Genetic Mutation Analysis in Patients with Congenital Hypothyroidism

[1]Tasnia Kawsar Konika, [2] Sadia Sultana, [3] Sharmin Rahman Madhuri, [4]Fatema Tuz Zohra, [1]Prianka Jabin, [5]Sutanu Roy, [6] Rejuana Purveen, [7]Ashrafi Anar

[1] Assistant Professor, Department of Nuclear Medicine & Molecular Imaging, Enam Medical College Hospital.
[2] Ex- Professor & Director, NINMAS, BSMMU
[3] Consultant, Ibn Sina Diagnostic Center, Uttara.
[4] Medical Officer, Institute of Nuclear Medical Physics, AERE, Savar
[5] RMO, Aliahat 20 Bed Hospital, Shibganj, Bogura
[6] Consultanat, Popular Diagnostic Center, Savar.
[7] Sonologist, Combined Military Hospital

Correspondence Address : Dr. Tasnia Kawsar Konika, Assistant Professor, Department of Nuclear Medicine & Molecular Imaging, Enam Medical College Hospital, Savar, Dhaka. Email: tasniakawsar@yahoo.com

ABSTRACT

Purpose: Congenial hypothyroidism (CH) is one of the most common inborn endocrine disorders in Bangladesh. Genetic mutation is one of the major causes of CH, which can lead to an absent, hypoplastic, or ectopic thyroid gland or defects in the hormone synthesis pathway. The purpose of this study is genetic mutation analysis in terms of mutations in different genes in already-diagnosed patients with CH who are attending Bangabandhu Sheikh Mujib Medical University (BSMMU).

Materials and Methods: This cross-sectional study was carried out at the National Institute of Nuclear Medicine and Allied Sciences (NINMAS) from July 2016 to June 2017. A total of 27 diagnosed patients with CH from 24 unrelated families were included. All laboratory procedures were conducted at the Institute for Developing Science and Health Initiatives (ideSHi). Blood samples were used for DNA extraction. Conventional PCR was done for DNA amplification. Direct sequencing of specific regions of PCR products was done. BLAST was used for mutation analysis, and the data were presented as numbers and percentages.

Results: This descriptive, cross-sectional study was conducted to see the most commonly found mutations in the TPO, TSHR, and PAX8 genes in diagnosed cases of congenital hypothyroidism. Out of a total of 27 patients, eight had dysgenesis, and 19 had dyshormonogenesis. A total of four TPO gene mutations, namely, 1117G > T, 1193G > C, 2145 C > T, and 2173A > C, were found to be involved with dyshormonogenesis. Among the four mutations, 1117G > T and 1193G >C were in exon 8, and they resulted in a change in the amino acid at protein positions 373 (Ala>Ser) and 398 (Ser>Thr). Other two mutations, namely, 2145C>T and 2173A>C, were found in exon 12. Substitution mutation 2145C>T did not change the amino acid proline to another amino acid at position 715. The substitution 2173A>C resulted in 725Thr>Pro. In the TSHR gene, all three mutations, namely, 2181G>C, 2161G>C, and 1523C>T, were found in exon 10. These mutations eventually lead to a change in the primary amino acid sequence of TSHR peptides. The amino acid substitutions that occurred in the TSHR protein were: 727Glu>Asp, 721Val>Leu, and 508Leu>Ser, respectively. Exon 3 of the PAX8 gene was analyzed, and no mutation was found.

Conclusion: This study aimed to conduct a genetic analysis on CH, a prevalent inborn endocrine disorder in Bangladesh, to better understand its causes and develop future management strategies.

