Genomic study of a large family with complex neurological phenotype including hearing loss, imbalance and action tremor

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A R T I C L E   I N F O

Article history:
Received 16 July 2021
Revised 15 October 2021
Accepted 17 December 2021
Available online 25 December 2021

Keywords:
Tremor
Hearing loss
Imbalance
MCM9
COCH

A B S T R A C T

Neurological disorders are often associated with a variety of symptoms, which can result from the combined action of genetic variants. We conducted a whole-genome analysis of a previously unreported unique multigenerational Dutch-Canadian family with a complex phenotype presenting with a combination of hearing loss, balance issues or action tremor. Ten family members were available for genetic study. The hearing loss and balance problems are explained by a pathogenic p.P51S substitution in COCH, which is a known founder mutation in Dutch and Belgian families affected by non-syndromic progressive sensorineural hearing loss often accompanied by vestibular dysfunction. Notably, p.P51S did not co-segregate with action tremor in our and reported kindreds. In our family, all 5 patients with tremor were carriers of the extremely rare p.R247W substitution in MCM9 (minor allele frequency in European population is 0.00003), which belongs to the top 0.1% of deleterious variants in the human genome. The MCM9 locus has not been previously associated with action tremor and deserves further investigation in future functional and genetic studies of action tremor.

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1. Introduction

Neurological disorders are often associated with a broad variety and variability of symptoms, which can result from the combined action of several variants in the human genome. Technological advances have provided an opportunity to examine the entire genome as a strategy for identifying rare risk alleles (Kuwano and Hara, 2013), which is especially valuable for conditions with multiple causal or risk genes. For instance, non-syndromic hereditary hearing loss is characterized by extreme heterogeneity with about 6000 causative variants identified in up to 110 genes (Shearer, et al., 1993). Vestibular disorders are also a clinically divergent group of balance problems that could arise from pathology of the inner ear, vestibulocochlear nerve, or central vestibular pathways; however, the genetics of vestibular disorders is still in its infancy (Mei, et al., 2021).

Another example is essential tremor - a common progressive movement disorder characterized by a bilateral upper limb ac-
action tremor (Bhatia et al., 2018), which can involve the head, legs, trunk, and voice (Louis, 2013), and shares features with some neurodegenerative diseases (e.g., Parkinson’s disease). Worldwide prevalence of essential tremor is 0.4%, which increases markedly with age, reaching 5% in individuals over the age of 65 (Louis and Ferreira, 2010). However, the prevalence of familial essential tremor in specific ethnic groups is not well-known. The high heritability of essential tremor is evident by its significant concordance in monozygotic (69%–93%) vs. dizygotic (27%–29%) twins (Lorenz et al., 2004). In general, the common disease (e.g., essential tremor) could be explained by either the combined action of low-risk common variants or rare pathogenic variant(s) with large risk-effect that is more expected in a familial form of the disease. About 50 genes or loci have been reported to be associated with essential tremor; however, their poor replication point to the high genetic and clinical heterogeneity of the disease (Siokas et al., 2020).

Tremors are classified along two main axes: etiology and clinical features (Bhatia et al., 2018). The etiology of tremors is divided into acquired, genetically defined and idiopathic causes. The clinical features are divided into four categories: (1) historical features (e.g., age at onset), (2) tremor characteristics (e.g., body distribution), (3) associated signs (e.g., additional neurological signs), and (4) additional laboratory tests (e.g., structural imaging). Activation of tremor is divided into rest and action tremors, the latter being further subdivided into kinetic, postural and isometric tremors. Examples of different tremor syndromes include essential tremor, task-specific tremors, dystonic tremors and parkinsonism-associated tremors (Bhatia et al., 2018).

Hence, the significant heterogeneity of neurological symptoms underscores the importance of using next-generation sequencing for genetic diagnosis. Here, we conducted a comprehensive whole-genome analysis of a large family affected by a complex phenotype, including symptoms of hearing loss, imbalance and action tremor.

