Semantic Analysis of Macedonian Medical Abstracts Indexed in the PubMed Database using GoPubMed

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Abstract

Aim: The aim of this study was to analyze semantically medical abstracts from the Republic of Macedonia indexed in the PubMed database with GoPubMed.

Material and Methods: The analysis was performed with GoPubMed on March 18, 2013 in order to identify indexed papers affiliated with the country Macedonia in the PubMed database. A total number of 1469 abstracts were identified for analysis.

Results: Macedonian medical scientists published papers in a total of 400 different journals which have been indexed in PubMed database. The largest number of published papers was in the domestic journal Prilozi. Top twenty Macedonian authors published 72.4% of the total number of abstracts indexed in PubMed. A significant increase of abstracted papers during the period of 1989-2012 was recorded (from 50 abstracts to 180 abstracts, respectively) and a significant increase of relative research interest (from 0.00006 to 0.00018, respectively). Top author networks of the Macedonian scientists have shown that the largest group was composed of scientists working in the field of nephrology and related disciplines.

Conclusion: Requirements for at least one paper to be published and indexed in PubMed before a candidate approaches to the Philosophy Doctor Degree defence at the Faculty of Medicine, Ss Cyril and Methodius University of Skopje was a trigger for an increased number of publications indexed in PubMed in the last decade. A larger number of Macedonian medical journals should be indexed in PubMed in order to increase the impact of Macedonian medical scientists in the world.

Introduction

PubMed is a free database accessing primarily the MEDLINE database of references and abstracts on life sciences and biomedical topics. The United States National Library of Medicine (NLM) at the National Institutes of Health maintains the database as part of the Entrez system of information retrieval. PubMed was first released in January 1996 [1]. In addition to MEDLINE, PubMed provides access to: (a) older references from the print version of Index Medicus back to 1951 and earlier; (b) references to some journals before they were indexed in Index Medicus and MEDLINE, for instance Science, BMJ, and Annals of Surgery; (c) very recent entries to records for an article before it is indexed with Medical Subject Headings (MeSH) and added to MEDLINE; and (d) a collection of books available full-text and other subsets of NLM records [2].

Many PubMed records contain links to full text articles, some of which are freely available, often in PubMed Central [3] and local mirrors such as UK PubMed Central [4]. Information about the journals indexed in PubMed is found in the NLM Catalog [5]. As of 29 March 2013, PubMed has over 22,622,619 records going back to 1966 and about 500,000 new records are added each year [6].
Several databases, programs or WEB sites use PubMed as a source for further analysis of the abstracted papers (Fig. 1). BiomedExperts is a publication-based scientific social network that allows researchers to collaborate virtually in order to increase biomedical research. It involves more than 3,500 institutions from more than 190 countries. One of the most attractive features of BioMedExperts is that members receive automatic alerts - by e-mail and/or when signing in to the web interface of the service - for publications authored by their contacts and by "bookmarked researchers", including authors that are not signed-up members of the service themselves [7]. Macedonian biomedical scientists are not adequately represented in BiomedExperts database and scientists are encouraged to register, log in, and correct personal profiles [8]. Pubget.com is a free service for non-profit institutions and their libraries and researchers. The site provides direct access to full-text content from 450 libraries around the world. In January 2012 it was announced that Pubget was acquired by Copyright Clearance Center [9].

GO2PUB was developed to automatically enrich PubMed queries with gene names, symbols and synonyms annotated by a Gene Ontology (GO) term of interest or one of its descendants. It processes the result and displays the PMID, title, authors, abstract and bibliographic references of the articles. GO2PUB is based on a semantic expansion of PubMed queries using the semantic inheritance between terms through the GO graph [10]. Web server GoPubMed was introduced, which allows users to explore PubMed search results with the GO, a hierarchically structured vocabulary for molecular biology. GoPubMed has several advantages. First, it gives an overview of the literature abstracts by categorizing them according to the GO and thus allowing users to quickly navigate through the abstracts by category. Second, it automatically shows general ontology terms related to the original query, which often do not even appear in the abstract. Third, it enables users to verify its classification because GO terms are highlighted in the abstracts and each term is labelled with an accuracy percentage. Fourth, exploring PubMed abstracts with GoPubMed is useful as it shows definitions of GO terms without the need for further look up. GoPubMed is online at www.gopubmed.org [11, 12].

