

Explorative Investigation on Thyroid Peroxidase and Sodium Iodide Symporter Gene Variants in Down Syndrome

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Abstract

Objective: Down syndrome (DS), the most common cause of intellectual disability, is frequently associated with thyroid malfunction. Normal thyroid function is essential for growth, neuronal development as well as cognition. Sodium iodide symporter (NIS) and thyroid peroxidase (TPO) are important enzymes for thyroid and to understand their role in DS, we investigated variants in these two genes in families with DS probands (N=183) and controls (N=222).

Methods: Genomic DNA isolated from peripheral blood was analyzed by sequencing for genotyping of target sites. Data obtained was analyzed by population- and family-based statistical methods. SNP-SNP interactions were analyzed by Multifactor dimensionality reduction (MDR) test.

Results: Out of twelve, only two (rs4808708 and rs4808709) functional SNPs in NIS and among eleven only one SNP, rs1126799 in TPO were polymorphic. Case-control analysis failed to show any statistically significant difference. Family-based analysis revealed significant over transmission of rs1126799 'C' allele to probands. MDR analysis showed synergistic effect of rs1126799 with both rs4808708 and rs4808709. rs4808708 and rs4808709 showed redundancy amongst themselves.

Conclusion: This pioneering association study on TPO and NIS gene variants in DS subjects showed synergistic interaction between the SNPs, in absence of any independent contribution, and together may regulate thyroid hormone metabolism.

Keywords: Down syndrome; Thyroid function; Sodium iodide symporter; Thyroid peroxidase; rs1126799; rs4808708; rs4808709

Abbreviations AD: Alzheimer's Disease; BC: Breast Cancer; CEU: Utah Residents with Western and Northern European Ancestry from the CEPH Collection; DS: Down Syndrome; fSNP: Functional Single Nucleotide Polymorphism; HCB: Han Chinese from Beijing, China; HSF: Human Splicing Finder; HWE: Hardy-Weinberg Equilibrium; IG: Information Gain; IND: Eastern Indian Control; JPT: Japanese from Tokyo, Japan; LD: Linkage Disequilibrium; MAF: Minor Allele Frequency; MDR: Multifactor Dimensionality Reduction Test; NIS: Sodium Iodide Symporter; SLC5A5: Solute Carrier Family 5 Member 5; TDT: Transmission Disequilibrium Test; TH: Thyroid Hormone; TPO: Thyroid Peroxidase; TRH: Thyrotropin Releasing Hormone; TSH: Thyroid Stimulating Hormone; YRI: Yoruba from Ibadan, Nigeria

Introduction

Occurring in about one in every 600-800 live births [1], Down syndrome (DS) is one of the most common disorders with survivable chromosomal aneuploidy. Partial triplication of human chromosome 21 (21q22), harbouring the DS critical region, is generally associated with DS. Among the endocrinopathies associated with DS, thyroid gland abnormality is the best-known and includes sub-clinical and

overt hypothyroidism, hyperthyroidism and positive thyroid antibodies [2]. Elevated thyroid stimulating hormone (TSH) along with normal tetraiodothyronine (T4) levels, identified in subjects with subclinical hypothyroidism and hyperthyrotropinemia, were reported in approximately 50% of children with DS [3]. Hypothyroidism is more prevalent than hyperthyroidism in individuals with DS, which increases with age [4]. Further, the type of abnormalities depends on the diagnostic criteria as well as age group and sample size of the population [4].

People with DS are at higher risk of developing early Alzheimer's disease (AD) like symptoms and dementia than the general population. Relationship between dementia and thyroid dysfunction in AD has been the focus of considerable research [5-7] and a significantly higher rate of antibodies to thyroid peroxidase (TPO) were noticed in AD patients as compared to controls [8]. Elevated serum TSH, without overt hypothyroidism, was also reported to confer three to four fold higher probability of dementia [9].

Out of different malignant conditions, DS patients frequently develop acute leukemia while solid tumors, especially breast cancer (BC), are rare [10]. Reports on cancer incidence and death rates in subjects with DS clearly show a strikingly lower rate of breast neoplasms in comparison to non-DS subjects [10-12]. Investigators have also reported that patients with BC have altered thyroid functions and increased serum level of TSH, thus indicating a possible

relationship between thyroid dysfunction and BC [13-15]. Sodium iodide symporter (NIS) expression has been observed in a large proportion of breast adenocarcinoma [16]. On the contrary, 87% of normal breast samples, excluding gestational/lactational changes, were negative for NIS expression [17].

