Down syndrome related muscle hypotonia: association with \textit{COL6A3} functional SNP rs2270669

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Down syndrome (DS), the principal cause for intellectual disability, is also associated with hormonal, immunological, and gastrointestinal abnormalities. Muscle hypotonia (MH) and congenital heart diseases (CHD) are also frequently observed. Collagen molecules are essential components for maintaining muscle integrity and are formed by the assembly of three chains, alpha 1-3. The type VI collagen is crucial for cardiac as well as skeletal muscles. The COL\textalpha\textsuperscript{1} (VI) and COL\textalpha\textsuperscript{2} (VI) chains are encoded by genes located at the 21st chromosome and are expected to have higher dosage in individuals with DS. The \textalpha\textsuperscript{3} (VI) chain is encoded by the \textit{COL6A3} located at the chromosome 2. We hypothesized that apart from \textit{COL6A1} and \textit{COL6A2}, \textit{COL6A3} may also have some role in the MH of subjects with DS. To find out the relevance of \textit{COL6A3} in DS associated MH and CHD, we genotyped two SNPs in \textit{COL6A3}, rs2270669 and rs2270668, in individuals with DS. Subjects with DS were recruited based on the Diagnostic and Statistical Manual for Mental Disorders-IV and having trisomy of the 21st chromosome. Parents of individuals with DS and ethnically matched controls were enrolled for comparison. Informed written consent was obtained for participation. Peripheral blood was used for isolation of genomic DNA. Target genetic loci were studied by DNA sequence analysis. Data obtained was subjected to population – as well as family-based statistical analysis. rs2270668 was found to be non-polymorphic in the studied population. rs2270669 showed significant association of the “C” allele and “CC” genotype with DS probands having MH ($P = 0.02$). Computational analysis showed that rs2270669 may induce structural and functional alterations in the COL\textalpha\textsuperscript{3} (VI). Interaction of COL\textalpha\textsuperscript{3} (VI) with different proteins, crucial for muscle integrity, was also noticed by computational methods. This pioneering study on \textit{COL6A3} with DS related MH thus indicates that rs2270669 “C” could be considered as a risk factor for DS related MH.

**Keywords:** Down syndrome, muscle hypotonia, congenital heart disease, functional SNP, \textit{COL6A3}

**INTRODUCTION**

Down syndrome (DS), the most common genetic cause for intellectual disability is characterized by the presence of one extra copy of the human chromosome 21 (Hsa21) (Roizen and Patterson, 2003; Rachidi and Lopes, 2007). The commonly accepted hypothesis to correlate the extra Hsa21 with DS pathobiology is overexpression of genes present in the Hsa21 (Chou et al., 2008). However, changes in the expression of other euploid genes, possibly due to the presence of an extra Hsa21, were also speculated to contribute to the phenotypic variations in DS (Chou et al., 2008).

Down syndrome, with a great variability in the penetrance level, is characterized by complex phenotypic features, including morphological abnormalities of head and limbs, short stature, hypotonia, and hyperlaxity of the ligament etc. Malfunction of organs, particularly the heart (50% of newborns with DS), gastrointestinal tract obstructions or dysfunctions (4–5% of newborns with DS), increased risk of leukemia, early onset of Alzheimer like neuropathology are also common in individuals with DS (Antonarakis and Epstein, 2006; Rachidi and Lopes, 2007). Almost all children with DS suffer from muscle hypotonia (MH), a state of reduced muscle tone, usually related to the skeletal muscles. Due to MH, delay in developmental milestones, mastication problems (due to poor neuromuscular control), muscular weakness and dental anomalies are also of common occurrence in DS (Faulks et al., 2008). Besides skeletal muscle abnormality, congenital heart disease (CHD) due to cardiac muscle malformation is also a frequent problem (Klewer et al., 1998; Gittenberger-de Groot et al., 2003). Nearly 70% of atrio-ventricular (AV) canal defects are diagnosed in infants with DS (Korenberg et al., 1992).