The aim of this study was to analyze semantically medical abstracts from the Republic of Macedonia indexed in the PubMed database with GoPubMed.

Material and Methods

A semantic analysis of PubMed database was performed with GoPubMed on March 8, 2013 in order to identify published papers from the field of medical sciences affiliated with the country Macedonia. The term was Macedonia[geo] with description "the country Macedonia" and with synonyms: Repubblica di Macedonia, Macedonia, Mazedonien, Republic of Macedonia, Macedonia, Repubblica, República de Macedonia, República da Macedónia, Macedônia, República da, Macédoine [12].

Relative research interest was calculated as: weighted publications per year (subject specific)/total weighted publications per year (in PubMed), where weighted publications per year = number of publications per year multiplied by relevance factors (as defined by PubMed) [13].

Results

Macedonian medical scientists published papers in a total of 400 different journals which have been indexed in PubMed database. The largest number of published papers was in the domestic journal Prilozi (272 abstracts or 18.5% of total number) followed by Med Arh (200 abstracts), Ugeskr Laeger (61 abstracts), Med Pregl (42 abstracts), Bratisl Lek Listy (37 abstracts), Int J Mol Sci (33 abstracts), Lijec Vjesn (23 abstracts), Acta Chir Iugosl (22 abstracts), and Croat Med J (21 abstracts). Less than 20 papers from the Republic of Macedonia have been published in each of the rest of the journals indexed in PubMed (Table 1).

The semantic analysis of terms in the abstracts from the Republic of Macedonia revealed that the majority of the terms were humans (1,005), followed by patient (748), adults (406), and middle aged (370). The rest of the publications with more than 100 terms belong to Evaluation Studies as Topic, Diagnosis, Aged, Child, Adolescent, Unknown term
default # fulltext, Macedonia (Republic), Methods, Hospitals, Hospitalization, Risk Factors, Surgery, Women, Syndrome, Arteries, and Kidney (in descending order) (Table 2). On the top of twenty Macedonian authors who are included in PubMed are "Polenakovic M" (156 abstracts or 10.6% of the total number) and "Efremov G" (154 abstracts or 10.5% of the total number). The rest of the 18 authors are: "Tasic V", "Spasovski G", "Gucev Z" or "Guech V", "Popov Z", "Ivanovski N", "Petrushevskaya G" or "Petrushevskaya G", "Spiroski M" or "Spiroski M", "Sikole A", "Stafilov A", "Pop-Jordanova N", "Spiroski: Macedonian Medical Abstracts in PubMed".
The first indexation of an abstract from the Republic of Macedonia in PubMed, analysed with GoPubMed, was in the year 1987. There was a very slow increase of indexed abstracts/papers in the next eleven years (till 1998 year). A significant increase of abstracted papers was recorded during the period of 1989-2012 (from 50 abstracts to 180 abstracts, respectively) and a significant increase of relative research interest (from 0.00006% to 0.00018%, respectively). The relative increase for the period between 1989 and 2012 was 27.8% for the abstracts and 33.3% for the relative research interest (Fig. 2).

Geographic distribution of the Macedonian authors affiliated with different parts of/countries in the world, whose papers have been indexed in the PubMed database and semantically analyzed with GoPubMed, revealed that they are mostly aggregated in Europe, North America and Japan (Fig. 3).

Top author networks of the Macedonian scientists indexed in PubMed and semantically analysed with GoPubMed have shown several groups.

Table 3: Top twenty authors from the Republic of Macedonia according to abstracts indexed in PubMed semantically analysed with GoPubMed (1469 abstracts identified on March 18, 2013).