Since some physiological abnormalities like, cognitive impairment, growth retardation, muscle hypoplasia, lethargy, dullness, as well as large tongue with underdeveloped nasal bridge and short neck are observed in subjects with DS as well as those with irregular thyroid functions, we speculated that abnormal function of the thyroid gland, due to defects in genes encoding for proteins involved in thyroid hormone (TH) synthesis, secretion, or recycling, may have a role in DS related thyroid abnormalities. Our preliminary investigation revealed that the thyrotropin releasing hormone (TRH) gene variant, rs13097335 "G" allele, in addition to having lower occurrence in the BC population than female control, was preferentially transmitted from parents to female DS probands and based on the observation we hypothesized that higher occurrence of rs13097335 "G" allele in female DS probands may confer protection to BC [18]. In the present study, we investigated two other TH biosynthesis pathway genes, Solute carrier family 5 member 5 (SLC5A5) or NIS [19] and thyroid peroxidase (TPO) [20,21], in nuclear families with DS probands to find out their role.

Materials and Methods

Subject recruitment

Total 685 individuals including control (N=222), probands with DS (N=183) and their parents (Father=117; Mother=163) from unrelated nuclear families were analyzed in the present study. Recruitment was based on the Diagnostic and Statistical Manual of Mental Disorders-IV [22]. Age range of the probands was 8 months-27 yrs (7.7 ± 0.51 ; Mean \pm SE). All the DS cases were recruited after confirmation for trisomy of the 21st chromosome by karyotyping. Ethnically matched healthy individuals without any history of intellectual disability were recruited as control. All the individuals were engaged for the study after obtaining informed written consent for participation. Institutional Human Ethical Committee approved the study protocol.

Sample collection and analysis of target sites

Peripheral blood samples were obtained from recruited subjects and genomic DNA was prepared using standard protocol [23]. Target sequences were amplified by polymerase chain reaction using oligonucleotides designed by online Primer3 software (http://www.embnet.sk/cgi-bin/primer3_www.cgi). Amplicons generated were subjected to genotyping by ABI prism 3130 Genetic Analyzer using Big Dye sequencing kit v3.1 followed by analysis using Sequencing Analysis software v5.2.

In silico analysis

Minor allele frequency (MAF) of the studied SNPs were retrieved from the Ensembl 1000 genomes project, Phase 1 for four major populations [Utah residents with western and northern European ancestry from the CEPH collection (CEU), Han Chinese from Beijing, China (HCB), Japanese from Tokyo, Japan (JPT) and Yoruba from Ibadan, Nigeria (YRI)]. For calculating the risk conferred by a SNP, Function Analysis and Selection Tool for Single Nucleotide

Polymorphisms (FASTSNP) were employed [24]. Functional effect of the SNP was detected by using a web based tools, F-SNP, which helps in measuring risk of a gene variant with respect to all categories like protein coding, splicing, transcriptional and post-transcriptional regulation, etc. Web based tool SNP-NEXUS was also used for functional analysis of the SNPs [25]. The Human Splicing Finder (<http://www.umd.be/HSF/>) [26] was used to locate alternative splice sites in amplified portion of the genes.

Statistical analysis

Genotypic data obtained for control, DS probands and their parents were tested for Hardy-Weinberg equilibrium (HWE) by using online program (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>). Allelic and genotypic distributions in the eastern Indian control (IND) population were compared with four major populations reported in the Ensembl 1000 genomes project, Phase 1. Difference in allelic and genotypic frequency distribution was calculated by the Unphased program (Version 3.1.7) [27]. Allelic odds ratio (OR) was calculated by the Odds ratio calculator (<http://www.hutchon.net/ConfidORnulhypo.htm>). Linkage disequilibrium (LD) values were measured by Haploview 4.1 using default settings [28]. Haplotype frequency distributions of polymorphic SNPs were inspected by the Unphased program (Version 3.1.7) [27]. Power of all the chi square tests was calculated by the Piface program [29]. Transmission disequilibrium test (TDT) to identify allelic transmission pattern from parent to DS probands was performed by the Unphased program (version 2.404) [30]. To identify SNP-SNP interaction, case-control dataset was subjected to multifactor dimensionality reduction (MDR) test using open-source MDR software package version 2.0 beta 8.1 [31]. The percentage of the information gain (IG) by each marker and pairwise markers was visualized in nodes and connections respectively. MDR helps to identify significant interactive mode of SNP-SNP interactions even in absence of any statistically significant independent main effects of those markers.