**Abbreviations:** AV, atrio-ventricular; BM, Bethlem myopathy; CEU, Caucasians from Utah with ancestry from western and northern Europe; CHD, congenital heart disease; CI, 95% confidence interval; COLVI, collagen type VI; DNA, deoxyribonucleic acid; DS, Down syndrome; HCB, Han Chinese from Beijing, China; Hsa21, Human chromosome 21; IND, Indian control population; IJT, Japanese from Tokyo, Japan; MH, muscle hypotonia; N, number of sample; OR, odds ratio; PCR, polymerase chain reaction; RE, restriction enzyme; SNP, single nucleotide polymorphism; UCMD, Ullrich congenital muscular dystrophy; YRI, Yoruba from Ibadan, Nigeria.
Collagen, the trimeric extracellular matrix protein, is an important component for the formation of skeletal as well as cardiac muscles (Gordon and Hahn, 2010). It is the most abundant protein in mammals and is found in large quantities in the tendon, ligament, fascia, and skin (Bader et al., 2009). Collagen forms 1–2% of muscle tissue and 6% of strong muscles with tendons (Sikorski and Zdzislaw, 2001). As a component of the extracellular matrix, it is also present in bone, cartilage, inter-vertebral disk, cornea, lens, blood vessels, and intestine (Gordon and Hahn, 2010). Collagen is comprised of three helices, α1, α2, α3, which differ from each other in the amino acid compositions (Eastoe, 1967). Thirty different types of collagens based on the supra-molecular assembly and functional properties were reported (Kumar et al., 2007).

Among different types of collagen, the type VI (COLVI) has a crucial role in the function and stability of skeletal (Sabatelli et al., 2001) and cardiac (Klewer et al., 1998) muscles. Assembly of COLVI is a complex multistep process; equimolar quantities of three genetically distinct subunits, α1(VI), α2(VI), and α3(VI), associate to form a triple helical monomer which is then bonded in an anti-parallel direction to form dimers. These dimers align to form tetramers with the help of disulfide linkage (Furthmayr et al., 1983). In humans, genes encoding for the α1 and α2 chain of type VI collagen (COL6A1 and COL6A2 respectively) are located on the long arm of Hsa21, 21q22.3, a region determined to be critical for CHD associated with trisomy 21 (Francomano et al., 1991). Investigators have shown that mutations in three COL6 genes result in CHD associated with trisomy 21, COL6A1 (De Fazio et al., 2003), COL6A2 (Dey et al., 2001), and COL6A3 (Baker et al., 2007). However, till date, no investigation on COL6A3 with hypotonia was first presented in 1990s (Oley et al., 1993; Wilson et al., 1993; Oley et al., 1996). Investigators have also reported deletion of 2q37 in cases exhibiting CHD/MH (Rauch et al., 1996).

A 410 bp sequence of the COL6A3 gene, including the two selected SNPs, was amplified by polymerase chain reaction using forward primer 5′ ATTCCCTTCTGCATG 3′ and reverse primer 5′ TGCTCCTTGTGTTCTAATGGTGA 3′. Amplicons generated were analyzed for restriction fragment length polymorphism of rs2270668 and rs2270669 with HindII and HaeIII restriction enzymes respectively (Table 1).

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Table 1 | Details of restriction fragment length polymorphism analysis.

| SNP IDs | Alleles  | RE used | Cut site of RE \(^a\) | Genotype | Length of fragments (bp) | Reaction condition |
|---------|----------|---------|-----------------------|----------|-------------------------|--------------------|
| rs2270668 | A/G | HinfI | 5’ CTNA G 3’ | AA | 410 | 5μl Amplicon incubated overnight at 37°C with 1 U Genei buffer C and 1 U HinfI in 20 μl reaction mixture, resolved in 2.5% agarose gel |
| rs2270669 | C/G | HaeIII | 5’ CCC 3’ | GG | 250/160/143/107 | 5μl Amplicon incubated overnight at 37°C with 1 U Genei buffer C and 1 U HaeIII in 20 μl reaction mixture, resolved in 15% polyacrylamide gel |

\(^a\) Bold and underlined bases are the SNP position and the black arrows are the cutting position of the restriction enzyme (RE).