<table>
<thead>
<tr>
<th>Rank</th>
<th>Author</th>
<th>Number of PubMed Abstracts</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&quot;Polenakovic M&quot;</td>
<td>156 (10.6 %)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&quot;Efremov G&quot;</td>
<td>154 (10.5 %)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&quot;Tasic V&quot;</td>
<td>93 (6.3 %)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&quot;Spasovski G&quot;</td>
<td>79 (5.4 %)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>&quot;Gucev Z&quot; OR &quot;Guchev Z&quot;</td>
<td>63 (4.3 %)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>&quot;Popov Z&quot;</td>
<td>58 (3.9 %)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>&quot;Ivanovski N&quot;</td>
<td>53 (3.6 %)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>&quot;Petershevski G&quot; OR &quot;Petrusevska G&quot;</td>
<td>52 (3.5 %)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>&quot;Siprovski M&quot; OR &quot;Sipirovski M&quot;</td>
<td>51 (3.5 %)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>&quot;Sicilie A&quot;</td>
<td>51 (3.5 %)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>&quot;Stafilov T&quot;</td>
<td>40 (2.7 %)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>&quot;Pop-Jordanova N&quot;</td>
<td>38 (2.6 %)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>&quot;Cakalaroski K&quot;</td>
<td>36 (2.4 %)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>&quot;Dzikova S&quot;</td>
<td>32 (2.2 %)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>&quot;Grcevska L&quot;</td>
<td>30 (2.0 %)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>&quot;Kolevski P&quot;</td>
<td>26 (1.8 %)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>&quot;Kocarev L&quot;</td>
<td>26 (1.8 %)</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>&quot;Georgievska-Ismail L&quot;</td>
<td>25 (1.7 %)</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>&quot;Petlichkovski A&quot; OR &quot;Petlikovski A&quot;</td>
<td>23 (1.6 %)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>&quot;Petlikovski A&quot; OR &quot;Petlichkovski A&quot;</td>
<td>23 (1.6 %)</td>
<td></td>
</tr>
<tr>
<td>Total number of abstracts</td>
<td>1063 (72.4 %)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

We present the semantic analysis of Macedonian medical-related documents indexed in the PubMed database with the GoPubMed. Macedonian medical scientists have published papers in a total of 400 different journals which have been indexed in PubMed database. The largest number of published papers was in the domestic journal Prilozi and the majority of terms used in the abstracts were humans, followed by patient, adults, and middle aged.

Two Macedonian authors whose papers are indexed in PubMed account for the largest number of included abstracts. These are "Polenakovic M" (156 abstracts or 10.6 % of the total number) and "Efremov G" (154 abstracts or 10.5 % of the total number). Top twenty Macedonian authors published 72.4% of the total number of abstracts indexed in PubMed. A significant increase of abstracted papers during the period of 1989-2012 was recorded. The relative increase during this period (1989-2012) was 27.8% for the abstracts and 33.3% for the relative research interest. Top author networks of the Macedonian scientists indexed in PubMed and semantically analyzed with GoPubMed have shown that the largest group was composed of scientists working in the field of nephrology and related disciplines and several other networks composed of four authors each.

The low number of abstracts in PubMed from the Republic Macedonia prior to 1989 could be a result of: (a) semantically unrecognized country term (Republic of Macedonia) before and immediately after secession from the Social Federal Republic of Yugoslavia; (b) a low number of Internet connections both individually and institutionally; (c) low pressure of the educational and scientific community to publish in the journals indexed in PubMed (Publish or Perish); and (d) permanent jobs of biomedical scientists independent of their scientific impact. Similar results have been obtained by analyzing Macedonian medical scientific papers in the Scopus database, where the number slowly, but steadily has increased and reached its plateau in the years 2008 and 2009 [14].

In spite of the long tradition of medical publishing in the Republic of Macedonia (Macedonian Medical Review has been published regularly since 1946) and a relatively large number of journals [15], they have very limited scientific influence [16] mainly because they do not comply with the international publication standards. Macedonian biomedical scientists have published their papers in 160 journals indexed in Scopus database [14] and in 400 journals indexed in PubMed from which only one is a domestic journal (Macedonian Journal of Medical Sciences in Scopus and Prilozi in PubMed).