2. Methods

2.1. Participants

The study was focused on a Canadian family with four consecutive generations affected by a complex neurological phenotype (Fig. 1A). It was conducted in accordance with the University Health Network Research Ethics Board approved protocol (UHN-REB 08-0615-AE). Informed consent was obtained from all study participants. The proband (II:2) sought medical attention at the Movement Disorders Research Center (Toronto Western Hospital), which initiated the collection of blood samples from ten family members (Fig. 1A). An affected subject (III:3) gathered detailed information from all family members, seven of which were directly examined by neurologists (JFB and/or AEL), including four patients who also had a detailed cochlear and vestibular assessment by an otorhinolaryngologist (JR).

2.2. Whole-genome analyses

Blood genomic DNA was isolated using a QIAGEN kit. All 10 study participants (Fig. 1A) were genotyped at the Clinical Genomics Centre (Toronto) on the Illumina NeuroX array that has the standard exome content of ~240,000 variants, as well as ~24,000 custom variants related to neurologic diseases (Nalls et al., 2015; Ghang et al., 2015). Genotype data were loaded to GenomeStudio (Illumina), which confirmed call frequencies of >0.96 for all samples, and GenTrain scores of >0.65 for all variants. Next, whole-genome sequencing (WGS) was conducted for the 6 most informative family members (Fig. 1A) at Genome Quebec (Montreal, Canada). In brief, 1 μg DNA was used for PCR-free library preparation using the NxSeq AmpFREE Low DNA Library Kit (Ludgen). WGS was performed using the NovaSeq 6000 (Illumina Inc.) PE150, with an average sequencing depth of >30. The raw fastq data was aligned to UCSC hg19 reference genome to generate SAM and BAM files, using the Burrows-Wheeler Aligner (http://bio-bwa.sourceforge.net) and SAMTOOL (http://samtools.sourceforge.net).

The Genome Analysis Toolkit pipeline (https://gatk.broadinstitute.org/hc/en-us) was used to call single nucleotide variants (SNVs) and insertion/deletions, which were analyzed using the ANNOVAR package (https://doc-openbio.readthedocs.io/projects/annovar/en/latest/). We selected rare exonic and splicing variants with a minor allele frequency <0.0001 in public datasets, including the Genome Aggregation Database v2.1.1 (non-Finnish European gnomAD_NFE and gnomAD_all), as well as the Exome Aggregation Consortium (EXAC_NFE and EXAC_all) (gnomad.broadinstitute.org) (Karczewski et al., 2020). The combined annotation dependent depletion (CADD) score was used to predict the deleterious effect of SNVs (Rentzsch et al., 2019). To genotype prioritised rare SNVs, DNA samples of 10 family members were amplified using primers targeting relevant regions of COCH, MCM9, RP11, CTUB or IFTGM (Supplemental Table S1), followed by Sanger sequencing.

Using the ExpansionHunter tool (https://github.com/Illumina/ExpansionHunter), we searched for repeat expansions in 24 genes known to cause neurological diseases (Supplemental Table S2). To find rare short tandem repeats, we used ExpansionHunter Denovo with outlier mode, and a Z-score > 10 (https://github.com/Illumina/ExpansionHunterDenovo), and compared against 150 profiles with short tandem repeats extracted from the Illumina Polaris database (https://github.com/Illumina/Polaris/wiki/HiSeqX-Diversity-Cohort). We also used cnvnamr (https://github.com/abyzovlab/CNVnamr) to call copy number variants (CNVs). We selected CNVs with a frequency < 0.001 in both Database of Genomic Variants and the gnomAD (> 500 tested samples). To analyze and filter out common CNVs, we used AnnotSV (https://lbgi.fr/AnnotSV/) that compiles relevant functional, regulatory and clinical information for structural variants (Geoffroy et al., 2018).

3. Results

3.1. Clinical findings

Table 1 contains a summary of all affected family members with their symptoms, including adult-onset tremor, hearing loss and a complaint of imbalance. A family pedigree is suggesting an autosomal-dominant mode of inheritance of either each symptom or their combination (Fig. 1A). The proband (II:2) and their parents were born in the same city in The Netherlands. The ethnicity of the proband’s parents and grandparents is Dutch and Flemish. The proband’s mother (I:1) suffering from a tremor starting in her 40s whereas the father (I:2) suffered from severe hearing loss starting in his 50s, eventually leading to deafness. This supports the possibility of two distinct genetic disorders (tremor and hearing loss) inherited in generations II-IV of the family. The proband was followed at Toronto Western Hospital, and complained of a head, neck and right arm tremor starting at age 40, followed by hearing loss at age 50 and the imbalance problem at age 70. On careful clinical assessment the proband was found to have very mild cervical dystonia (mild left torticollis, shoulder elevation and jerky head tremor) without associated complaints apart from the head tremor. No other family member had complaints suggestive of dystonia.