Table 2 | Comparative analysis of allelic and genotypic frequencies in different populations.

| Populations | Allelic frequency | Genotypic frequency | \(\chi^2\), \(p\) value |
|-------------|-------------------|---------------------|---------------------|
|              | C     | G     | CC   | GC   | GG   | CC   | GC   | GG   | \(\chi^2\), \(p\) value |
| CEU         | 0.793 | 0.207 | 0.603 | 0.379 | 0.017 | 11.1, 0.004 |
| HCB         | 0.756 | 0.244 | 2.91, 0.088 | 0.600 | 0.311 | 0.089 | 6.53, 0.038 |
| JPT         | 0.784 | 0.216 | 4.15, 0.042 | 0.591 | 0.386 | 0.023 | 10.6, 0.005 |
| YRI         | 1.000 | 0.000 | 42.4, 0.000 | 1.000 | 0.000 | 0.000 | 81.7, 0.000 |
| IND         | 0.649 | 0.351 | –    | 0.419 | 0.459 | 0.122 | –    |

CEU, Caucasians from Utah with ancestry from western and northern Europe; HCB, Han Chinese from Beijing, China; JPT, Japanese from Tokyo, Japan; YRI, Yoruba from Ibadan, Nigeria; IND, Eastern Indian control population.

Significant differences are mentioned in bold.

**RESULTS**

rs2270668 was found to be monomorphic for the “A” allele in the studied eastern Indian population (\(N = 189\)) and no further analysis was carried out for this SNP.

rs2270669 was polymorphic and data obtained for this site was subjected to population – as well as family-based analysis. Genotype frequency was in Hardy–Weinberg equilibrium for all the groups. Comparison of allelic and genotypic frequencies revealed significant difference for the studied IND population as compared to the CEU and JPT (Table 2), principally due to an increase in the “G” allele with a concomitant decrease in the “C” allele in the IND population. Comparison with HCB also revealed a trend toward increase in the “G” allele frequency in the IND population along with a significant difference in genotypic frequencies (Table 2). As appeared from the HapMap data, in the YRI population this site was monomorphic for the “C” allele.

Case-control association analysis revealed an increase in the “C” allele frequency in probands with DS as compared to eastern Indian control subjects; however, the difference was statistically insignificant (Table 3). Analysis of subjects sorted on the basis of gender failed to reveal any significant difference for male probands while female probands showed a trend toward increase in the “C” allele and “CC” genotype (Table 3). Comparative analysis of probands with DS sub-grouped on the basis of phenotypic characteristics revealed significant increase in the occurrence of the “CC” allele (\(\chi^2 = 4.86, p = 0.0277\)) as well as “CC” genotype (\(\chi^2 = 10.7, p = 0.005\)) in probands with DS having MH (\(N = 29\)) as compared to the controls. However, there was no significant difference in allelic or genotypic frequencies in probands with DS having CHD (\(N = 22\)). Probands with DS having MH and/or CHD (\(N = 47\)) also showed significantly higher occurrence of the “CC” genotype with concomitantly low GC/GG genotypes in comparison to controls (\(\chi^2 = 7.32, p = 0.026\)).