Results

Population based analysis

Out of twelve functional SNPs studied in the NIS gene (detailed in Table 1), four (rs144973943, rs4808708, rs148626179 and rs4808709) were polymorphic in other populations. However, in the IND population, only two (rs4808708 and rs4808709) were found to be polymorphic. Out of eleven SNPs in the TPO gene (Table 1), polymorphic data was available for six (rs28913012, rs28913013, rs1126799, rs61734476, rs28913015 and rs28913016) in other populations and only one, rs1126799 was polymorphic in the IND population. The polymorphic SNPs were subjected to further statistical analysis.

Genotypic data of all the polymorphic SNPs followed the HWE in all the study groups. Comparative analysis of allele frequencies obtained from other studied populations (CEU, HCB, JPT and YRI) and IND population (Table 2) revealed that the IND population followed the pattern of CEU and JPT populations for both NIS SNPs (rs4808708 and rs4808709), while differed significantly from the HCB ($p=0.002$). rs4808709 also differed from the YRI population ($p=0.004$). Allelic frequency of the TPO gene rs1126799 was significantly different from the CEU and HCB populations ($p=0.018$ and 0.038 respectively).

Gene	SNP ID	Position	Alleles (A1/A2)	MAF (A2) in other populations			
				CEU	CHB	JPT	YRI
NIS	rs188032589	Intron 13	A/G	No data			
	rs144973943		A/C	0.000	0.000	0.000	0.023
	rs201365655		A/G	No data			
	rs4808708		G/A	0.152	0.000	0.053	0.106
	rs140356148	Exon 14	G/A	No data			
	rs149937279		G/A	No data			
	rs201835225		C/T	No data			
	rs146477312		A/G	No data			
	rs200308966		G/C	No data			
	rs148626179	Intron 14	A/C	0.000	0.015	0.019	0.000
	rs4808709		A/G	0.167	0.000	0.053	0.227
	rs200646716		C/T	No data			
	TPO	rs28913012	Intron 14	C/T	0.000	0.000	0.000
rs28913013		C/T		0.005	0.000	0.000	0.056
rs201659055		Exon 15	G/A	No data			
rs143001064			G/A	No data			
rs28913014			C/T	No data			
rs141598673			G/A	No data			
rs1126799			C/T	0.449	0.422	0.332	0.167
rs61734476			G/A	0.000	0.000	0.000	0.023
rs201576336			C/T	No data			
rs28913015			C/T	0.000	0.000	0.000	0.023
rs28913016			G/A	0.000	0.000	0.000	0.116

Table 1: Description on studied SNPs of sodium iodide symporter and thyroid peroxidase genes; MAF=Minor Allele Frequency, CEU=Utah Residents with Western and Northern EUROPEAN Ancestry from the CEPH Collection, HCB=Han Chinese from Beijing, China, JPT=Japanese from Tokyo, Japan, YRI=Yoruba from Ibadan, Nigeria.

Gene	SNP ID	Allele	IND	CEU	χ^2 , P	HCB	χ^2 , P	JPT	χ^2 , P	YRI	χ^2 , P
NIS	rs4808708 (G/A)	G	0.91	0.83	2.83,	1.00	9.42,	0.95	1.23,	0.9	0.58E ⁻⁰¹ ,
		A	0.09	0.17	0.093	0.00	0.002	0.05	0.268	0.1	0.809
	rs4808709 (A/G)	A	0.91	0.82	3.47,	1.00	9.42,	0.95	1.23,	0.76	8.17,
		G	0.09	0.18	0.063	0.00	0.002	0.05	0.268	0.24	0.004
TPO	rs1126799 (C/T)	C	0.72	0.56		0.58		0.66		0.84	
		T	0.28	0.44	5.560,	0.42	4.310,	0.34	0.842,	0.16	4.200,

				0.018		0.038		0.359		0.041
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Table 2: Comparative analysis on allele frequencies obtained for worldwide populations and the Indian control population (IND); CEU=Utah Residents with Western and Northern European Ancestry from the CEPH Collection, HCB=Han Chinese from Beijing, China, JPT=Japanese from Tokyo, Japan, YRI=Yoruba from Ibadan, Nigeria; statistically significant differences are presented in bold.

