replication of viruses such as Foot and mouth disease virus infection in cattle and HIV virus in human beings. RNA helicases A (DHX9) has been associated with cattle FMD disease (Radi et al. 2012; Lawrence and Rieder 2009). We can reveal prognostic and diagnostic markers and identify potential drug targets by characterizing these helicases.

Cattle are economically important domesticated ungulates. Phylogenetic analysis has shown a distant clad for cattle as compared to humans and rodents (Murphy et al. 2004) and around 800 breeds have been established serving as resource for the genetics of complex traits studies. The genome sequence for domesticated cattle (Bos taurus) was assembled and published in 2009 (The Bovine Genome Sequencing and Analysis Consortium 2009). The sequence reveals presence of a minimum 22,000 genes in cattle. In the present study, sequenced cattle genome was used to evaluate the number of DEAD-box and related family proteins which might be present, along with their phylogeny. The composition of these bovine motor proteins have also been analysed. In silico analysis of bovine DExH/D helicases provided the putative role of these proteins in various RNA metabolism processes which might be operating in Bos taurus.

**Methods**

**Database search and enlistment of RNA helicases**

The sequences for DExH/D family members encoded by Bos taurus were downloaded from NCBI/BLAST (http://www.ncbi.nlm.nih.gov). Amino acid sequence of eIF4A1 (Swiss-Prot Id-Q3SZ54) was obtained first from Swiss-Prot using the key words eIF4A1 Bos taurus. The input sequence so obtained was used in the Cow RefSeq protein database available at NCBI/BLAST home. The cow genome sequences were searched using program BLASTP-Compare protein sequence against ‘BLAST Cow sequences’ resource. Finally tentative lists of DExH/D family members were compiled and all proteins (DExH/D family members) were assigned unique Swiss-Prot IDs, protein names and gene names. After identification of bovine RNA helicases their phylogenetic analysis was carried out along with helicases of other animals of veterinary importance like horse, pig and sheep. For this key words DEAD and DEAH helicase along with animal name were used to download homologs from pig, horse and sheep from Swiss-Prot database for phylogenetic analysis of these DExH/D helicases vis a vis bovine helicases. The amino acid sequences of both families of RNA helicases were aligned and the neighbour-joining method in MEGA 5.0 was applied to examine their evolutionary relationship (Tamura et al. 2011).

Specific sequences of Bos taurus were used for BLASTP search against human homologs as described above to compare their homology. Protein sequences were validated by the presence of signature motifs. Predictive molecular weight and isoelectric point for the RNA helicases were calculated from Sequence Manipulating Suite (http://www.bioinformatics.org/sms2/). Protein localization was studied using WoLF PSORT (http://www.gen-script.com/psort/wolf_psort.html) program.

**Motif identification and phylogenetic analysis**

The signature motifs for the protein family were identified. Protein sequences of DEAD and DEAH members were first aligned using ClustalW2 program available at http://www.ebi.ac.uk/Tools/msa/clustalw2/ and alignment files were downloaded. Conserved motifs in bovine DExH/D were also identified using the MEME suite (version 4.9.1) at meme.ncbi.nlm/meme/cgi-bin/meme.cgi. Finally list of signature motifs was generated. Phylogenetic analysis was performed using MEGA5 program (http://www.megasoftware.net/) by the Neighbour-Joining method (NJ) with parameters; complete deletion option, p-distance and bootstrapping method with 1000 replicates (Tamura et al. 2011). Final image was obtained using the MEGA5 program. Domain analysis was performed using the program Scan Prosite (http://expasy.org) and these domain structures were used in the figures.

**Results and discussion**

**Identification and validation of Bos taurus DExH/D family members**

Genomes of all organisms have genes encoding RNA helicases. Although various comprehensive analyses of these helicases are available in various organisms, limited studies have been conducted on the role of RNA helicases in cattle. The studies of biological function of cattle RNA helicases can unravel their roles and can help in understanding different diseases in cattle and also help in improving economically important traits. Fifty four DEXH/D family members of RNA helicases were identified in Bos taurus in the present study, amongst which 38 members belonged to DDX family (DEAD) (Table 1) and 16 members to DHX family (DEAH) of RNA helicases (Table 2). Further analysis of cattle helicase sequences with MEME suite suggested the pattern of amino acids occurrence in signature motifs validating the protein family members. Besides characteristic residues of
motifs, some residues were found to be conserved around each motif of various DExH/D family members. The 38 bovine DDX members identified were DDX1, DDX3X, DDX3Y, DDX4, DDX5, DDX6, DDX10, DDX17, DDX18, DDX19A, DDX19B, DDX20, DDX21, DDX23, DDX24, DDX25, DDX27, DDX28, DDX31, DDX39A, DDX39B, DDX41, DDX42, DDX43, DDX46, DDX47, DDX49, DDX50, DDX51, DDX52, DDX53, DDX54, DDX55, DDX56, DDX57, DDX58, DDX59, DDX60, DDX61, DDX62, DDX63, DDX64, DDX65, DDX66, DDX67, DDX68, DDX69, DDX70, DDX71, DDX72, DDX73, DDX74, DDX75, and DDX76.