Masic I and Sivic S compared the ratio of using Medline in Bosnia and Herzegovina (B&H), with the basics for health education at biomedical faculties of five universities. The results showed that only 11.6% of professors use Facebook type of social network, 49.3% of them have a profile on BiomedExperts scientific social network and 79% have available articles in the largest biomedical database system for spreading medical information MEDLINE [17]. Our findings have shown that twenty biomedical scientists from the Republic of Macedonia have published 72.4% of the total number of abstracts indexed in PubMed from which one half are from the nephrology field and related disciplines. Prevalence of the scientists from the nephrology field can be seen more clearly from the top author networks of the Macedonian scientists indexed in PubMed, which is the largest group composed of scientists working in this field. This prevalence of scientists from the field of nephrology is also visible in the Scopus database where the biggest H-index of 10 for the period between 2007 and 2008 in the Republic of Macedonia is obtained for the nephrology subject category, followed by medicine (miscellaneous) with H-index of 7, hematology and endocrinology, diabetes and metabolism with H-index of 6, transplantation, oncology and pathology and forensic medicine with H-index of 5 [18].

Several explanations can be offered with reference to predominance of the nephrology subject category in the biomedical sciences of the Republic of Macedonia, such as: long-standing tradition of scientific publishing; a very large international scientific network of Macedonian nephrologists; a very large number of kidney diseases in the Republic of Macedonia; a very big percentage of finances allocated for kidney diseases used from the Health insurance funds for the nephrology and similar. However, we should not underestimate the monopoly of nephrologists in the Macedonian scientific medical journals: the Editor-in-Chief of the most prominent journal Prilozi is a nephrology expert, the Editor-in-Chief of the Macedonian Medical Review is a nephrology expert, and the Editor-in-Chief of Vox Medici was nephrologists also. It has to be mentioned that currently the Vice Dean for Science at the Faculty of Medicine is also a nephrology expert. I hope that this situation will be changed in the near future.

Introduction of more strict criteria for submission and/or defense of Master of Science
(MSc) and Doctor of Philosophy (PhD) degrees at the Faculty of Medicine in Skopje in the 2000’s, as well as more strict criteria for election of mentors for Doctor of Philosophy at the Ss Cyril and Methodius University of Skopje applied five years ago, have significantly increased the number of biomedical scientific papers published in foreign journals either indexed in Scopus [14] or in PubMed. The pressure of Publish or Perish on the Macedonian biomedical scientists should be continuous and increasing in the coming years.

In conclusion, I can say that a requirement for publication of at least one paper, which is to be indexed in PubMed prior to the Philosophy Doctor Degree defence at the Faculty of Medicine, Ss Cyril and Methodius University of Skopje, was a trigger for an increased number of publications indexed in PubMed in the last decade. A larger number of Macedonian medical journals should be indexed in PubMed in order to increase the impact of Macedonian medical scientists in the world.

References

A Novel Mutation in the GJB2 (Connexin 26) Gene in Egyptian Children with Non-syndromic Sensorineural Hearing Loss

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¹National Research Center - Research on Children with Special Needs Department, Giza, Egypt; ²National Research Center - Biomedical Technology, Giza, Egypt; ³Hearing and Speech Institute - Department of Audiology, Giza, Egypt

Abstract

This study aimed to investigate the frequency of any novel gene mutations, in human GJB2 gene among Egyptians with familial sensorineural non-syndromic hearing impairment.

PCR amplifying the entire coding region of GJB2 gene and direct DNA sequencing to analyze mutations in this gene among 78 cases with autosomal recessive congenital non syndromic hearing loss was used.

We describe for the first time a novel mutation in the coding region of the GJB2 gene. A deletion mutation of T at position 59, in the intracellular domain of connexin 26, resulting in a frameshift at the 20th amino acid leading to a premature termination of the protein was detected in 9% of the studied cases.

These data provide a novel molecular explanation for the role of GJB2 mutation in hearing loss to be taken into consideration in the genetic diagnosis and counseling of non-syndromic sensorineural hearing loss in Egyptians.

Introduction

Hearing loss is the most common sensory deficit in humans and most auditory system dysfunctions resulting in hearing loss are genetically inherited [1]. It affects approximately 10 per cent of the world population, which is significant enough to compromise the development of normal language skills and social development. It can appear at any age with varying degrees of severity [2].

Over 100 loci, both nuclear and mitochondrial, are already implicated in the development of this most common sensory disorder of humans [3].

