Abstract

Objective: To study the orthologs of the five genes of congenital hypothyroidism NIS, PAX8, DUOX2, FOXE1, NKKX2-1 that are involved in the development of the thyroid gland.

Methods: The study was conducted at INMOL Cancer Hospital, Lahore in September 2017 and comprised of finding gene orthologs, phylogenetic tree and domains of NIS, PAX8, DUOX2, FOXE1, NKKX2-1 which were studied using different bioinformatics tools, including FASTA, BLAST, ENSEMBL, UniProt, MultiAlin, to find out the important domains involved in the mutations of these genes.

Result: Genes showed consensus sequence / motifs involved in congenital hypothyroidism. Phylogenetic results showed that these genes shared some common motifs. Phylogenetic trees revealed sub-clusters with high protein homology.

Conclusion: Genes involved in congenital hypothyroidism were found to have a consensus sequence motifs.

Keywords: Thyroid gland, Bioinformatics tools, PAX8, SLC5A5, DUOX2/THOX2, NKKX2-1, FOXE1, Congenital hypothyroidism (JPMA 70: 427; 2020).

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Introduction

An important part of the body is the thyroid gland which is butterfly-shaped, located below the larynx in front of the neck. The thyroid gland consists of two lobes that are connected by a strip of tissue which is called isthmus. The weight of the thyroid gland is 25g in adult. The thyroid hormones result in bone maturation, growth and brain development. The deficiency of thyroid hormone can cause growth disorder, mental retardation and major thyroid gland disease like congenital hypothyroidism, or low production of thyroid hormone-4 (T4).1 Several cases of congenital hypothyroidism were seen at a cancer hospital, and low level of thyroid hormones at birth in new-borns, if not treated within 24 hours, can cause neurodevelopmental impairment throughout the whole life, and infertility.2 It is more common in females than males. Previously it was reported that the symptoms of congenital hypothyroidism are constipation, fatigue, umbilical hernia, sleeping, feeding difficulties, poor appetite, poor muscle tone, low body temperature, infrequent bowel movement, intelligent quotient (IQ) <80 in most children, skin moulting, enlarged tongue, and low bodyweight.3 It was also reported that in most cases, congenital hypothyroidism may be transient or permanent.2 Permanent is the type of congenital hypothyroidism in which if the infant is not diagnosed early and the treatment is not started early, then it results in mental retardation. Transient is a type of congenital hypothyroidism in which early diagnosis and early treatment turns T4 back to its normal level, and the child can be protected against mental retardation. Around the world congenital hypothyroidism is caused due to iodine deficiency, absence or poor development of thyroid gland, cousin marriages, and sometime the thyroid glands do not move to their proper place during the gestation period, and there is transfer of maternal antibodies through the placenta.4 Also, data conclude that in Pakistan, 80% of the deliveries take place in midwife clinics and villages, and most of the births were conducted by unskilled birth attendants which means no check and balance system in there to keep on the thyroid level of new-borns.2 No such lab work is practised in poor areas of Lahore city, no appropriate apparatus, lack of data storage, lack of screening programmes and lack of education are all involved in the spread of this disease.5 When this disease is confirmed in a new-born, then the thyroid replacement therapy is initiated. The infant is given levothyroxine either mixed in milk or

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water, or by syringe. The level of the dose can be checked according to the symptoms of the infant and can be checked up after few months interval. The goal is to restore the level of T4 and prevent the child from being mentally retarded. It was also reported that 2% cause of congenital hypothyroidism is due to genetic mutation and can be detected in the genes transcription factor that regulates the development of thyroid gland. Genes associated with thyroid gland dysgenesis include those causing non-syndromic congenital hypothyroidism (thyroid stimulating hormone [TSH] receptor) and those causing syndromic congenital hypothyroidism (TITF-1(NK2 homeobox 1), PAX-8(Paired Domain Box Gene 8)). Genes associated with dysshormonogenesis include those for thyroid peroxidase (TPO), thyroglobulin (TG), the sodium iodide symporter (NIS), pendrin (PDS), and, most recently, thyroid oxidase 2 (THOX2). When the normal thyroid gland does not develop properly then its means that mutation occurs in the genes like NIS, PAX8, DUOX2, FOXE1, NKX2-1 that are involved in the development of the thyroid gland.