Case-control analysis for all the three sites failed to reveal any statistically significant difference in allelic and genotypic frequencies (Table 3) for families with DS probands (DSF, DSM and DSP) when compared to the control. Probands and the control groups, stratified on the basis of gender, revealed a trend for lower occurrence of the derived allele in the male probands as compared to the male control. On the other hand, the female probands showed higher occurrence of

the derived alleles as compared to female controls for all the three studied SNPs, though the differences were statistically insignificant. NIS rs4808708-rs4808709 haplotype frequency also failed to show significant difference. Lack of any potential risk by any allele was also observed by OR (Table 3). Pair wise LD analysis revealed that SNP pairs of NIS was in complete LD for the control group and in strong LD ($r^2 > 0.90$) in all other study groups.

SNP ID	Allele	Control	DSF	χ^2, P	DSM	χ^2, P	DSP	χ^2, P	OR-1 [95% CI]	OR-2 [95% CI]			
rs4808708 (G/A)	G	0.906	0.879	1.176,	0.889	0.592,	0.898	0.146,	0.913	1.095			
	A	0.094	0.121	0.278	0.111	0.442	0.102	0.702	[0.680-1.227]	[0.815-1.471]			
	GG	0.817	0.767	1.249,	0.785	0.626,	0.802	0.154,	-	-			
	GA	0.178	0.224		0.209		0.731				0.192	0.926	
	AA	0.005	0.009		0.535		0.006				0.005		
rs4808709 (A/G)	A	0.907	0.881	1.065,	0.886	0.858,	0.898	0.163,	0.903	1.108			
	G	0.093	0.119	0.302	0.114	0.354	0.102	0.687	[0.672-1.213]	[0.824-1.489]			
	AA	0.818	0.771	1.130,	0.778	0.914,	0.802	0.171,	-	-			
	AG	0.177	0.22		0.568		0.215				0.633	0.192	0.918
	GG	0.005	0.009		0.006		0.006						
rs1126799 (C/T)	C	0.717	0.686	0.689,	0.682	1.061,	0.708	0.071,	0.957	1.045			
	T	0.283	0.314	0.407	0.318	0.303	0.292	0.79	[0.789-1.162]	[0.861-1.268]			
	CC	0.53	0.451	2.753,	0.461	1.864, 0.394	0.489	1.993,	-	-			
	CT	0.374	0.469		0.253		0.442				0.439	0.369	
	TT	0.096	0.08		0.097		0.072						

Table 3: Allelic and genotypic frequencies observed in the control, DS probands (DSP), father of DS probands (DSF) and mother of DS probands (DSM); OR 1=Odds Ratio with A1 as the risk allele for DSP group; OR 2=Odds Ratio with A2 as the risk allele for DSP group; CI=Confidence Interval.

Family based transmission analysis

Family based analysis failed to identify any biased transmission for rs4808708 and rs4808709. However, rs1126799 'C' allele exhibited statistically significant over transmission from parents to probands (Table 4, LRS=4.801, p value=0.028).

SNP ID	Proband	Allele	Transmitted	Not-transmitted	LRS, P
rs4808708 (G/A)	Both	G	0.52	0.48	0.095, 0.758
		A	0.48	0.52	

rs4808709 (A/G)	Male	G	0.5	0.5	0.000, 1.000
		A	0.5	0.5	
	Female	G	0.57	0.43	0.430, 0.512
		A	0.43	0.57	
	Both	A	0.55	0.45	0.364, 0.546
		G	0.45	0.55	
	Male	A	0.48	0.52	0.048, 0.827
		G	0.52	0.48	

rs1126799 (C/T)	Female	A	0.64	0.36	1.657, 0.198
		G	0.36	0.64	
	Both	C	0.64	0.36	4.801, 0.028
		T	0.36	0.64	
	Male	C	0.65	0.35	3.320, 0.068
		T	0.35	0.65	
	Female	C	0.63	0.37	1.516, 0.218
		T	0.37	0.63	

Table 4: Allelic transmission frequency analyzed by transmission disequilibrium test for nuclear families with DS probands; LRS=Likelihood Ratio Statistic; statistically significant differences are mentioned in bold.