Family-based analysis by TDTphase failed to show any bias in transmission of any allele from the parents (from

\(^3\)http://fastsnp.ibms.sinica.edu.tw
\(^4\)http://compbio.cs.queensu.ca/F-SNP/
\(^5\)http://string-db.org/
Table 3 | Allelic and genotypic frequencies observed in different groups.

| Types                     | Study group       | Allelic frequency | χ², p value | OR (CI)          | Genotypic frequency | χ², p value |
|---------------------------|-------------------|-------------------|-------------|------------------|---------------------|-------------|
|                           |                   | C     | G     |                   | CC    | GC    | GG    |                   | CC    | GC    | GG    |                   |
| All subjects              | Control (N = 205) | 0.649 | 0.351 | −                  | 0.419 | 0.459 | 0.122 | −                  | 3.16  | 0.206 |
| Father                   | (N = 106)         | 0.708 | 0.292 | 0.827, 0.363      | 1.318 | 0.73–2.39 | 0.462 | 0.491 | 0.047 | 1.46, 0.481 |
| Mother                   | (N = 151)         | 0.688 | 0.312 | 0.362, 0.547      | 1.199 | 0.66–2.16 | 0.450 | 0.477 | 0.074 | 1.46, 0.481 |
| Proband                  | (N = 174)         | 0.724 | 0.276 | 1.14, 0.287       | 1.38  | 0.76–2.52 | 0.512 | 0.425 | 0.063 | 2.97, 0.226 |
| Subjects categorized     |                   |       |       |                   |       |       |       |                   |       |       |       |                   |
| based on sex             |                   |       |       |                   |       |       |       |                   |       |       |       |                   |
| Male                     | Control (N = 70)  | 0.693 | 0.307 | −                  | 0.5   | 0.386 | 0.114 | −                  | 3.6  | 0.507 |
| Male                     | Proband (N = 89)  | 0.708 | 0.292 | 0.952E–01, 0.758  | 1.1   | 0.60–2.01 | 0.483 | 0.449 | 0.067 | 1.36, 0.507 |
| Female                   | Control (N = 121) | 0.649 | 0.351 | −                  | 0.397 | 0.504 | 0.099 | −                  | 5.69  | 0.058 |
| Female                   | Proband (N = 61)  | 0.754 | 0.246 | 2.38, 0.123       | 1.62  | 0.88–2.98 | 0.557 | 0.393 | 0.050 | 5.69, 0.058 |
| DS with MH and/or CHD    | Proband with CHD  | 0.727 | 0.273 | 1.50, 0.221       | 1.46  | 0.80–2.66 | 0.500 | 0.455 | 0.045 | 4.70, 0.096 |
| as compared to control   | (N = 22)          |       |       |                   |       |       |       |                   |       |       |       |                   |
| Proband                  | with MH (N = 29)  | 0.793 | 0.207 | 4.86, 0.027       | 2.03  | 1.08–3.81 | 0.621 | 0.345 | 0.034 | 10.7, 0.005 |
| Proband                  | with MH and/or    | 0.766 | 0.234 | 3.50, 0.061       | 1.80  | 0.97–3.35 | 0.574 | 0.383 | 0.043 | 7.32, 0.026 |
| MH (N = 47)              |                   |       |       |                   |       |       |       |                   |       |       |       |                   |
| Control                  | (N = 205)         | 0.649 | 0.351 | −                  | 0.419 | 0.459 | 0.122 | −                  | 3.16  | 0.206 |

OR, odds ratio; CI, 95% confidence interval; MH, muscle hypotonicity; CHD, congenital heart disease; DS, Down syndrome.

Significant differences are mentioned in bold.

father/mother/both the parents) to the DS probands, irrespective of the gender of the proband (Table 4). Further analysis of DS probands having MH and/or CHD also failed to exhibit any bias in transmission of any allele from the parents (Table 4).

Functional assessment of rs2270669 by SNPeffect revealed that it may be responsible for changing solvent accessibility of the protein. A potential change in splicing regulation by SC35 (in presence of C) and SF2 (in presence of G) was also observed. In silico analysis using String revealed that a number of proteins, important for muscle development as evidenced from Panther pathway tool7, can interact with COLα3 (VI) (Table 5).