### Table 1 Summary of the features of the Bovine DDX member proteins

| Bos Taurus  | Human    | Isoelectric point | Molecular weight (kDa) | Localization | % Coverage with human | % Identity with human |
|-------------|----------|-------------------|------------------------|--------------|-----------------------|-----------------------|
| DDX1        | DDX1     | 7.23              | 82.43                  | C,N          | 100                   | 97                    |
| DDX3X       | DDX3X    | 7.2               | 73.15                  | N            | 100                   | 99                    |
| DDX3Y       | DDX3Y Isoform2 | 7.39            | 73.17                  | N            | 100                   | 91                    |
| DDX4        | DDX4 Isoform1 | 5.96            | 79.46                  | N,C          | 100                   | 91                    |
| DDX5        | Dead box polypeptide S | 9.21          | 69.16                  | N            | 100                   | 100                   |
| DDX6        | DDX6     | 8.93              | 54.39                  | N            | 99                    | 99                    |
| DDX10       | DDX10    | 9.17              | 101.18                 | N            | 100                   | 89                    |
| DDX17       | DDX17 Isoform1 | 8.75            | 72.33                  | N,C          | 100                   | 99                    |
| DDX18X1     | DDX18    | 10.04             | 75.13                  | N,M          | 100                   | 90                    |
| DDX19A      | DDX19A   | 6.72              | 54.00                  | C,N          | 100                   | 97                    |
| DDX19B      | DDX19B Isoform1 | 8.54          | 54.46                  | M,N,C        | 95                    | 98                    |
| DDX20       | Dead box polypeptide 20 | 6.77          | 92.71                  | N,C          | 100                   | 88                    |
| DDX23       | DDX23    | 10.22             | 95.67                  | N            | 100                   | 99                    |
| DDX24       | DDX24    | 10.01             | 94.53                  | N            | 100                   | 81                    |
| DDX25       | DDX25    | 6.33              | 54.63                  | C,N          | 100                   | 93                    |
| DDX27       | DDX27    | 9.89              | 87.10                  | N            | 100                   | 95                    |
| DDX28       | DDX28    | 10.75             | 60.02                  | M,C,N        | 99                    | 85                    |
| DDX31       | DDX31    | 10.43             | 80.87                  | N            | 99                    | 79                    |
| DDX39A      | DDX39A   | 5.39              | 49.15                  | C,N          | 100                   | 96                    |
| DDX39B      | DDX39B   | 5.38              | 48.97                  | C,N          | 100                   | 99                    |
| DDX41       | DDX41    | 6.94              | 69.83                  | C,N,M        | 100                   | 99                    |
| DDX42       | DDX42    | 7.28              | 107.56                 | N,C          | 96                    | 95                    |
| DDX43       | Dead box polypeptide 43 | 8.77          | 72.04                  | N            | 99                    | 76                    |
| DDX46       | DDX46IsoformX1 | 9.87          | 117.46                 | N,C          | 100                   | 99                    |
| DDX47       | DDX47IsoformX1 | 9.64          | 50.92                  | N            | 100                   | 96                    |
| DDX49       | DDX49    | 9.82              | 44.39                  | C,N,M        | 99                    | 91                    |
| DDX50       | Dead box polypeptide 50 | 9.64          | 82.60                  | N,C          | 100                   | 97                    |
| DDX51       | DDX51    | 7.56              | 60.69                  | N,C          | 98                    | 82                    |
| DDX52       | DDX52    | 10.32             | 67.52                  | N,C          | 100                   | 91                    |
| DDX53       | DDX53    | 9.88              | 68.47                  | N            | 99                    | 68                    |
| DDX54       | DDX54    | 10.68             | 102.72                 | N            | 94                    | 90                    |
| DDX55       | DDX55    | 9.83              | 68.61                  | N,C          | 100                   | 94                    |
| DDX56       | DDX56Isoform1 | 9.02          | 61.27                  | N,C,M        | 100                   | 93                    |
| DDX59       | DDX59    | 8.03              | 67.45                  | N,C          | 100                   | 77                    |
| EIF4A1      | EIF4A1Isoform1 | 5.12          | 46.15                  | N            | 100                   | 100                   |
| EIF4AII     | EIF4AII  | 5.13              | 46.41                  | N            | 100                   | 100                   |
| EIF4A-III   | EIF4A-III | 6.69           | 46.85                  | N,M          | 100                   | 99                    |
| Nucleolar RNA Hel2 | Isoform1(DDX21) | 9.87          | 87.25                  | N,C          | 100                   | 89                    |