Analysis of interaction between the sites

As evidenced from nodal IG values, MDR analysis revealed subtle independent effects of rs4808708 (0.57%), rs4808709 (0.42%) and rs1126799 (0.44%) (Figure 1). rs1126799 showed synergistic interaction with rs4808709 (IG=0.58%) as well as with rs4808708 (IG=0.43%) for the studied data set. NIS SNP pairs showed redundant interactions between them (IG=-91%) (Figure 1).

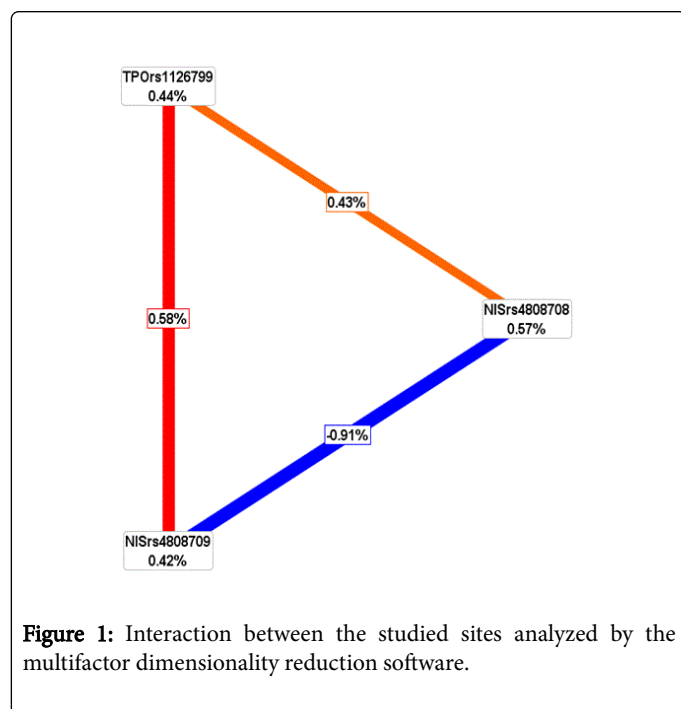


Figure 1: Interaction between the studied sites analyzed by the multifactor dimensionality reduction software.

Functional analysis

HSF analysis of the NIS gene exon 14 and its adjacent intronic region containing 12 fSNPs predicted that, apart from the natural acceptor and donor splice sites, there are an additional 32 potential acceptor splice sites and 13 potential donor splice sites. The NIS exon 14 has a strong donor splice site with a consensus value of 94.02 and a strong acceptor site with a consensus value of 90.04. The two polymorphic SNPs, rs4808708 and rs4808709, were intronic variants

and situated at the opposite end of the exon 14. rs4808708 is located closer to the acceptor site than to the donor site but do not have any regulatory role in splicing. On the other hand, rs4808709, closer to donor site than the acceptor site, is predicted by the ESE finder software to disrupt binding sites of regulatory SRp40 protein. F-SNP analysis revealed that both SNPs also have a predicted function in transcriptional regulation with a FS score 0.176.

HSF analysis of the TPO gene exhibited that, in the exon 15 and its adjacent intronic regions, apart from the natural acceptor and donor splice sites, there are an additional 16 potential acceptor splice sites and 8 potential donor splice sites. The exon 15 has a strong donor splice site with a consensus value of 93.49 and a moderately strong acceptor site with a consensus value of 86.95. A potential cryptic acceptor splice site exists at nucleotide position -31 with a stronger score than that of the wild type (89.91 versus 86.95). rs1126799 found to be polymorphic in the studied population is a coding, non-synonymous SNP. The cDNA position of the SNP is c.2541. The SNP, which is located closer to the acceptor site than to the donor site, is predicted by the ESE finder software to disrupt binding sites for two regulatory SR proteins: SF2/ASF and SRp40 at c.2535 and c.2541, respectively. rs1126799 (C/T) is responsible for Alanine (A) to Valine (V) substitution at the 847th position of the polypeptide with a predicted FS score of 0.129. SNPeffect predicted an alteration in aggregation and secondary structure of the protein due to the substitution. However, other prediction tools (Polyphen, SIFT) predicted the SNP as a benign one.