Keywords: Congenital hypothyroidism, Thyroid dysgenesis, Thyroid dyshormonogenesis, Genetic mutation

Bangladesh J. Nucl. Med. Vol. 26 No. 2 July 2023
DOI: https://doi.org/10.3329/bjnm.v26i2.71469

INTRODUCTION

Congenital hypothyroidism (CH) is the most common inborn endocrine disorder, with a prevalence of 1 in 3000 to 4000 live births worldwide (1). In Bangladesh, CH has a prevalence of 1 in 2000 live births (2). CH may broadly be categorized into permanent and transient types. Thyroid dysgenesis and dyshormonogenesis are considered as permanent primary CH, where the thyroid hormone deficiency is persistent and requires lifelong treatment. Apart from the primary causes, secondary or central CH, peripheral CH, and syndromic CH are also included in permanent CH. Whereas, transient CH is characterized by temporary thyroid hormone deficiency usually for a short period and can be corrected back to normal level. Transient CH is usually associated with maternal iodine deficiency or the maternal intake of antithyroid drugs (3, 4). Genetic causes constitute around 15-20% of cases of CH, which includes mostly thyroid dysgenesis and dyshormonogenesis (4,5).

The genes associated with congenital hypothyroidism are classified into two main groups: those involved with thyroid gland dysgenesis and those with dyshormonogenesis. Genes

that are responsible for thyroid gland dysgenesis include thyroid transcription factors like TTF-1, TTF-2, and PAX8; the TSH receptor (TSHR) gene; and the Gsα gene. Mutations in genes that are found in thyroid dyshormonogenesis include the TPO (thyroid peroxidase), TG (thyroglobulin), PDS (Pendred syndrome), NIS (sodium iodide symporter), and THOX2 (thyroid oxidase 2) genes (6–8).

So far reported, the prevalence of congenital hypothyroidism in Bangladesh is higher than in other parts of the world (2). But till now, there has been no published data on the genetic analysis of congenital hypothyroid patients. Gene mutation is one of the important factors that can play a vital role in the developmental process of this disease. Under this circumstance, the present study is undertaken to explore the genetic mutations of congenital hypothyroidism in terms of mutations in the TPO, TSHR, and PAX8 genes in patients with CH attending NINMAS and BSMMU hospitals.

PATIENTS AND METHODS

This cross-sectional study was carried out at the National Institute of Nuclear Medicine & Allied Sciences (NINMAS) from July 2016 to June 2017, as part of the fulfillment of an M.Phil degree in Nuclear Medicine under Bangabandhu Sheikh Mujib Medical University (BSMMU). A total of 27 diagnosed patients with CH from 24 unrelated families were included in the BSMMU outpatient department. Ethical approval was obtained from the Medical Research Ethics Committee (MREC) of NINMAS. Informed written consent was obtained from the study subjects with strict confidentiality of the procedure and study results. All laboratory procedures were conducted in ideSHi. Blood samples were used for DNA extraction. Conventional PCR was done for DNA amplification. Direct sequencing of specific regions of PCR products was done using the BigDye Chain Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and the ABI PRISM 310 automated sequencer (Applied Biosystems, USA). BLAST was used for mutation analysis, and the data were presented as numbers and percentages.

STATISTICAL ANALYSIS

The collected data was analyzed using computer-based, programmed Statistical Package for Social Sciences (SPSS) software (Version 26) for Windows (SPSS Inc., Chicago, Illinois, USA). Qualitative variables were expressed as frequencies and percentages. Quantitative variables were expressed as mean ± standard deviation.

RESULT

The total number of study participants was 27, and among them, 8 (29.6%) and 19 (70.4%) patients had thyroid dysgenesis and dyshormonogenesis, respectively. The total number of male patients was 15; those included 11 dyshormonogenesis participants and four dysgenesis participants. On the other hand, among the 12 female participants, eight had dyshormonogenesis and four had dysgeneses.