_N, M and C represent Nuclear, Mitochondrial and Cytoplasmic localization, respectively_
Table 2 Summary of the features of the Bovine DHX member proteins

| Bos Taurus | Human | Isoelectric Point | Molecular weight (kDa) | Localization | % Coverage with human | % Identity with human |
|------------|-------|-------------------|------------------------|--------------|-----------------------|----------------------|
| DHX8       | DHX8  | 8.33              | 140.28                 | N            | 99                    | 98                   |
| DHX9       | Helicase A | 6.88 | 141.97                 | N            | 90                    | 95                   |
| DHX15      | DHX15 | 7.48              | 90.95                  | N            | 100                   | 99                   |
| DHX16      | DHX16 Iso1 | 6.39 | 119.88                 | N,C          | 100                   | 98                   |
| DHX29      | DHX29 | 8.67              | 155.28                 | N            | 99                    | 93                   |
| DHX30      | DHX30 Iso1 | 8.61 | 135.97                 | M,C,N        | 100                   | 97                   |
| DHX32      | DHX32 | 4.79              | 83.88                  | C,N          | 100                   | 89                   |
| DHX33      | DHX32 Iso1 | 9.23          | 79.75                  | N            | 98                    | 92                   |
| DHX34      | DHX34 | 7.96              | 128.80                 | N            | 100                   | 88                   |
| DHX35      | DHX35 Iso1 | 8.66          | 78.89                  | N            | 99                    | 96                   |
| DHX36      | DHX36 Iso1 | 7.87          | 114.85                 | N,M          | 100                   | 92                   |
| DHX37      | DHX37 | 8.93              | 129.02                 | N,C,M        | 100                   | 85                   |
| DHX38      | PRP16 | 6.55              | 140.19                 | N            | 100                   | 95                   |
| DHX40      | DHX40 Iso1 | 8.83          | 88.52                  | N            | 100                   | 99                   |
| DHX57      | DHX57 | 7.69              | 155.76                 | N            | 96                    | 91                   |
| DHX58      | DHX58 | 8.63              | 77.19                  | C,N          | 100                   | 83                   |

N, M and C represent Nuclear, Mitochondrial and Cytoplasmic localization, respectively.

DDX56, DDX59, eIF4AII, eIF4AIII and eIF4A1II (Table 1). In all, 9 motifs (Q, I, Ia, Ib, II, IV, V and VI) were identified in these proteins which are shown in Fig. 1. The signature motifs in DDX protein showed consensus sequences as GFxxPxxIQ (Q), AxxGxGKT (I), PTRELA (Ia), TGGR (Ib), DExD (II), SAT (III), FVxT (IV), RGxD (V) and HRxGRxxR (VI). In the case of DDX49 three motifs namely; TGGR, DExD and SAT were found missing (Fig. 1). The 16 DHX members that could be identified were DHX8, DHX9, DHX15, DHX16, DHX29, DHX30, DHX32, DHX33, DHX34, DHX36, DHX37, DHX38, DHX39, DHX40, DHX57 and DHX58 (Fig. 2). Consensus sequences GxxGxGKT (I), TQPRRV (Ia), TDGML (Ib), DExH (II), SAT (III), FVxT (IV), RGxD (V) and HRxGRxxR (VI) were found in the members of DHX proteins. Some motifs in two DHX members i.e. DHX32 and DHX58 were not found (Fig. 2). In protein DHX32, SAT, TNIASET and QRxGRAGR motifs were absent, and instead of motif DExH; DDIIH motif was observed. In DHX58 conserved motif DECH was observed and remaining motifs were missing. QRxGRAGR motif was not observed in the DHX38 protein (Fig. 2). Four members i.e. DHX32, DHX58, DHX38, and DDX49 showed variable conserved motifs and need biochemical evidence for confirmation. Figure 3 describes patterns in different motifs of DDX and DHX helicases using Hidden Markov Model (HMM). In Fig 3a, b position specific probability is represented by the size of particular amino acid residue in different motifs, larger the size more will be probability of occurrence.