PAX8 has chromosomal location at 2q14.1. In the thyroid gland, PAX8 is essential for the formation of thyroxine-producing follicular cells, which are of endodermal origin. Carcinoma and thyroid dysgenesis have been associated with the mutation occurs on these amino acids of PAX8 protein. Mutation disrupt the protein production, interaction between the other transcription with PAX8 protein and also preventing the deoxyribonucleic acid (DNA) binding with the PAX8 protein. DUOX2 (Dual Oxidase 2) (THOX2) gene has chromosomal location at 15q21.1, and produces an enzyme dual oxidase 2 that are found in the thyroid gland, salivary gland, digestive tract and airway tract of throat and lung. Grasberger and Refetoff found that the DUOX / DUOX2 arrangement is highly conserved. The bidirectional association of DUOX and DUOX2 emerged before the divergence of echinoderms. Hydrogen peroxide generated by the enzyme Dual oxidase 2 is required for the synthesis of thyroid hormones. Thyroid hormone production is reduced due to mutation in the DUOX2 gene which limits the enzymes for the synthesis of hydrogen peroxide and leads to congenital hypothyroidism. FOXE1 (Forkhead box protein E1) chromosomal location 9q22.33, is intron-less gene encoded in the protein of the transcription factor forkhead family. This encoded protein plays an important role in the thyroid morphogenesis. Mutation in this gene results in thyroid cancer, thyroid agenesis, cleft palate and choanal atresia. Wild FOXE1 with forkhead (FH) domain mutations, directly bound FOXE1-binding motifs in the MSX1 (msh homeobox 1) and TGFβ3 (transforming growth factor beta 3) promoters and drove expression of MSX1 and TGFβ3 reporter genes. NKX2-1(NK2 homeobox 1) has cyto genetic location at 14q13.3, and the gene makes a protein called homeobox protein NKX2-1, and functions as a transcription factor. This protein is important for the function and development of the thyroid gland, lung and brain. For the production of the thyroid gland, this protein also controls those genes that are involved in the development of the thyroid gland and mutation in this gene results in brain-thyroid-lung syndrome and in the thyroid gland leads to congenital hypothyroidism. NIS (SLC5A5) has cyto genetic location at 19p13.11, and is a glycoprotein which biosynthesis the thyroid hormones by transporting 1 iodide ion and 2 sodium ion into the thyroid follicular cells. The mutation in NIS gene results in no access of iodide to move into the epithelial cell of thyroid gland and results in the increase of TSH and decreased T3, T4, which also interferes with the morphological and biochemical changes in the thyroid gland and leads to congenital hypothyroidism, cretinism etc. All of these mutations impair the normal function of the thyroid gland can be studied by the bioinformatics tools.

The current study was planned to study the orthologs of the five genes of congenital hypothyroidism NIS, PAX8, DUOX2, FOXE1, NKX2-1, and to analyse the similarity and dissimilarity between these genes.

Materials and Methods
The study was conducted at INMOL Cancer Hospital in September 2017 and comprised orthologs, phylogenetic tree and domains of NIS, PAX8, DUOX2, FOXE1, NKX2-1 which were studied using different bioinformatics tools. The genes which we study are PAX8, DUOX2, SLC5A5, NKX2-1, and FOXE-1. First the genes of the thyroid gland were collected from the National Centre for the Biotechnology Information (NCBI). Secondly, from the FASTA (Fast Alignment Search Tool) format the nucleotide sequences were collected, to search the homology then run on BLAST (Basic Local Alignment Search Tool). Orthologous sequences of these genes were studied by using the OTTH and Ensemble tool from
the NCBI. To find the function of genes OMIM (online Mendelian Inheritance in Man), Uniprot (Universal Protein resource) and HGNC (Human Gene Nomenclature Committee) tools were used. Multiple alignments were performed by Multalin (Multiple sequence alignment with hierarchical clustering) and Alibee tools, to align the multiple sequences at a time which is given in the form of table. For protein structure domains and motifs analysis Conserved domain database (CDD) was used as shown in figure-1.