Analysis by BioGPS revealed significant expression correlation of COL6A3 with COL6A1, COL6A2, COL12A1, N1D1, MMP2, and MFAP5 (Table 6).

DISCUSSION
The α3 (VI) chain is a crucial component for skeletal as well as cardiac muscles. Terminal deletion in chromosome 2q harboring the COL6A3 has been reported to lead to various disease phenotypes (Oley et al., 1993; Wilson et al., 1995; Rauch et al., 1996). DS related AV canal defect was reported to be associated with abnormalities in the COLVI and thus the molecule was speculated as an important candidate to study cardiac muscle development also (Davies et al., 1995; Baptista et al., 2000). Muscle related disorders like BM were reported to be associated with mutation in exon numbers 4, 5, 6, 7, 40, etc. of the COL6A3 (Pepe et al., 1999; Lampe et al., 2005). Mutation in this gene was also reported to be associated with UCMD (Demir et al., 2002). A "A > G" transition in the splice-donor site of intron 29 was reported to cause deletion of the exon 29 (Demir et al., 2002). A nonsense mutation in the exon 5 (R465X), resulting in a shorter N-terminal domain of COLα3 (VI), was also reported. Another nonsense mutation (R2342X), leading to absence of collagen VI in muscle and fibroblasts, was reported to induce a severe phenotype of UCMD (Demir et al., 2002). However, investigation on rs2270669 in five UCMD patients revealed presence of the derived allele in heterozygous condition in only one individual; control individuals also showed presence of the SNP and it was inferred that this site may not have significant contribution in the disease etiology (Baker et al., 2005; Baker and Rowland, 2007). The current investigation is the first genetic association study on COL6A3 rs2270669 and rs2270669 in subjects with DS.

7http://www.pantherdb.org/pathway/
Table 4 | Frequency of rs2270669 allele transmission in families with DS probands.

| Sex                  | Parent     | Allele | DS probands | DS probands with CHD and/or MH |
|----------------------|------------|--------|-------------|--------------------------------|
|                      |            |        | Transmitted | Not-transmitted | $\chi^2$, $p$ value | Transmitted | Not-transmitted | $\chi^2$, $p$ value |
| All DS probands      | Both       | C      | 0.479       | 0.521           | 0.167, 0.683 | 0.523       | 0.476           | 0.048, 0.827 |
|                      |            | G      | 0.521       | 0.479           |            | 0.476       | 0.523           |                  |
|                      | Father     | C      | 0.515       | 0.485           | 0.030, 0.862 | 0.500       | 0.500           | 0.000, 1.000 |
|                      |            | G      | 0.485       | 0.515           |            | 0.500       | 0.500           |                  |
|                      | Mother     | C      | 0.424       | 0.576           | 0.761, 0.383 | 0.556       | 0.444           | 0.111, 0.739 |
|                      |            | G      | 0.576       | 0.424           |            | 0.444       | 0.556           |                  |
| Only male probands   | Both       | C      | 0.500       | 0.500           | 0.000, 1.000 | 0.429       | 0.571           | 0.143, 0.705 |
|                      |            | G      | 0.500       | 0.500           |            | 0.571       | 0.429           |                  |
|                      | Father     | C      | 0.625       | 0.375           | 1.011, 0.315 | 0.500       | 0.500           | 0.000, 1.000 |
|                      |            | G      | 0.375       | 0.625           |            | 0.500       | 0.500           |                  |
|                      | Mother     | C      | 0.400       | 0.600           | 0.805, 0.369 | 0.333       | 0.667           | 0.340, 0.560 |
|                      |            | G      | 0.600       | 0.400           |            | 0.667       | 0.333           |                  |
| Only female probands | Both       | C      | 0.500       | 0.500           | 0.000, 1.000 | 0.571       | 0.429           | 0.287, 0.592 |
|                      |            | G      | 0.500       | 0.500           |            | 0.429       | 0.571           |                  |
|                      | Father     | C      | 0.429       | 0.571           | 0.287, 0.592 | 0.500       | 0.500           | 0.000, 1.000 |
|                      |            | G      | 0.571       | 0.429           |            | 0.500       | 0.500           |                  |
|                      | Mother     | C      | 0.600       | 0.400           | 0.403, 0.526 | 0.667       | 0.333           | 0.680, 0.410 |
|                      |            | G      | 0.400       | 0.600           |            | 0.333       | 0.667           |                  |

MH, muscle hypotonia; CHD, congenital heart disease.