Phylogenetic analysis

Phylogenetic analysis of DExH/D helicases was performed to elucidate evolutionary relationship. On analysing bovine helicase with that of horse, pig and sheep (Fig. 4a, b) it was observed that some DEAD box helicase family members could be subdivided into nine subgroups in all the species taken into consideration. However, DDX 6, DDX 10, DDX 11, DDX 24, DDX 26, DDX 27, DDX28, DDX 31, DDX 41, DDX 47, DDX49, DDX 51, DDX52, DDX 54, DDX 56, DDX 58 and DDX 59 members of DEAD box of all these species could not be included in above nine subgroups (Fig. 4a). Similarly, DHX family members could also be subdivided into four subgroups for all the species (Fig. 4b). However, DHX15, DHX32 and DHX40 could not be included in the any of these four subgroups (Fig. 4b). The extent of similarity indicates toward conserved structure of DExH/D helicases in all the species studied during evolution but their functions remained to be defined by biochemical analysis. In second analysis, relationship amongst bovine helicases was carried out (Fig. 5a, b for DDX and DHX respectively). Phylogenetic analysis established close relationship between different members. The closely related members within DDX subfamily are DDX17-DDX5, DDX43-DDX53, DDX42-DDX46, DDX43-DDX3X-DDX3Y, DDX41-DDX59, DDX39A-DDX39B, DDX19A-DDX19B, eIF4A members, DDX10-DDX18, DDX56-DDX51, DDX47-DDX49, DDX27-DDX54 and DDX50-DDX21. Similarly, within DHX members DHX8-DDX16, DHX33-DDX35, DHX15-DDX32 and
### Protein ID and Conserved Motifs

| Protein ID | I | La | Ib | II | III | IV | V | VI |
|------------|---|----|----|----|-----|----|---|----|
| DXA6       |   |    |    |    |     |    |   |    |
| DXA10      |   |    |    |    |     |    |   |    |
| DXA18      |   |    |    |    |     |    |   |    |
| DXA25      |   |    |    |    |     |    |   |    |
| DXA27      |   |    |    |    |     |    |   |    |
| DXA28      |   |    |    |    |     |    |   |    |
| DXA31      |   |    |    |    |     |    |   |    |
| DXA34      |   |    |    |    |     |    |   |    |
| DXA38      |   |    |    |    |     |    |   |    |
| DXA41      |   |    |    |    |     |    |   |    |
| DXA42      |   |    |    |    |     |    |   |    |
| DXA43      |   |    |    |    |     |    |   |    |
| DXA44      |   |    |    |    |     |    |   |    |
| DXA45      |   |    |    |    |     |    |   |    |
| DXA49      |   |    |    |    |     |    |   |    |
| DXA50      |   |    |    |    |     |    |   |    |
| DXA51      |   |    |    |    |     |    |   |    |
| DXA52      |   |    |    |    |     |    |   |    |
| DXA53      |   |    |    |    |     |    |   |    |
| DXA54      |   |    |    |    |     |    |   |    |
| DXA55      |   |    |    |    |     |    |   |    |
| DXA56      |   |    |    |    |     |    |   |    |
| DXA57      |   |    |    |    |     |    |   |    |
| DXA58      |   |    |    |    |     |    |   |    |
| DXA59      |   |    |    |    |     |    |   |    |
| DXA60      |   |    |    |    |     |    |   |    |

**Fig. 1** The amino acid sequence of conserved motifs constituting the RNA helicases of bovine DDX proteins.
DHX36-DHX57 show close relationship. All these members occur as separate clades.