GJB2 (Gap Junction protein β Type 2), encodes Connexin 26 (Cx26), a member of a highly conserved protein family found throughout the animal kingdom [4]. Six connexins oligomerize to form pore-like plasma membrane hemichannels called connexons, they form gap junction channels which facilitate electrical and biochemical coupling between cells. Ultrastructural studies have identified two gap junction networks, including that of the epithelial tissue and that of the connective tissue, in the mammalian cochlear duct and vestibular system [5]. The gap junction system is the most likely pathway for cochlear K+ recirculation following hair cell depolarization maintaining cochlear homeostasis [6].

The GJ channels have diverse functions, including electrical signal propagation, metabolic cooperation, growth control, spatial buffering of ions, and cellular differentiation [7]. Mutations in one gene,
GJB2 are responsible for more than half of all cases of recessive non-syndromic deafness [8].

The mutation spectra of this gene vary among different ethnic groups. The greatest problem for the genetic diagnosis of hearing impairment is the high genetic heterogeneity of this disorder. Because of the high frequency of GJB2 mutations, mutation analysis of this gene is widely available as a diagnostic test. Certain Cx26 gene mutations are ethnic-specific such as, 35delG mutation prevalent in Caucasians, R143W in Ghana, 167delT in Ashkenazi Jews, 235delC in Orientals and W24X in Indians [9]. In all these populations, Cx26 gene mutations were highly observed in heterozygous condition, which proves the heterogeneity of non syndromic hearing impairment.

The 35delG mutation, which accounts for the majority of GJB2 mediated hearing loss among Caucasians, is a deletion of a single guanine residue (G) in a stretch of 6 Gs at nucleotide position 30–35 of the coding region of the GJB2 gene. This single deletion shifts the reading frame, resulting in a premature chain termination product comprising only 12 amino acids. The 35delG mutation in GJB2 gene among Egyptians with recessive nonsyndromic congenital sensorineural hearing impairment was studied and revealed that 10.17% had deletion of G at position 35 [10].

Understanding the underlying causes of the variability in GJB2 gene is of major importance in terms of genetic counseling. The purpose of this study was to investigate the frequency of any novel gene mutations, other than 35delG, in human GJB2 gene among Egyptian children with familial sensorineural non-syndromic hearing impairment.

Patients and Methods

Studied Population

Seventy eight Egyptian children (38 males and 40 females) with prelingual non-syndromic hearing loss were enrolled in this study. Their ages ranged between 1 to 15 years. Parental consanguinity was present in all studied families (34 families). All patients were recruited from the genetic counseling service for deaf people at the Clinic of Children with Special Needs, National Research Center, and the outpatient clinic of the Hearing and Speech Institute, Cairo, Egypt.

The study was approved by the Ethics Committee of The National Research Center, Cairo, Egypt, and written informed consent was obtained from all parents or caregivers. All cases were subjected to the following:

1. Detailed history was taken for each patient that included: Parental consanguinity, family history of similar condition, pregnancy history, perinatal history, history of drug intake, recurrent ear infections, ear trauma, and history of any chronic illness. Onset, course and duration of the hearing loss were obtained.
2. Three generations pedigree was constructed for each case.
3. Careful clinical examination had been done to exclude syndromic deafness, and other associated anomalies.
4. Ear, nose and throat (ENT) examination had been carried out to check for any ear malformations, signs of trauma or scars of previous operations.
5. Full audiological assessment: Auditory brain stem response (ABR) for cases ≤ 4 years old and pure-tone audiometry (PTA) for cases > 4 years old to determine the degree of hearing for both ears.

Molecular Analysis

Genomic DNA Extraction: Five ml venous blood was obtained from each patient in a sterile tube containing EDTA as an anticoagulant for genomic DNA extraction. Extraction was carried out by using a ready-made QIAGEN kit (QIAaamp DNA Mini kit and, QIAGEN; Germany), Genomic DNA was diluted 1:100 in TE buffer and the A260 absorbance was read and the concentration was calculated.

