Research Article

Detection of Neurofibromatosis 2(NF2) Associated Vestibular Schwannoma-A Genetic Counseling Approach

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Vestibular Schwannoma (VS) also called as Acoustic Neuromas the benign tumors, resulting from the mutations that occur in the NF2 gene on chromosome 22. Mutation in the NF2 gene accounts for inherited autosomal dominant disorders that predispose into non-malignant central nervous system (CNS) disorders like vestibular schwannomas (VS) and meningiomas. VS usually grow slowly with tinnitus, hearing loss and balance dysfunction like symptoms. Management of such a condition becomes crucial as the development of tumors remains a significant reason behind morbidity and mortality of the patient. Awareness and early detection pertaining to the symptoms can restrict the disease manifestations to an extent and this could be achieved by performing a genetic test. In this study, the researchers report a classic case of recurrent headache and strong family history of VS. Germline mutation test by Next Generation Sequencing (NGS) detected a pathogenic (p.Gln538Ter) nonsense mutation in NF2 gene. Post-test counseling was offered and the family members were referred for prediction testing to understand their likelihood of developing the tumors.

Key Words: *Autosomal Dominant; Genetic Testing; Hereditary Cancer; Multigene Panel; Next Generation Sequencing; Pedigree Analysis*

INTRODUCTION

Acoustic neuromas now termed as Vestibular Schwannomas (VS) are benign tumors that are made out of Schwann cells of the vestibulocochlear nerve (VIII cranial nerve), that arises either from the internal auditory canal (IAC) or the cerebellopontine angle (CPA) leading from an individual's inner ear to the brain [1]. They are classified into sporadic VS and those associated with the neurofibromatosis type 2 (NF2) [2]. It is an uncommon type of tumor that usually affects middle-aged people, contributing to nearly 90 percent of all intracranial schwannomas [3]. The tumor arises due to the overproduction of Schwann cells present around the peripheral nerves that directly influence a person's hearing, with sensory nerves being frequently affected than motor nerves. Affected individuals might also develop schwannomas of other cranial and peripheral nerves, meningiomas, ependymomas, and cataract [4]. It can be either unilateral which is the most common type among the age group from 30-60 years and affects only one ear, or, bilateral which is an inherited form that affects both the ears [5]. Bilateral lesions result in disturbances in hearing and deafness that later compress the brainstem, thereby inducing abnormality and ataxia [6].

VS is a classic feature of the Neurofibromatosis type 2 (NF2); a rare genetic disorder characterized by the non-cancerous tumors of the nerves. NF2 has an estimated incidence of 1 in 33,000 people worldwide [7]. It is inherited in an autosomal dominant pattern wherein every child of an affected parent may have a 50 percent possibilty of inheriting the condition. It may be underrecognized in the children with initial manifestations like skin tumors and ocular findings because NF2 is categorized as an adult-onset disease [8, 9]. Continuous exposure to loud noise as well as radiations that affect the neck and face invariably leads to VS over a period of time. It is reported that patients with NF2 are prone to acquiring VS with most of such cases are sporadic (90%) with nearly 5–10 percent attributed to NF2 [10]. The combined lifetime risk for developing a unilateral tumor is 1:1,000 [11]. Younger population between 18 to 24 years are frequently prone to the risk for NF2 with higher mortality rates due to poor prognosis [12]. The NIH diagnostic criteria for NF2 includes bilateral VS with a family history of NF along with either unilateral VS associated with meningioma, glioma, schwannoma, neurofibroma, subcapsular lenticular opacities. This criterion has undergone several modifications, and other diagnostic criteria such as National Neurofibromatosis Foundation Criteria and Manchester criteria were recommended. The Manchester clinical diagnostic criteria for NF2 includes bilateral VS; first degree relatives with NF2 and unilateral VS or any two of meninigioma, glioma, neurofibroma, posterior subcapsular lenticular opacities; meningiomas and unilateral VS or any two of shwannoma, glioma, neurofibroma and cataract [4]. The most characteristic features of NF2 include- bilateral vestibular schwannoma of the superior vestibular branches of the cranial nerve (VIII); first degree relative with NF2 and unilateral VS with any two of Schwannomas, Meningiomas and Ependymomas and Multiple meningiomas with unilateral VS [13]. The clinical presentation and location of the tumor vary based on the expression levels of NF2; yet a large proportion of patients still present with the involvement of the eighth cranial nerve [14]. However, early diagnosis and efficient treatment strategies have improved survival rates [15]. Hearing and imaging tests help in the diagnosis of the NF2. Since the treatment of Vestibular Schwannoma is primarily surgery or stereotactic radiosurgery, the use of radiation therapy should be carefully considered as they are more susceptible to radiation induced tumors[16]. Though malignant transformations of NF2 tumours are quite rare, better treatment options are required, as numerous tumors might lead to early morbidity and mortality [4]. But if the hearing function is intact, it can still be managed. Although surgery is ideal, complications such as hearing loss, facial nerve palsy and ataxia are commonly seen [6]. Since NF2 patients suffer typically from multiple CNS tumors, the long term outcome is usually poor. A Japanese retrospective study on NF2 in the their population from the year 2009 to 2013, has shown the risk factors for the disability to be young age of onset, family history of NF2 and interventional therapy [17]. Plotkin et al [18] has shown that targeting vascular endothelial growth factor (VEGF), an angiogenic factor involved in physiological and pathological conditions by a humanized monoclonal antibody bevacizumab can help in reducing the volume of VS. A recent study of NF2 associated VS in Japanese patients has investigated the therapeutic effects of bevacizumab therapy where the patients achieved a radiologic response with a reduction of 20 percent or more from tumor baseline volume in 41 percent of patients [19].