Table 5 | Biological function of proteins having interaction with $\alpha$3 (VI).

| Proteins interacting with COL $\alpha$3 (VI) | Biological processes involved |
|--------------------------------------------|------------------------------|
| COL6A1                                     | Macrophage activation, blood circulation, intracellular protein transport, receptor-mediated endocytosis, signal transduction, cell–cell adhesion, cellular component morphogenesis, ectoderm development, mesoderm development, skeletal system development, regulation of liquid surface tension, defense response to bacterium, asymmetric protein localization |
| LAMA2                                      | Immune system process, muscle contraction, neurological system process, intracellular protein transport, endocytosis, signal transduction, cell–cell signaling, cell–matrix adhesion, protein modification process, cell motion, ectoderm development, mesoderm development, nervous system development, muscle organ development |
| ITGA7                                      | Cell adhesion               |
| COL1A2                                     | Macrophage activation, blood circulation, intracellular protein transport, receptor-mediated endocytosis, signal transduction, cell–cell adhesion, cellular component morphogenesis, skeletal system development, angiogenesis, regulation of liquid surface tension, defense response of bacterium, asymmetric protein localization |
| GP6                                        | Natural killer cell activation, cell surface receptor linked signal transduction, cell–cell signaling, blood coagulation |
| SDC1                                        | Macrophage activation, signal transduction, cell adhesion, mesoderm development, skeletal system development |
| ITGA1                                      | Cell adhesion               |
| CD44                                       | Immune system process, cell communication, cell adhesion |
| ITGA2B                                     | Cell adhesion               |

rs2270668 (A/G) is responsible for a non-synonymous amino acid change [Lys (A) to Arg (G)] at amino acid position 3006 (codon 2) in the $\alpha$3 (VI) chain. In eastern Indian subjects ($N=189$), rs2270668 was non-polymorphic for the “A” allele. In other populations studied in the 1000 genome project ($N=208-218$), MAF of this SNP was found to be only 0.0041. It may be inferred from the present study that this SNP does not have any significant role in the disease etiology.

rs2270669 (C/G) is located at amino acid position 3012 (codon 1) and is responsible for a missense amino acid change from proline (C) to alanine (G) in $\alpha$3 (VI). In silico functional assessment of rs2270669 by SNPeffect revealed that it may be responsible for changing solvent accessibility of the protein. Substitution of residues in solvent accessible surface of a protein was reported to cause change in surface area affecting the backbone torsion.
Table 6 | Genes showing expressional correlation with COL6A3.

| Gene   | Correlation coefficient |
|--------|-------------------------|
| COL6A1 | 0.9071                  |
| COL6A2 | 0.8835                  |
| NID1   | 0.8377                  |
| MMP2   | 0.8291                  |
| MFAP5  | 0.8139                  |
| COL12A1| 0.8107                  |
| MFAP5  | 0.8090                  |

Minor allele frequency of rs2270669 in the IND population showed severe dissimilarity with CEU and JPT. A significant difference in genotype frequencies of the control population was also noticed with the HCB. On the other hand, eastern Indian subjects with DS showed an allelic distribution pattern similar to the HCB and JPT. Population of the Indian subcontinent is highly heterogeneous (Indian Genome Variation Consortium, 2005). Subjects recruited for the present study are Indo-Caucasoid population from of the eastern part of India and we have noticed stark dissimilarities in genotypes of Indian population belonging to different geographical regions (Bhaduri et al., 2007). The Indo-Caucasoid subjects principally descend from the Euro-Caucasoid population, while migrants from mongoloid and Afro-Negroid population are prevalent still in the north-eastern and southern regions of India respectively (Ghosh and Seshadri, 2005). Allele frequency of a given SNP may get diluted by population admixture or by natural selection pressure, thus changing significantly from the ancestral populations. Whether this is the case for rs2270669 or is a genuine characteristic feature is a matter of conjecture at the moment and needs further validation in extended number of samples.