DNA amplification using polymerase Chain Reaction (PCR): Amplification of the connexin 26 (Cx 26) coding region was achieved by using the following primers:

Forward Primer:  5' tct tt cca gag caa acc gc 3'
Reverse Primer:  3'act cgt gcc caa cgg agt ag 5'

PCR reaction was performed in a final volume of 50 µl, containing 200 ng genomic DNA, 10mM Tris- HCl (pH 9.0 at 25°C), 50mM KCl and 0.1% Triton® X-100, 1.5 mM MgCl₂, 200 µM dNTPs, 10 µM of each primer and 2.5 units of Taq polymerase (Promega USA). Then the thermal cycler was programmed according to the following steps to undergo the amplification reaction for Cx26 gene coding region. First; samples were denatured at 94 °C for 5 minutes. Subsequently; 40 cycles of denaturation were achieved at 94 °C for 15 seconds, annealing was carried out at 55 °C for 30 seconds and extension was done 72 °C for 1 minute, followed by 5 minutes of post extension.

DNA Purification: Amplified fragments on agarose gel were purified by using a ready-made extraction kit (Invisorb® Spin DNA, Invitek; Germany).

Sequencing: Direct DNA sequencing was carried out in both directions using the forward and reverse primers on the Automated Sequencer “ABI Prism 310 Genetic Analyzer”. The cycle–sequencing reaction was performed in a volume of 20 µl containing: 8 µl of the terminator ready reaction mixture 3.2 pmole of either the forward primer or the reverse primer and 30 ng of purified PCR product.
The thermal cycling protocol was followed on the “Perkin Elmer, Gene Amp PCR System 9700”; 95°C for 5 minutes followed by 25 cycles of (96°C for 10 seconds, 50°C for 5 seconds and 60°C for 4 minutes). Centri-Sep Columns (Princeton Separations, Philadelphia) were used for effective and reliable removal of excess Dye Deoxy™ terminators from completed DNA sequencing reactions. Data were compared and aligned with different sequences of different strains using the Clustal W software for multiple alignments http://www.genome.jp/tools/clustal.

Table 1: Different strains that showing alignment with the coding sequence of Cx26 gene in Egyptian patients.

|----------|-----------------------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|

Results

We studied 78 Egyptian patients with prelingual non-syndromic hearing loss. Parental consanguinity was present in all studied families (34 families). Forty eight cases (61.5%) had severe hearing loss, 16 cases (20.5%) showed moderate hearing loss and 14 cases (17.9%) had mild hearing loss.

After direct sequencing of all samples using the primers in both directions, forward and reverse primers, the obtained sequence was aligned with human Cx26 gene on NCBI database revealing a novel GJB2 deletion frameshift mutation (Figure 1) in 3 out of 34 families.

A novel mutation was detected, which was the deletion of T at position 59 resulting in a frameshift at the 20th amino acid, leading to a premature termination of the protein (Table 2). The mutation was segregated in the family according to a recessive mode of inheritance. No other homozygous loss-of-function mutation segregating with affected status was identified in other family members. This variant was not present in more than 150 ethnically-matched, healthy, in our in-house dataset. The sequence is shown in the reverse direction. All patients with the novel mutation showed severe hearing loss.

Table 2: A novel mutation in Cx26 gene in Egyptian patients with non-syndromic sensorineural hearing loss.

<table>
<thead>
<tr>
<th>Nucleotide Change</th>
<th>Effect</th>
<th>Type of Mutation</th>
<th>Protein Domain</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del of T at position 59</td>
<td>Stop Codon (Term.)</td>
<td>Framshift</td>
<td>IC1</td>
<td>3 out of 34 families (71/78 cases, 9%).</td>
</tr>
</tbody>
</table>

Discussion

Deafness is a worldwide prevalent disease that seriously impairs human quality of life. The deafness-associated gene mutation spectrum as well as dominant gene profile varies greatly among regions and races [11]. 35de1G, 167de1T, and 235de1C are reported to be the most prevalent mutant genes among Caucasian, Jewish, and Asian populations, respectively [12-14]. Contemporary studies of the causes of congenital and early-onset deafness have incorporated the tools of genetic epidemiology, including molecular testing, to identify a growing number of potential genetic and environmental causes.