Diagnosis of NF2 is on the basis of clinical indications, which usually is detected by physical assessment, and audiogram (test to measure the hearing ability). Secondly, the diagnosis can be made by the identification of a heterozygous pathogenic variant in the NF2 gene through molecular genetic testing. The mainstream of genetic counseling infiltrates many areas of healthcare, rendering strong support to the uncertainties faced by families towards adaptation to risk aversion and timely diagnosis. As a part of the healthcare team,

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genetic counselors help to identify families that are at risk of such syndromes by collecting medical and family histories, understand the pattern of inheritance, and provide the information about the genetic test and the implication of their results. They also discuss the available options for the risk identified, offer referrals for education support groups, and other healthcare professionals. Vestibular Schwannoma is considered as a major health issue across many parts of the world. In such scenarios, a genetic counselor can serve as a pivotal source of information for other health care professionals and patient families to identify the patients and at-risk relatives; and discuss the options available for them. They also help the families in determining the psychosocial tools which are needed to cope with the outcomes of the testing. Hence, there is a need for genetic counseling and testing while diagnosing and addressing VS.

OBJECTIVES

1. To provide genetic counseling to the proband and recommend genetic testing for germline mutations.
2. Based on the proband's test results, a recommendation of genetic testing for the family members to analyze the presence of a hereditary syndrome.
3. To explain the preventive and surveillance measures to the family.

METHODOLOGY

In this study, the researchers have discussed a case of VS, which was based genetic counseling and germline testing by Next Generation Sequencing (NGS). Based on the proband's genetic test result, mutation confirmation in relatives was confirmed by Sanger sequencing.

Germline Mutation Analysis by NGS

The Institutional Ethics Committee of Health Care Global Enterprises, Bangalore (EC Registration No: ECR/386/Inst/KA/2013/RR-19) has approved genetic testing for this study. Pre-test counseling was offered, and written consent was obtained for performing the germline mutation testing based on Illumina Trusight Cancer sequencing panel by NGS. The panel included 94 genes that are associated with inherited cancers and also few rare cancer types. The genes evaluated were NF1, NF2 and SMARCB1 along with 25 American College of Medical Genetics and Genomics (ACMG) recommended genes (APC, BMPR1A, BRCA1, BRCA2, MEN1, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, RB1, RET, SDHAF2, SDHB, SDHC, SDHD, SMAD4, STK11, TP53, TSC1, TSC2, VHL, WT1) to identify secondary findings.

Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Germany) from the proband's blood sample. The DNA was quantified using Qubit Fluorometer, and 50ng was subjected to the Nextera DNA library preparation kit (Illumina, USA) for library preparation. After transposon shearing of the genomic DNA, the tagmented DNA was converted into adapter-tagged indexed libraries on which limited PCR cycles were performed to add specific index sequences or barcodes to enable multiplexing of samples. The individual libraries (500ng from each) were pooled and hybridized to biotin labelled probes and were enriched by streptavidin beads which binds to the probes. Enriched library was then subjected to the second hybridization, and after amplification, the pooled library was quantified, and about 6 to 10 pM was loaded onto the Illumina MiSeq platform using the standard V2 kit, as per the manufacturer's guidelines. The variants were detected by Strand NGS v2.1.6 variant detection algorithm. The results were interpreted using the StrandOmics v3.0, proprietary analysis software of Strand Life Sciences. The identification and labelling of the variants with a read quality >Q30 and confidence score >50 was made based on the standards recommended by ACMG [20]. The variants identified were classified and categorized according to the ACMG guidelines as a) benign, b) likely benign, c) variant of unknown significance d) likely pathogenic and e) pathogenic.

After receiving test reports, post-test counselling sessions were scheduled for the patient and the family members. The results were discussed with the patient, and after obtaining the consent to share the results, the family members were counselled to address the further issues based on the test results.

Mutation Specific Testing (MST) by Sanger Sequencing

MST was offered for all at-risk relatives to confirm the presence of the variant detected in the proband. Specific flanking primers for the variant detected in the proband were designed. The blood sample was processed for Genomic DNA extraction and then quantified. PCR using the big dye terminator sequencing kit (Lifetech Inc., USA) was used to amplify the genomic region encompassing the variant of interest. Further, the Gene Jet PCR purification kit (Thermo Fischer) was used to purify the PCR products and specific forward and reverse primers by Big Dye Terminator v3.1 (Life Technologies) kit was used for sequencing. The samples were further subjected to capillary electrophoresis on the genetic analyzer (3500 DX, Lifetech Inc., USA). The sequence electropherogram was visualized using the Finch TV software (Geospiza, USA) and the sample sequence was compared with the reference sequence to evaluate the presence of the variant c.notation (p.notation).

Case Report

A 25-year-old female presented with complaints of recurrent headaches, giddiness for almost a year. She had difficulty in walking and was swaying towards either side at the time of presentation. It was observed that she had a strong history of VS in her maternal side of the family which included the proband's mother, maternal aunt and maternal uncle. MRI brain was recommended for the patient, which revealed bilateral lesions in cerebellopontine (CP) angles extending into internal auditory canal suggestive of bilateral VS. Though the proband had no complaints of hearing loss, an audiological evaluation was recommended to rule out the possibility of loss of hearing, and was within normal limits. A DOTA PET CT scan (Fig.1 and Fig.2) revealed metabolically active lesions in bilateral CP angles with compression on brainstem (Fig. 1). Sagittal image of the spine also showed multiple schwannomas in the spinal cord as well as in the cauda equine (Fig. 2a and b). The patient was treated with cyberknife guided hypofractionated stereotactic radiosurgery to both CP angle lesions and also spine lesions (Fig. 2c). Considering the bilateral nature of the disease, 25Gy/5# was administered. A follow up MRI revealed stable lesions in bilateral CP angle and spine. It was observed that the patient improved symptomatically after treatment.

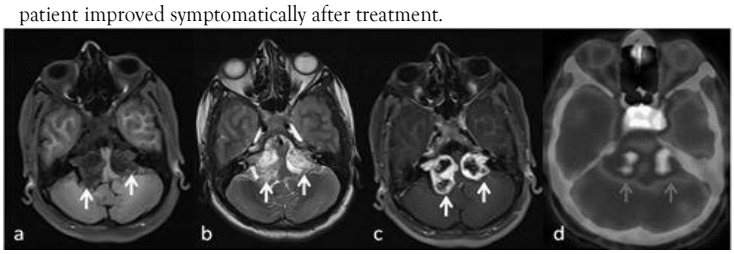

Figure 1) a) Axial T1W, b) axial T2W and c) axial post contrast T1W images of the brain at the level of internal auditory meatus shows T1 hypointense and T2 hyperintense heterogeneously enhancing lesions in the bilateral CP angle cisterns, extending to internal auditory canals (white arrows). 68Gallium Dotanoc PETCT image (d) shows metabolically active lesions in the posterior fossa, suggestive of bilateral acoustic schwannomas (red arrows).