Discussion

Thyroid dysfunction is one of the main reason for intellectual and developmental disability which can result from a number of different causes, either individually or in combination. These include iodine deficiency or excess, defects in the structure or function of the thyroid gland, micronutrient imbalances (including iron, selenium and zinc), genetic causes, structural abnormalities in the hypothalamus and/or pituitary that affect normal functioning of the hypothalamic-pituitary-thyroid axis, autoimmune disease associated with production of antibodies to thyroglobulin, TPO and/or TH etc. After the worldwide introduction of neonatal screening for congenital hypothyroidism, it goes beyond doubt that early diagnosis and thyroxine supplementation can prevent brain damage to a great extent. However, in spite of well sought out evidences for association between thyroid dysfunction and DS, the reason still remains unknown. Clinical manifestations of hypothyroidism are very nonspecific and quite often are masked by the symptoms of DS. We hypothesized that identification of components crucial for maintaining normal thyroid function may aid in recognizing subjects who are at risk of developing hypo-/hyperthyroidism, thus helping in providing remedial intervention.

At birth 0.7% of infants with DS exhibit persistent primary congenital hypothyroidism [32] which rises to at least 12% in adults [33]. Clinical forms of hypothyroidism found in subjects with DS include transient and primary hypothyroidism, pituitary-hypothalamic hypothyroidism, thyroxin-binding globulin deficiency and chronic lymphocytic thyroiditis. There are very few studies about the thyroid functions of new-borns with DS. A study on thyroid function test in infants with DS during the first 24 months claimed a direct relationship with the trisomic state of chromosome 21, hypothetically through genomic dosage imbalance of dosage-sensitive genes interfering with TH production [34].

NIS localized at the basolateral plasma membrane of the thyrocytes initiate the first step of thyroid metabolism by uptaking iodide from the blood. The human NIS gene (SLC5A5) is localized at chromosome 19p12-13.2 with an open reading frame of 1929 nucleotides encoding a glycoprotein of 643 amino acids and has a molecular mass of ~ 70-90 kDa. The coding region of human NIS contains 15 exons interrupted by 14 introns [35]. The proposed secondary structure of NIS is an intrinsic membrane protein with 13 putative transmembrane domains, an extracellular amino-terminus and an intracellular carboxyl-terminus with three putative N-linked glycosylation sites at position 225, 485 and 497 [36].

Iodide transport defect was identified as a cause for congenital hypothyroidism mediated by NIS mutations which shows an autosomal recessive inheritance pattern. After cloning of NIS, 13 mutations have been identified in the NIS coding region: V59E, G93R, R124H, deletion 143-323, Q267E, C272X, deletion 287-288, T354P, G395R, deletion 439-443, 515X, Y531X, G543E [37-44]. They were nonsense, alternative splicing, frame shift, deletion, or missense mutations. A variable degree of hypothyroidism, goitre, reduced or absent iodide uptake and low iodide saliva:plasma ratio was observed in all these patients. These mutations were reported to lead to either in the synthesis of truncated proteins, proteins with partial deletions or amino acid substitutions [36-46]. Many potential regulation sites that are subject to post-translational modification (phosphorylation, sumoylation, ubiquitinylation) or sites of protein-protein interaction (SH3 or SH2 binding motifs, PDZ domain protein binding sites, etc.) were predicted by in silico analysis to be present on the NIS protein, mainly at the C-terminal domain. Exon 14 and 15 of the NIS gene encodes the intracellular C-terminal portion of the protein. Exon 14 and its adjacent intronic region of the NIS gene containing 12 fSNPs were studied in the present investigation and only two (rs4808708 and rs4808709) were found to be polymorphic. Both were intronic variants and situated at the opposite end of the studied exon. Both were predicted to have a function in transcriptional regulation. The splice distance of rs4808708 (G/A) and rs4808709 (A/G) is 9 bp and 29 bp respectively. Both the SNPs failed to show any association in the present study by population- as well as family-based analysis. A sexual dimorphism in the distribution of the both SNPs in the DSP group was noticed, although the differences were statistically insignificant. The SNP pair was in complete LD in the studied population and therefore has a higher chance of being transmitted together. The results may indicate that studied NIS gene variants may not contribute to the DS related thyroid abnormalities.