In most cases, inherited HL is monogenic. In 70% of neonates who fail newborn hearing screens and are presumed to have inherited HL, there are no other distinguishing physical findings and the HL is classified as nonsyndromic. In the remaining 30%, the HL is accompanied by other physical findings and is said to be syndromic [15]. Of the more than 400 syndromes in which HL is a recognized feature, Usher syndrome, Pendred syndrome and Jervell and Lange-Nielsen syndromes are the most frequent [16]. Monogenic hearing loss can be inherited in different ways. Autosomal recessive HL (ARNHL) occurs in 80% of cases and is typically prelingual, while autosomal dominant HL (ADNSHL) accounts for about 20% of cases and is most often postlingual. In less than 1% of cases, the inheritance occurs through the X-chromosome or the mitochondria [17]. Monogenic hearing loss is an extremely heterogeneous trait, with

Figure 1: The sequence of a novel deletion frameshift mutation. An arrow indicates the mutation site (Deletion of T at bp 59).
over 100 mapped loci and 46 causally implicated genes (Hereditary Hearing Loss Homepage; http://webh01.ua.ac.be/hhh/).

The important role of intercellular communication via gap junctions has been confirmed by findings that certain CX gene mutations, particularly those of GJB2 and GJB6, cause hearing loss [18].

Gap junctions (GJs) are the only known intercellular channels linking the cytoplasm of adjacent cells. They facilitate intercellular exchanges of ion, metabolite and signaling molecules. GJs in all vertebrates are assembled from connexins (Cxs). Connexins are small transmembrane proteins that belong to an extensive protein family found in most metazoans. Six connexins can oligomerize to form a hemichannel (connexon) in the cell membrane [4]. The Cx gene family has twenty-one members in human genome [19]. New information on the physiological roles of vertebrate connexins has emerged from genetic studies. Mutations in connexin genes underlie a variety of human diseases, including deafness, demyelinating neuropathies, and lens cataracts [20].

To date, more than 154 GJB2 mutations have been identified in the coding exon of GJB2 (http://davinci.crg.es/deafness), but a single chain-termination mutation, 35delG, accounts for up to 70% of pathologic alleles in many populations. Although this mutation is common in Western Europe and the Middle East [21], much lower frequencies have been observed in Asia [22]. The 35delG mutation exhibits linkage disequilibrium, and haplotype analysis suggested that it arose from a single individual in the Middle East approximately 10,000 years ago [23].

The GJB2 mutation spectrum diverges substantially among populations, as reflected by specific ethnic biases for common mutations like 235delC in the Japanese (carrier rate of 1%–2%) [24], 167delT in the Ashkenazi Jewish population (carrier rate of 7.5%) [25], V37I in Taiwan (carrier rate of 11.6%) [26], R143W in Ghana and W24X in Indians [9]. Despite this variability, the combined frequency of all GJB2 mutations is sufficiently high in most populations to make mutation analysis of this gene a clinically useful, and therefore widely available, genetic test. Among the Caucasians, populations of Slovak Romany, Italy and Northeastern Hungary show very high rate of Cx26 mutations. On the contrary, relatively low frequency rate was observed in a few African populations; Kenya (2%) and Sudan (7%) while high frequencies in Tunisia (17%) [2].

In Egypt, Meguid et al. (2008) [10] reported that the frequency of 35delG mutation in GJB2 gene among cases with recessive nonsyndromic congenital sensorineural hearing impairment was 10.17%. All cases were homozygous for the mutation. Compared to other populations, 35delG mutation comprised a lower frequency among Egyptians which confirms the genetic heterogeneity and ethnic variation of nonsyndromic sensorineural hearing impairment. They stated that audiological evaluation of cases with 35delG mutation revealed that severe hearing loss was detected in 66.7% of cases, and moderate hearing loss in 33.3%. In the present study, we aimed to characterize the mutation profiles, of 78 patients with autosomal recessive nonsyndromic hearing loss in Egypt to find out any other novel mutations. The entire coding region of the GJB2 gene was directly sequenced in all patients.

We describe for the first time a new mutations in GJB2 gene, which also has not been previously described, is a deletion of T at position 59 (c.59delT), in the intracellular domain of connexin 26 among 9% of the studied cases, resulting in a frameshift at the 20th amino acid, leading to a premature termination of the protein that would severely disrupt the protein structure.