**In Silico Characterization of Bovine DExH/D family members**

Putative molecular weights and isoelectric points of bovine DExH helicases were determined in silico (Tables 1 and 2). Similarly predictive subcellular localizations of these proteins were examined (Tables 1 and 2). These helicases varied in their isoelectric point and molecular subunit mass. Isoelectric point of DDX members varied from 5.12 (EIF4AI) to 10.68 (DDX54) whereas pI for DHX members ranged between 4.79 (DHX32) and 9.23 (DHX33). 24 DDX and 8 DHX members had pI above 8. Molecular mass for these helicases ranged between 44.39 kDa (DDX49) and 117.46 kDa (DDX46) in case of DDX members and between 77.19 kDa (DHX58) and 155.76 kDa (DHX57) for DHX members. The predictive pI value and molecular mass will help in isolation and purification leading to further characterization of these helicases. Analysis with WoLF PSORT program indicated that cattle RNA helicases are localized in the nucleus, cytoplasm and mitochondria (Tables 1 and 2).

**Comparative analysis of human and bovine DExH/D family members and putative function assignment**

*Bos taurus* has a 2.86 billion bp long genome with a minimum of 22,000 genes (The Bovine Genome...
Sequencing and Analysis Consortium 2009). Similarly, 2.91 billion bp long human genome has around 20,000–25,000 genes (International Human Genome Sequencing C 2004). Cattle genome encodes all orthologs of human DExH/D family members. Bovine DEAD box RNA helicases has typically Q motif, ATP binding and Helicase C-terminal domains as found in human helicases. Domain structures of bovine DExH/D RNA helicases as compared with that of human helicases indicated high similarity between the two species (Figs. 6 and 7). Despite this identity DDX17, DDX18, DDX24, DDX27, DDX31, DDX42, DDX49, DDX51, DDX53 and DDX54 show difference in positions of domains as compared to human helicases (Fig. 6). In bovine DDX49 typically overlapping of ATP binding and Helicase domain was observed. Interestingly, both bovine and human DHX32 showed only ATP binding domain and no other domain was observed. Further, levels of homology amongst human and bovine DExH/D RNA helicases are shown in Tables 1 and 2. Bovine DEAD helicases showed high similarity with their human counterpart (identity 76–100 %).

The higher similarity of these bovine helicases with well characterized human helicases can help to predict their functions in cattle developmental processes also. The putative functions of these helicases have been summarized in Tables 3 and 4. The importance of DExH/D RNA helicases in environmental stress is becoming evident (Shih and Lee 2014). DDX1, 3, 5, 6, 17, 21, 24, 47, DHX9 and DHX36 are associated with various viral infections. Similarly DDX6 and DDX19 are associated with neurological disorders, as summarised previously (Steimer and Klostermeier 2012). This manuscript presents first report on genome-wide comprehensive analysis of bovine DExH/D helicases providing valuable information regarding classification and putative function of these RNA helicases, essential for growth and development. Identification of bovine counterparts of helicases associated with various stress and diseases can be exploited as prognostic and diagnostic markers.