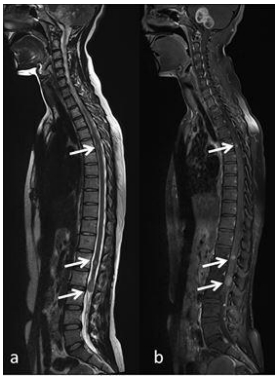

Figure 2a, 2b) Sagittal T2 W and post contrast T1 W images of the spine (a and b). There are multiple T2 hyperintense intensely enhancing intramedullary lesions in the spinal cord as well as in the cauda equina (arrows).

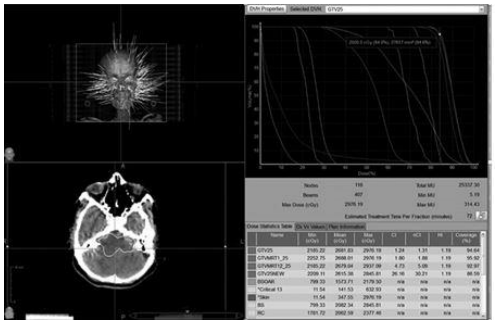

Figure 2c) CK Planning Image.

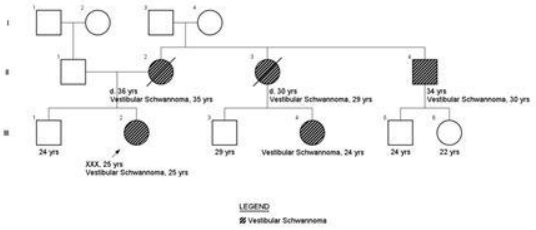

Figure 3) Pre-test pedigree analysis.

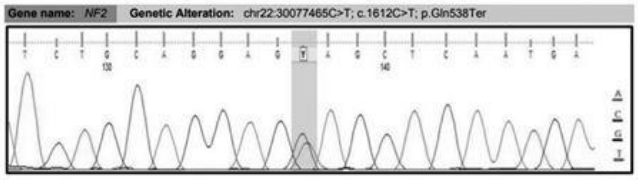

Figure 4) Sanger sequencing data (electropherogram) from the individual showing heterozygous nucleotide change 'C>T' at the position c.1612 in the NF2 gene.

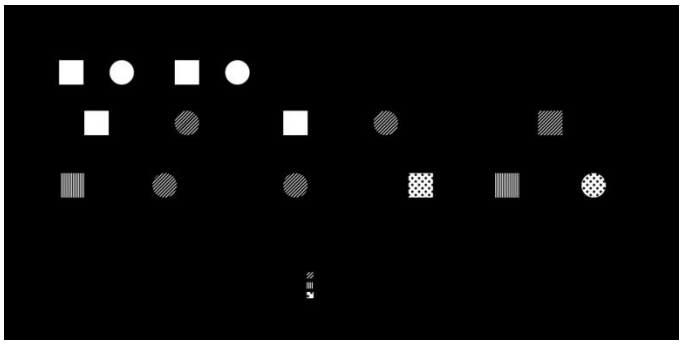

Figure 5) Post-test pedigree analysis.

Family History

The proband (Fig. 3, III-2) was referred for genetic counseling by their treating physician with a personal medical history of VS at 25 years accompanied by a cousin (Fig. 3 III-4). In the pre-test counseling session, the pedigree chart using scientific symbols was drawn to understand the family history [21]. The pedigree analysis revealed that the proband's mother, deceased at 36 years, (Fig. 3, II-2) was diagnosed with VS at 35 years. The maternal aunt, who died at 30 years (Fig. 3, II-3) and their 34 year old maternal uncle (Fig.3, II-4) were also diagnosed with VS at 29 and 30 years respectively. The proband's first cousin (Fig. 3, III-4) had symptoms of VS at the age of 24 years and was confirmed to have the same condition.