Even though there are several genes involved with dyshormonogenesis, the TPO gene was primarily targeted, as mutations in this gene have been found to occur most commonly in dyshormonogenesis patients (5, 6). In this study, a total of 4 TPO gene mutations, namely, 1117G >T, 1193G >C, 2145C>T, and 2173A >C, were found to be involved with dyshormonogenesis (Table 1). Among the 4 mutations, 1117G >T and 1193G >C are in exon 8, and they result in point mutations resulting in a change in the amino acid at protein positions 373 (Ala>Ser) and 398 (Ser>Thr). These substitutions result in changes in the protein catalytic site and consequently hamper normal TPO enzyme activity. Other two mutations namely, 2145C>T and 2173A>C are in exon 12. Substitution mutation 2145C>T does not change the amino acid proline to another amino acid at position 715 and might not have any impact on TPO enzyme activity. However, the substitution 2173A>C results in 725Thr>Pro and decreases TPO enzyme activity.

Table 1: Mutation analysis in dyshormonogenesis samples (TPO gene)

Serial no.	Mutation	Exon	Functional change
1	1117G → T	8	373Ala → Ser
2	1193G → C	8	398Ser → Thr
3	2145C → T	12	715Pro → Pro
4	2173A → C	12	725Thr → Pro

Among the four mutations, 1117G >T and 1193G >C were the most frequent, found in 13 and 14 participants, respectively (Table 2). On the other hand, there were four participants for 2145C>T and 2173A>C substitutions, respectively.

Table 2: Frequency of different patterns of mutations in study subjects (TPO gene)

Types of mutations	Mutation	Number of samples
1	1117G>T	13
2	1193G>C	14
3	2145C>T	4
4	2173A>C	4

Table 3 represents the frequency of mutations in homozygous and compound heterozygous states in study participants. Compound heterozygous states 1117G>T and 1193G>C were most frequent, which was found in 63.2% of study participants. The second frequent mutation status was 2145C>T plus 2173A>C compound heterozygous state, and this state was present in 21% of the study participants. However, the homozygous 1193G>C and homozygous 1117G>T states were found in 10.5% and 5.3% of participants, respectively.

Table 3: Distribution of mutation combination in dyshormonogenesis samples (TPO gene)

Mutation combination	Number of samples(N=19)	Percentage
1117G → T*	12	63.2%
1193G → C*		
2145C → T*	4	21%
2173A → C*		
1193G → C#	2	10.5%
1117G → T#	1	5.3%

*indicates heterozygous state, # indicates homozygous state.

Similar to the selection of the most common responsible gene namely, the TPO gene for mutation analysis in dyshormonogenesis patients, TSHR and PAX8 genes were selected for mutation analysis in dysgenesis patients. Table 4 shows the mutation analysis and distribution of dysgenesis samples for the TSHR gene. In the TSHR gene, all three mutations namely, 2181G>C, 2161G>C, and 1523C>T were found in exon 10. These mutations eventually lead to a change in the primary amino acid sequence in TSHR peptides. The substitutions that occurred due to 2181G>C, 2161G>C and 1523C>T substitution mutations were 727Glu>Asp, 721Val>Leu and 508Leu>Ser, respectively. No mutation was found in the PAX8 gene.

Table 4: Mutation analysis and distribution of dysgenesis samples (TSHR gene)

Serial no	Mutation	Exon	Functional change	Percentage
1	2181G>C	Exon 10	727 Glu→Asp	75% (6 samples)
2	2161 G>C	Exon 10	721 Val→Leu	12.5% (1 sample)
3	1523 C >T	Exon 10	508 Leu→Ser	12.5% (1 sample)

All of TSHR gene mutations were found in homozygous states in the respective genes of the study participants. The most frequent homozygous mutation was 2181G>C which was present in 6 dysgenetic samples (75% of dysgenetic samples). 2161G>C and 1523C>T were the least frequent mutations each of which was found in 12.5% of the total dysgenesis participants.

DISCUSSION

Genetic causes constitute around 15-20% of cases of CH, which includes mostly thyroid dysgenesis and dyshormonogenesis (4). Thyroid dysgenesis occurs in almost 85% of CH cases, which includes the absence of thyroid glands or thyroid agenesis (40%), abnormally located or ectopic glands (40%), and hypoplastic glands (5%). The remaining 15% of cases are associated with defects in thyroid hormone synthesis, also called dyshormonogenesis (5).