The structure and function of human connexins have been extensively studied at the biochemical and physiological levels. Studies of mutant forms of connexin have preceded their connection to human disease. Site-directed mutagenesis and domain replacement forms of human Cx26 have aided in the definition of important and essential elements of connexin primary structure [27]. Connexins contain four transmembrane domains (TM1-TM4), two extracellular domains (EC1-EC2), one cytoplasmic loop (CL), and N- and C-cytoplasmic termini (NT-CT). The N-terminal domain is involved in the process of membrane integration and hexamer formation [28] and, together with the first transmembrane domain, determines voltage gating. The extracellular loops regulate the connexon-connexon interactions, including heterotypic channel formation. Each loop contains three cysteine residues, conserved across all connexins and that form essential intramolecular disulfide bonds [29]. The intracellular loop and C-terminal domains regulate pH gating [30]. The TM domains are important for protein folding.

Many Hl-causing mutations of GJB2 gene have been reported in the connexin-deafness database (http://davinci.crg.es/deafness/). However, after reviewing the literature, our described novel mutation had not been previously identified for cases with autosomal recessive nonsyndromic hearing loss. One novel mutation at position 59 (c.59T>C) affecting the highly conserved residue within the N-terminal domain of GJB2, was previously described in an adult with apparently sporadic congenital HL in Austria [31]. These findings may indicate that this site is a mutational hot spot.

Summing up, in addition to the 35delG mutation which was previously reported to occur in almost 10.17% of Egyptian individuals suffering from HL [10], this study emphasises the importance of GJB2 gene mutations in inherited cases of nonsyndromic sensorineural hearing loss in Egypt. A
novel mutation was identified. The characterization of novel mutant alleles may contribute to a better understanding of the function of the connexin 26 domains. Moreover, these data show the importance of further investigations and future genetic counseling for GJB2 gene mutations.

References

Calf Muscle (Triceps Surae Complex) Thickness in an Adult Nigerian Population: An Imaging Based Normographic Study

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Abstract

Objective: The thickness of the triceps surae muscle complex was assessed by ultrasound in a young adult Nigerian population. Its relationship with some anthropometric variables and sexual dimorphism was also assessed.

Subjects and Methods: Sixty young adults (30 males and 30 females) between the ages of 19 and 30 years were recruited for the study. Anthropometric protocols for height, weight, foot length (FL), lower leg length (LLL) and mid calf circumference (MCC) were observed.

Results: Results show that the mean thickness of the calf muscle complex (CMT) was 81.65 ± 8.92 mm for all the subjects; 83.70 ± 8.73 mm for males; 79.95 ± 8.56 mm for females. The mean mid calf circumference (MCC) was 35.26 ± 2.61 cm for all subjects; 36.41 ± 3.56 cm for males; 34.10 ± 3.33 cm for females. Pearson's correlation coefficient showed that CMT correlated positively with height, weight, BMI and BSA in the sample subjects. However, such correlations were not observed among the male subjects. MCC also correlated with some anthropometric parameters (height, weight, BMI, FL, lower leg length (LLL) and mid calf circumference (MCC) were observed.

Conclusion: The findings from this study will form the bases for further research on relationships of the calf muscle complex. It also indicates that males have thicker Calf muscles and CMT has a positive relationship with height, weight, BMI and BSA especially among the female subjects.

Introduction

The skeletal muscle architecture is the primary determinant of muscle function [1, 2]. The knowledge of muscle architecture is of great practical importance in understanding the relationship between muscle structure, force and extension ability [3]. Muscle architecture has been mainly characterized by Fascicle length, Pennation angle and the thickness of the muscle [4-6].

Muscle thickness can be described as the distance between aponeurosis [5]. This muscle parameter is very important clinically in the assessment of atrophy and hypertrophy in neuromuscular diseases [7]; in the estimation of general muscle volume [8]; in the quantification of amyotrophic lateral sclerosis [9]; categorizing the intensity of relationship with activity levels in Cerebral Palsy [10] and in the monitoring of lean tissue mass.

Most assessments of muscles are done with the use of ultrasound since real-time sonography enables in vivo muscle scanning and offers an assurance for a realistic determination of changes in muscle architecture [6, 11]. In 1980, it was first discovered that diseased muscle showed a different ultrasound appearance compared to healthy muscles.

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