The segregation of family members in the maternal side with an early onset of VS supported a hereditary cancer syndrome evaluation according to Manchester diagnostic criteria for NF2 [13]. To evaluate NF2 associated VS, a hereditary multigene panel test was recommended for the patient. As both of her maternal aunt and uncle (Fig. 3 II-3, 4) had passed away with the condition, proband's maternal cousins (Fig. 3 III-1, 3, 4, 5, 6) were recommended to consult a genetic counselor.

RESULTS AND DISCUSSION

In this report, the researchers have discussed the importance of genetic testing for the patient suspected to have a hereditary syndrome. Based on the proband's test result, early detection in the unaffected family members is the primary purpose of screening; leading to educating the at-risk individuals based on their risk of having the genetic mutation and available surveillance options.

Mutations in the NF2 gene are associated with hearing impairments in patients with Vestibular Schwannoma. The NF2 gene gives instructions for the production of merlin, a protein (schwannomin), which belongs to a protein related family called ezrin-radixin-moesin-(ERM). Merlin helps in regulating cell to cell adhesion, controlling the cell shape, cell growth and its function as a tumor suppressor [22]. Nearly 88 percent of mutations in the NF2 gene are reported to be familial while 59 percent are sporadic among the patients. This is indicative of the functional loss of the NF2 protein that leads to tumor development [23].

Here, the researchers report a case of VS confirmed based on the genetic testing recommended through genetic counseling. The family history taken during the session revealed that the proband's mother, deceased at 36 years, (Fig. 3 II-2) was diagnosed with VS at 35 years. Her maternal aunt, who died at 30 years (Fig. 3 II-3) and her 34 year old maternal uncle (Fig.3 II-4) were also diagnosed with VS at 29 years and 30 years, respectively. The proband's first cousin (Fig. 3 III-4) had symptoms of VS at the age of 24 years and was confirmed to have the same condition. Based on the presentation of the proband and the history of VS in family members, germline mutations analysis by NGS was recommended. The genetic evaluation showed that the proband was carrying a heterozygous 'pathogenic' (disease-causing) variant c.1612C>T (p.Gln538Ter) detected in exon 15 of the NF2 gene which is associated with autosomal dominant NF2. The most common mutations reported in the NF2 gene is C>T transitions [24], with nonsense mutations being common among NF2 germline cases than frameshift mutations. The predicted cause for premature termination of the protein is due to the identified heterozygous nonsense substitution p.Gln538Ter of NF2 gene. The length of the truncated protein is predicted to be 537 amino acids instead of its original length of 595 amino acids. The resultant protein is likely to partially lack the C-terminal domain of the protein that results in loss-of-function [25]. Moreover, due to the introduction of a premature stop codon, this aberrant transcript will likely be targeted by the nonsense-mediated mRNA decay (NMD) mechanism [26]. PIK3-kinase/Akt, Raf/MEK/ERK and mTOR are the major signalling pathways that are affected by mutations in NF2 protein (Evans 2009b). Selvanathan et al. studied the impact that age of onset has on several NF2-related symptoms being identified, and patients with frameshift or non sense mutation have shown a younger age of onset [27]. It was hypothesized that the onset of hearing loss (tinnitus) at a young age could be explained by the onset of VS at young age. The evolution of loss of hearing cannot be predicted by any reliable methods in patients with VS. Like Methylation of DNA, deregulation of genes, and mutation of the NF2 genes have been associated with loss of hearing in VS patients [1].