Genes that are responsible for thyroid gland dysgenesis include the thyroid transcription factors (TTF-1, TTF-2, and Pax-8), the TSH receptor (TSHR) gene, and the Gsα gene. Mutations in genes that are found in thyroid dyshormonogenesis include the TPO (thyroid peroxidase), TG (thyroglobulin), PDS (Pendred syndrome), NIS (sodium iodide symporter), and THOX2 (thyroid oxidase 2) genes (5, 6).

This descriptive, cross-sectional study was conducted to identify TPO, TSHR, and PAX8 mutations in diagnosed cases of CH. A total of 27 samples were collected from diagnosed cases of congenital hypothyroidism whose age was below 18 years. Among the 27 patients, 5 were previously enrolled for mutation analysis in mRNA, and the rest, 22 cases, were newly collected. Samples were collected from the Pediatric Endocrinology Outdoor of BSMMU and also from NINMAS. Among the 27 patients, eight had dysgenesis, and 19 had dyshormonogenesis.

A TPO gene defect is the most frequent cause responsible for inherited dyshormonogenesis (9). In a cohort study done on 2000 by Bakker et al on 45 Dutch patients with CH due to total iodine organification defect, sixteen different mutations were found which included eight novel mutations. Majority of the mutations were found in exon 8,9,10 and 12. The most frequent mutation was GGCC insertion in exon 8 at nucleotide 1277 (12-14). In this study, a total of four TPO gene mutations, namely, 1117G >T, 1193G >C, 2145C> T, and 2173A> C, were found to be involved with dyshormonogenesis (Table 1). Among the 4 mutations, 1117G >T and 1193G >C are in exon 8, and they result in point mutations resulting in a change in amino acid at protein positions 373 (Ala>Ser) and 398 (Ser>Thr). These substitutions result in changes in the protein catalytic site and consequently hamper normal TPO enzyme activity. The other two mutations, namely, 2145C>T and 2173A>C, are in exon 12. Substitution mutation 2145C>T does not change the amino acid proline to another amino acid at position 715 and might not have any impact on TPO enzyme activity. However, the substitution 2173A>C results in 725Thr>Pro and decreases TPO enzyme activity. Among the four mutations, compound heterozygous states 1117G>T and 1193G>C were the most frequent, which were found in 63.2% of study participants. The second frequent mutation status was 2145C>T plus 2173A>C compound heterozygous state, and this state was present in 21% of the study participants. However, the homozygous 1193G>C and homozygous 1117G>T states were found in 10.5% and 5.3% of participants, respectively (Table 3).

The TSHR gene is located on chromosome 14q31 and contains 11 exons (5). Some of the mutations that are found in the TSHR gene are as follows: Cys390 > Trp substitution results in loss of hormone binding activity; Asp410> Asn; and Phe525> Leu substitution results in impaired adenylate cyclase activation (10,11). In this present study, in the TSHR gene, 2181G>C, 2161G>C, and 1523C>T were the three mutations that were found in exon 10 of the TSHR gene. These mutations eventually lead to the functional changes of 727Glu>Asp, 721Val>Leu, and 508Leu>Ser, respectively (Table 4).

PAX8 stands for paired box gene eight. Heterozygous PAX8 mutations are found to be inherited in an autosomal dominant manner in some of the patients. These patients either had hypoplastic or ectopic thyroid glands. In a study done by Macchia et al. in 1998, it was found that common sites for the PAX8 mutation are in exons 2 and 3 (11). In our present study, we searched for mutations in exon three. But no mutations were found.

CONCLUSION

Genetics significantly affects CH, the most common inborn endocrine disorder, with higher prevalence in Bangladesh. This study aims to evaluate factors responsible for CH development, improve management, and determine carrier status. Large-scale genetic analysis could represent mutational status in the entire population.