In human, TPO is a 110 kDA membrane-bound, glycosylated, heme-containing protein that catalyzes iodination of thyroglobulin and the coupling of iodotyrosyl residues to generate functionally active TH, ttriiodothyronine (T3) and T4. The gene encoding for TPO is located on chromosome 2p25 which spans ~ 150 kb and contains 17 exons [47]. The full length of TPO mRNA transcript (TPO1) is 3152 bp encoding for a protein consisting of 933 amino acids [48,49]. Apart from TPO1 seven different TPO transcripts, generated through alternate splicing, have also been discovered. Differential splicing generates shorter transcripts which lack one or multiple exons [50]. Among these eight types of transcripts, only TPO1, TPO3 (lacking exon 16) and TPO4 (lacking exon14) are expected to produce enzymatically active protein and can play important roles in TH synthesis [50,51]. Further, isoforms have a shorter half-life as compared to TPO1 [50].

TPO abnormality is the most common cause for congenital dysmorphogenetic hypothyroidism [52] where more than 60 mutations affecting the enzymatic activity have been described [53]. Being a key enzyme for TH synthesis, TPO gene defect (especially non-synonymous SNPs) can potentially lead to severe defects in hormone production. The first reported mutation in TPO gene was a homozygous GGCC insertion-duplication at position c.1186 in the exon 8 [54]. A homozygous TPO deletion (c.2422delT), associated with total iodine organification defect and abolition of TPO enzyme function [55], was detected in CH affected siblings from a consanguineous family [56]. A c.2268dup nonsense mutation in the exon 13 of the TPO gene had been reported to be common amongst dysmorphogenetic congenital hypothyroidism patients from Taiwan [57,58]. Screening and identification of mutations in the TPO gene has been done on human subjects from Argentina [59], Netherlands [60], Japan [61], Brazil [62], Portugal [63] and China [58].

A study on adult hypothyroid subjects from West Bengal, India identified single nucleotide changes at exon 7 and 14 of TPO gene such as Ala373Ser, Ser398Thr, Asp620Asn, Glu641Lys, Asp668Asn, Thr725Pro [64]. Another study on the same population identified six different previously known SNPs and two novel deletions after sequencing of 17 exonic regions. Among them, two SNPs (Thr725Pro and Asp666Asp) were found to be significantly associated with hypothyroidism [65]. In the present study, eleven SNPs in the exon 15 were analyzed in families with DS and only one non-synonymous change, rs1126799, was polymorphic in the studied population. SNP effect predicted an alteration in aggregation and secondary structure of the protein due to the substitution. The analyzed stretch harbors a potential cryptic acceptor splice site at nucleotide position - 31 with a stronger score than that of the wild type (89.91 versus 86.95). However, in the studied population, we have noticed only the wild type. rs1126799, which is located closer to the acceptor site than to the donor site, was predicted by the ESE finder software to disrupt binding sites of two regulatory SR proteins: SF2/ASF and SRp40 at c.2535 and c.2541 respectively; the "T" allele acts as a crucial enhancer motif for both regulatory proteins. Though population-based analysis failed to show significant difference, TDT analysis revealed a bias in transmission of the wild type "C" allele from parents to probands. The "C>T" substitution is responsible for alanine > valine substitution which change the size and hydrophobicity at the transmembrane domain of the protein. The observed over transmission of "C" in DS probands indicate higher chances for the native protein, containing alanine, to be present in the studied DS population.

By MDR analysis, the studied SNPs failed to show any significant independent contribution in probands with DS. However, synergistic effects were noticed for the gene variants. On the basis of the observation, it can be speculated that the studied gene variants of NIS and TPO may not contribute to DS related thyroid abnormalities, though may regulate the TH metabolism in an interactive way.

Conclusion

As per our knowledge, this is the first genetic association study on TPO and NIS gene variants in individuals with DS. TPO rs1126799 and NIS rs4808708 and rs4808709 failed to reveal any significant contribution in DS. However, a significant synergistic effect of these SNPs was observed in thyroid biosynthesis pathway. Major limitations of the current study are (1) small number of samples, (2) analysis of few SNPs and (3) lack of information regarding participant's thyroid status. Extensive research in the field would help in deciphering the

contribution of TH pathway genes in DS related thyroid anomaly. This pioneering investigation on Indo-Caucasoid DS subjects though did not show any contribution of TPO and NIS gene variants in DS pathophysiology, it unraveled significant positive interaction between the two genes which may aid in understanding their function in vivo.

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