Each first-degree relative (parents, siblings and children) of the proband has a 50 percent possibility of inheriting the same variant. Hence, based on this result, the family members (proband's sibling and maternal cousins) were tested by the Sanger Sequencing method for the known variant in the NF2 gene (Fig. 4). A total of five family members (Fig. 5 III-1, III3, III-4, III-5 III-6) were tested from the maternal side for the known NF2 pathogenic variant. In this analysis, three family members (Fig. 5 III-3, III4, III-6) tested positive for the c.1612C>T p.Gln538Ter variant detected in exon 15 of the NF2 gene, which is depicted in the pedigree (Fig. 5). Further, the asymptomatic family members who tested positive for the familial variant were kept under surveillance by their physician. New instances of hereditary NF2 can be avoided with careful genetic counseling. Guidelines for early detection and prevention were conveyed considering the psychological and social impact on the proband and the family members. This included an annual MRI to be started at a young age, around 10-12 years of age, and continued at least till the fourth decade of life, along with hearing evaluation- BAER testing and annual eye examination. The Proband and the family members were made aware of risk of recurrence.

A person with NF2 is educated about each of his or her offspring having a 50 percent chance of having NF2 gene. To determine if the baby will be born with the same condition a test can be performed on the fetus during pregnancy. Techniques like Amniocentesis and chorionic villus sampling allow small amounts of fetal DNA to be extracted for analysis. This sample can then be tested for the presence of the variant identified in the parent. A few families choose to utilize this information to prepare for the arrival of a child with a severe medical condition and to make an informed decision about the pregnancy.

CONCLUSION

NF2 is an autosomal dominant condition with about 50 percent possibility of transmission of a mutation from an affected individual to their offspring. People with this condition develop symptoms by the age of 60 years. Management of NF2 associated VS requires a multidisciplinary approach of early diagnosis and exploring new targeted therapies to revolutionize the outcomes of the condition. Detection of pathogenic variants at an beginning phase is effective in improving the clinical administration of the disease. Consequently, pre-symptomatic hereditary testing should be an integral part of the management of NF2 families. Though early intervention and diagnosis are improving the survival rate of those affected, many patients with NF2 still die at young age. Genetic clinics provide a model of collaborative care which exemplifies the integration of genetics into primary care. Integrative teams of Oncologists and Genetic Counselors play a significant role in the identification of hereditary genetic diseases as well as providing the right genetic test to help them understand the complexity of the disease. Here, the researchers have reported a family of VS confirmed by genetic testing, recommended through counseling. The proband's mother, maternal aunt and maternal uncle were diagnosed with VS at the age of 35, 29 and 30 years respectively. Her mother had succumbed to the disease a year later. One of the first cousins was also diagnosed at the age of 24 years. Genetic testing revealed the presence of a pathogenic nonsense substitution p.Gln538Ter of NF2 gene which is associated with VS. Screening of five family members to check the presence of the same variant confirmed that three of them were carriers and were classified as 'at-risk' individuals. Given the numerous problems affecting many patients, management of Neurofibromatosis Type 2 patients by a multidisciplinary team in specialized centres is highly recommended. Since NF2 remains a life-limiting condition, early diagnosis and management is important to achieve the best outcomes of the facial nerve, hearing preservation, and postoperative complications. Observation, microsurgical resection, stereotactic radiosurgery, medical therapy, and/or a combination of these which should maximize tumor control and minimize functional deficit.

RECOMMENDATIONS

This work emphasizes on the importance of identification of affected individuals through genetic counseling and testing for 'at risk' family members for hereditary diseases. Counseling provides information about hereditary predisposition risk, informed screening, and helps the families to decide on treatment planning and other management options. Among the multitude of tests available in the market, a genetic test provides more information about the inheritance pattern and helps to develop the best practice of treating or mitigating the disease.

ABBREVIATIONS

- AS: Acoustic Schwannoma
- ACMG: American College of Medical Genetics and Genomics
- BAER: Brainstem Auditory Evoked Response
- CNS: Central nervous system
- CP: Cerebello Pontine
- Gy: Gray
- MRI: Magnetic Resonance Imaging
- NF2: Neurofibromatosis 2
- NGS: Next generation sequencing
- NMD: Nonsense Mediated mRNA Decay
- PCR: Polymerase Chain Reaction
- VS: Vestibular Schwannomas

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