**Conclusions**

*Bos taurus* genome encodes 54 DExH/D family members (38 DDX and 16 DHX). Present work describes their evolutionary relationship, putative functions, p1, molecular weight and localization. Despite high similarity with well characterized counterparts, for some members, functions could not be predicted which needs further analysis. Hence, this study emphasises towards some bovine DExH/D members requiring further biological characterisation. Similarly, bovine DDX49 and DHX32 need biochemical characterization as they showed unique properties. Association analysis of these members with different abiotic and biotic stress may facilitate new diagnostic markers and drug targets.
Fig. 5 Phylogenetic analysis of Bovine DExH/D helicases. a and b represent analysis of bovine DEAD and DEAH respectively.
Fig. 6  Schematic diagrams of domain organisation in bovine DEAD helicases. Domain analysis was conducted using Scan Prosite (http://expasy.org). The domain structures were downloaded and used for figure generations. The number shown in black and red colour indicates the amino acids spanning motifs in bovine and Human DEAD box proteins.
Fig. 7  Schematic diagrams of domain organisation in bovine DEAH helicases. Domain analysis was conducted using Scan Prosite (http://expasy.org). The domain structures were downloaded and used for figure generations. The number shown in black and red colour indicates the amino acids spanning motifs in bovine and Human DEAH box proteins.
| Protein  | Function                                                                 | Ref.                                      |
|----------|---------------------------------------------------------------------------|-------------------------------------------|
| DDX1     | Associated with ARE mediated mRNA decay                                    | Chou et al. (2013)                        |
| DDX3X, DDX3Y | DDX3X can bind with DNA, RNA splicing, nuclear transport of RNA and translational regulation | Franca et al. (2007); Rosner and Rinkevich (2007) |
| DDX4     | Bovine vasa homolog (BVH) and is expressed in gonads                      | Bartholomew and Parks (2007)              |
| DDX5, DDX17 | Splicing and transcriptional regulation                                   | Aboeuf et al. (2002)                     |
| DDX6     | Spermatogenesis and localized in spermatogenic cells                       | Kawahara et al. (2014)                    |
| DDX10    | Ribosome assembly                                                          | Savitsky et al. (1996)                    |
| DDX18    | Hematopoiesis and deletion resulted into p-53 depended cell arrest in G1   | Payne et al. (2011)                       |
| DDX19    | m-RNA nuclear transport by remodelling of RNP particles through nuclear pore complex | Collins et al. (2009)                    |
| DDX20    | Transcriptional regulation, splicing process and mi-RNA pathway            | Takata et al. (2012)                      |
| DDX23    | Pre-mRNA splicing                                                          | Ismaili et al. (2001)                     |
| DDX24    | Innate immune signalling regulation                                        | Ma et al. (2013)                         |
| DDX25    | Posttranscriptional regulations of genes for spermatid elongation & completion of spermatogenesis | Dufau and Tsai-Morris (2007)             |
| DDX27    | ND                                                                        |                                           |
| DDX28    | Cellular division                                                          | Loo et al. (2012)                        |
| DDX31    | Transcription of rRNA gene and assembly of 60 s ribosomal subunit          | Bish and Vogel (2014)                     |
| DDX39    | mRNA splicing, genome integrity and telomere protection                    | Yoo and Chung (2011)                     |
| DDX41    | Type 1 interferon response                                                 | Zhang et al. (2011a)                     |
| DDX42    | Function as chaperon                                                       | Uhlmann-Schiffler et al. (2006)          |
| DDX43    | ND                                                                        |                                           |
| DDX46    | Pre-mRNA splicing                                                          | Hozumi et al. (2012)                     |
| DDX47    | Pre-RNA processing                                                         | Sekiguchi et al. (2006)                  |
| DDX49    | ND                                                                        |                                           |
| DDX51    | Ribosome synthesis and formation of 3′end of 28S rRNA                     | Srivastava et al. (2010)                  |
| DDX52    | ND                                                                        |                                           |
| DDX53    | ND                                                                        |                                           |
| DDX54    | Maintenance of central nervous system                                      | Zhan et al. (2013)                       |
| DDX55    | ND                                                                        |                                           |
| DDX56    | Assembly of pre-ribosomal particles                                        | Zirves et al. (2000)                     |
| DDX59    | Pathogenesis of orofaciiodigital syndrome                                  | Shamseldin et al. (2013)                 |
| eIF4A    | eIF4F complex formation and facilitates translation                       | Harms et al. (2014)                      |
| Nucleolar RNA Hel2 (DDX21) | RNA processing during interphase of mitosis                                    | De Weyer et al. (2012)                   |
Table 4 Putative functions of DHX members

| Protein | Function | Ref. |
|---------|----------|------|
| DHX8    | Mitosis and involved in mRNA splicing | English et al. (2012) |
| DHX9    | RNA induced silencing complex (RISC) loading factor | Fu and Yuan (2013) |
| DHX15   | RNA virus sensing and activating immune system | Lu et al. (2014) |
| DHX16   | Splicing | Gencheva et al. (2010) |
| DHX29   | Protein synthesis | Pisareva et al. (2008) |
| DHX30   | Mitochondrial DNA replication | Zhou et al. (2008) |
| DHX32   | Lymphocyte differentiation and T cell apoptosis | Huang et al. (2009) |
| DHX33   | rRNA transcript and nucleolar organizer | Zhang et al. (2011b) |
| DHX34   | NMD (nonsense-mediated mRNA decay) | Anastasaki et al. (2011) |
| DHX35   | ND | |
| DHX36   | Viral nucleic acid sensors, affinity towards G4-quadruplex | Fullam and Schroder (2013) |
| DHX37   | Glycinergic synaptic transmission and associated motor behaviour | Hirata et al. (2013) |
| DHX38   | Associated with retinitis pigmentosa | Ajmal et al. (2014) |
| DHX40   | Pre mRNA splicing and ribosome biogenesis | Xu et al. (2002) |
| DHX57   | ND | |
| DHX58   | Innate antiviral immune response | Li et al. (2009) |

Authors' contributions
MKS designed, performed experiments, analysed data and prepared manuscript; MP performed experiments; SM analysed and reviewed manuscript data; SKK supervised all experiments. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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