Result
Genes showed consensus sequence / motifs involved in congenital hypothyroidism. PAX8 gene had 61 orthologous in which 10 species were primates (Gene ID 7849, HGNC # 8622, Accession ID L19606.1, Ensembl: ENSG00000125618). In transcription regulators, most of the family members of the alpha-helical protein domain functioned as a sequence-specific DNA binding domain. Winged turn-helix domain was also included in this superfamily. Point mutations were located in the paired (Prd) domain of PAX8 and resulted in minimising DNA-binding activity of this transcription factor. These genetic alterations implicated PAX8 in the pathogenesis of thyroid dysgenesis and in normal thyroid development. DUOX2 (Gene ID: S0506, Accession ID AF267981.1, HGNC#13273, Ensembl: ENSG00000140279) gene had 72 orthologous in which 10 species were primates and 6 paralogues. Lactoperoxidase, peroxinectin, linoleate diol synthase, thyroid peroxidase myeloperoxidase, peroxidasa were included in this diverse family of enzyme. This member was also found in bacteria and fungi and the family was not restricted to the metazoans alone. FOXE1 had 41 orthologous in which 10 spices were primates and 39 paralogues (Gene ID: 2304, Accession ID BC152744.1, HGNC #3806, Ensembl: ENSG00000178919). It was also recognised as winged helix. It is the name for the FH protein Drosophila which played a role in transcription factor and rather than promoting segmental development, it promoted terminal development. As a monomer, the transcription factor domain of this family bound with the B-DNA, and also occurred in the hepatocyte nuclear factor protein (HNF), provide regulation of tissue-specific gene. FH is called winged helix. NKX2-1 had 70 orthologous in which 10 species were primates and 11 paralogues (Gene ID: 7080, Accession ID BC006221.2, HGNC# 11825, Ensembl: ENSG00000136352). SLC5A5 NIS had 112 orthologous in which 10 were primates and 10 paralogues (Gene ID: 6528, Accession ID KR712174., HGNC#11040, Ensembl: ENSG00000105641). Solute binding domain of SLC5 proteins, also called solute sodium symporter, nucleobase-cation-symport-1 (NCS1) and SLC6(soluble carrier 6) protein were included in the NIS superfamily. SLC5 co-transported sodium ion with the vitamins, amino acids, sugars or inorganic ion in disease and physiology of human the members of this superfamily were important. Na ion / chloride-dependent plasma membrane transport for the glycine, dopamine, monoamine neurotransmitters serotonin, amino acid neurotransmitter, norepinephrine and GABA (gamma-Aminobutyric acid) were included in SLC6. Related metabolites and salvage pathways formed nuclease that were essential component of the NCSIs. Anions, including (perchlorate)CLO4-, (bromide)Br- (thiocyanate) SCN- and iodide ion were transported by the NIS (product of SLC5A5). Multiple alignment of sequences were noted (Figure-2). Alibee alignment tool was used and results were obtained in form of phylogenetic tree showing the relation of evolution among biological species about
Insilico study of genes involved in Congenital..........

their phylogeny. Gene L19606.1 (PAX8) and gene BC006221.2 (NKX2-1) in their phylogeny showed close similarity to each other, while gene BC152744.1 (FOXE1) and gene AF267981.1 (DUOX2) in their phylogeny showed close similarity (Figure-3). Consensus sequences present in all five genes showed some important regulatory motifs that were involved in hypothyroidism.

1: "ACGGTTTCCTGCCCCCAGGCCAGGGACAA
GACATGTCA"

2: "CTTCTCTGCAGAAAACAC"

Discussion

Two active domains were found in most of the genes, central well-conserved DNA binding domain (DBD) which is supported by literature.1 Also, a C-terminal ligand binding domain was found as reported earlier.2 These domains can serve as important targets for drug designing. Study of their protein and their domains provided insight into the disease. Motif analysis furnished details about structurally important locus present within these genes. The TITF1 gene contained 3 exons. Two regions have been identified that mediate basal promoter activity in lung epithelial cells, one within the first intron, and the other 5-prime to the first exon.21

Gene domains and gene motif study of these genes along with orthologous study gives deep insight into finding new disease locus's for drug designing. Polymorphism of these genes will help in studying thyroid-related problems in different populations. The environmental factors and endocrine disruptors affecting thyroid hormones have not been studied extensively; it's an open field for researchers. It is also observed that thyroid level may differ due to climatic changes as well as food habit, iodine intake, race and socio-economic
conditions of people belonging to different areas of the world. This phenomenon and signalling mechanism can also be studied according to our own geographical area. We can find the new novel mutations in thyroid hormones. Further studies will help in understanding enzymatic mechanism. Relationship between different orthologus will help in understanding convergent evolution. Peroxidase-like domain, EF-hand domain, ferric oxidoreductase domain and (flavin adenine dinucleotide) FAD-binding (ferredoxin reductase)FR-type domain were mainly involved in congenital hypothyroidism. Phylogenetic analysis for identifying genes that are correlated with evolutionary changes in morphological, physiological and developmental conditions. The PAX8 proteins are transcriptional regulators that recognise specific DNA sequences via a conserved element, namely, the paired domain. This will provide entirely new opportunities to identify genes related to particular phenotypes.

Conclusion
Bioinformatic analysis of origin and function of each gene which utilises 3D motif structure provides insights into sequence / structure / function relationships of different genes.

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References
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