Though the present investigation showed a mild increase in the “C” allele and “CC” genotype as well as a concomitant decrease in the “GG” genotype in DS probands as compared to the controls, the difference was statistically insignificant. No preferential allelic distribution was found when the probands were categorized on the basis of gender; only a slightly higher trend was noticed for the “CC” genotype in the female probands. TDT analysis also failed to show any bias in transmission of any allele to DS probands from their parents. We may conclude from the present data that probands with DS have a higher trend of occurrence of the rs2270669 “C” allele. Whether this increase in the “C” allele is conferring any risk needs further corroboration with large cohort of samples.

Muscle hypotonia and CHD are common phenotypes in DS (Morris et al., 1982; Spicer, 1984; Gittenberger-de Groot et al., 2003). Our analysis in a limited number of subjects revealed a highly significant increase in the “C” ($\chi^2 = 4.86, p = 0.027, Power = 82.5\%$) allele [with a high odds ratio (OR = 2.03, CI = 1.08–3.81)] and “CC” genotype ($\chi^2 = 10.7, p = 0.005, Power = 97.74\%$) in probands with DS having MH as compared to the controls. Among the DS probands with CHD, an increase in “C” allele and “CC” genotype was also noticed. Comparison of the probands having MH and/or CHD with control also revealed a genotypic association [with increased “CC” and decreased “CG” and “GG” genotype; $\chi^2 = 7.32, p = 0.026$]. Whether the “CC” genotype is conferring any advantage to probands with DS having MH/CHD warrants further investigation in the field.

Expression analysis showed that COL6A3 has higher expressional correlation with COL6A1, COL6A2, COL12A1, NID1, MMP2, and MFAP5. In silico interaction analysis also showed significant interaction of COL6A3 (VI) with COL6A1, LAMA2, ITGA7, SDC1, etc. COL6A1 is involved in mesoderm development, skeletal muscle development, cell–cell adhesion etc. and may play an important role in cardiac and skeletal muscle development. COL1A2 and SDC1 also have roles in mesoderm and skeletal muscle development while LAMA2 regulates muscle contraction and muscle organ development. COL6A1, COL6A2, LAMA2, and SDC1 are also essential for cardiac functioning as they are involved in events like mesoderm development and angiogenesis. Thus COL6A3 (VI) along with these proteins may have important attribute in CHD and MH. Few other genes, viz. DMD, HLA-A, HLA-B, HLA-DRB1, SPP1, PABPN1, COL6A1, DUX4, CAPN3, FCMD, ATXN3, retrieved from the Genetic disease association database, may also have role in the development of muscle disorders.

In silico analysis in the present investigation revealed that allelic substitution of rs2270669 may alter globular domain formation, solvent accessibility, and splicing of the protein. We have also noticed an increase in the “C” allele in the DS probands, especially in those with MH/CHD. This may lead to an alteration in function of the COL6A3 (VI) thus modifying the coordinate functional tetrameric structure of COLVI and play a vital role in DS related MH/muscle dysfunction. Major drawback of the current study is the limited number of DS probands with MH/or CHD. Additionally, hypothesis based on computational methods need to be validated by experimental evidences. To understand the actual role played by COL6A3, or in other words rs2270669, in the etiology of DS related MH further investigation is warranted in an extended cohort of subjects with DS.

[^9]: http://geneticassociationdb.nih.gov/
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