The authors whose names are listed certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest.

REFERENCES

1. Toublanc, J.E., 1992. Comparison of epidemiological data on congenital hypothyroidism in Europe with those of other parts of the world. Hormone Research in Paediatrics, 38(5-6), pp.230-235.
2. Hasan, M., Nahar, N., Moslem, F., Begum, NA., 2008. Newborn screening in Bangladesh. Annals Academy of Medicine Singapore, 37(12),p 112. http://Autorecessive.svg.png.info; retrieved 10 February 2016.
3. Rastogi, M.V. and LaFranchi, S.H., 2010. Congenital hypothyroidism. Orphanet Journal of Rare Diseases, 5(1), p.17.
4. www.Genetics Home Reference. Info, retrieved 9 October 2016.
5. Park, S.M. and Chatterjee, V.K.K., 2005. Genetics of congenital hypothyroidism. Journal of Medical Genetics, 42(5), pp.379-389.
6. Grasberger, H. and Refetoff, S., 2011. Genetic causes of congenital hypothyroidism due to dyshormonogenesis. Current Opinion in Pediatrics, 23(4), p. 421.
7. Moreno, J.C., Bikker, H., Kempers, M.J., Van Trotsenburg, A.P., Baas, F., de Vijlder, J.J., Vulsma, T. and Ris-Stalpers, C., 2002. Inactivating mutations in the gene for thyroid oxidase 2 (THOX2) and congenital hypothyroidism. New England Journal of Medicine, 347(2), pp.95-102.
8. Krude, H., Schütz, B., Biebermann, H., von Moers, A., Schnabel, D., Neitzel, H., Tönnies, H., Weise, D., Lafferty, A., Schwarz, S. and DeFelice, M., 2002. Choreoathetosis, hypothyroidism, and pulmonary alterations due to human NKX2-1 haploinsufficiency. The Journal of Clinical Investigation, 109(4), pp.475-480.
9. Bikker, H., Vulsma, T., Baas, F. and de Vijlder, J.J., 1995. Identification of five novel inactivating mutations in the human thyroid peroxidase gene by denaturing gradient gel electrophoresis. Human Mutation, 6(1), pp.9-16.
10. De Roux, N., Misrahi, M., Brauner, R., Houang, M., Carel, J.C., Granier, M., Le Bouc, Y., Ghinea, N., Boumedienne, A., Toublanc, J.E. and Milgrom, E., 1996. Four families with loss of function mutations of the thyrotropin receptor. The Journal of Clinical Endocrinology & Metabolism, 81(12), pp.4229-4235.
11. Macchia, P.E., Lapi, P., Krude, H., Pirro, M.T., Missero, C., Chiovato, L., Souabni, A., Baserga, M., Tassi, V., Pinchera, A. and Fenzi, G., 1998. PAX8 mutations associated with congenital hypothyroidism caused by thyroid dysgenesis. Nature Genetics, 19(1), pp.83-86.
12. Bakker, B., bikker, H., Vulsma, T., de Randamie, J.S., Wiedijk, B.M. and de Vijlder, J.J.,2000. Two decds of screening for congenital hypothyroidism in The Netherlands: TPO gene mutations in total iodide organification defects (an update). The Journal of Clinical Endocrinology & Metabolism, 85(10), pp. 3708- 3709.
13. Grant, D.B., Smith, I., Fuggle, P.W., Tokar, S. and Chapple, J., 1992. Congenital hypothyroidism detected by neonatal screening: relationship between biochemical severity and early clinical features. Archives Of Disease In Childhood, 67(1), pp.87-90.
14. Fu, C., Xie, B., Zhang, S., Wang, J., Luo, S., Zheng, H., Su, J., Hu, X., Chen, R., Fan, X. and Luo, J., 2016. Mutation screening of the TPO gene in a cohort of 192 Chinese patients with congenital hypothyroidism. BMJ open, 6(5).