The proband passed away at age 80. She was the mother of ten children, two of whom (patients III:3 and III:4) have been regu-
Fig. 1. (A) Pedigree of the family with complex phenotype, including action tremor (in red), hearing loss (in green) and imbalance complaints (in blue). Sex is masked to protect confidentiality of the family members. The black arrow points to the proband and a diagonal line indicates deceased relatives. Age or age at death is shown above the symbol. Each generation is marked by roman numbers with DNA numbers below for the 10 participants of the genetic study. The red arrow indicates family members selected for whole genome sequencing (WGS). Genotypes for the 5 rare variants obtained by Sanger sequencing are listed beneath each tested individual (the minor allele is in red) in the same order as the five genes and identified variants listed on the left of the figure. (B) Sanger sequencing chromatograms of the COCH p.P51S and MCM9 p.R247W variants for all 10 study participants. (C) Clustal Omega alignment showing the conservation between MCM9 proteins from different species, including human. The box shows the position of the p.R247W mutation. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

Table 1
Clinical characteristics of the affected family members in the Dutch and/or Canadian kindred with complex phenotype.

| ID (DNA#) | Age | Symptoms (age at onset) | Additional information (age) |
|-----------|-----|-------------------------|------------------------------|
| I:1       | 65  | yes, deaf (50)          | yes (40)                     |
| I:2       | 80  | yes (50)                | no                           |
| II:1      | 94  | yes, profound (70)      | yes, head, neck & right hand (40) |
| II:2      | 80  | yes, profound (50)      | yes, head, neck & right hand (40) |
| II:3      | 89  | yes (70)                | yes, head & hands (50)       |
| II:4      | 89  | yes (50)                | yes (50)                     |
| II:5      | 93  | yes (50)                | yes (50)                     |
| III:1     | 70  | yes (40)                | yes, head & hands (50)       |
| III:2     | 68  | yes (50)                | yes (50)                     |
| III:3     | 67  | yes (53)                | yes (55)                     |
| III:4     | 67  | yes (53)                | yes (55)                     |
| III:5     | 66  | yes (52-55)             | yes (50)                     |
| III:6     | 63  | yes (49)                | yes (50)                     |
| III:7     | 63  | yes (49)                | yes (50)                     |
| III:8     | 62  | yes (50)                | yes (54)                     |
| III:9     | 61  | yes (57)                | yes (55)                     |
| III:10    | 62  | yes (50)                | yes (50)                     |
| IV:2      | 44  | yes (38)                | yes, acute vertigo (35)      |
| IV:9      | 46  | yes (34)                | yes (40)                     |
| IV:11     | 43  | yes, unilateral right   | yes, hand (36)               |
| IV:15     | 33  | yes (32)                | no                           |

Key: Nil, no information.

a current age or age at death.
larly followed at Toronto Western Hospital for similar complaints. Patient III:3 developed tremor and hearing loss at age 50 followed by balance issues in their late fifties (improved with physiotherapy). The head tremor had questionable dystonic features (fine jerky horizontal head tremor and minor left turn) but there was no posturing in the extremities, writer’s cramp, voice tremor or gait difficulties (with good arm swing and stride length). The tremor remained well-controlled (around 90% improvement) with long-acting propranolol at 120 mg daily. A cochlear implant was used at age 65.

Patient III:4 developed a head and arm tremor at age 45, hearing loss at age 53 (with need for a cochlear implant at age 65), and imbalance at age 55. On exam, the patient had a mild ‘no-no’ head tremor without dystonic features, a slight voice tremor, and a mild postural and kinetic action tremor moderately affecting her writing and spirals test. Total Essential tremor rating scale was 14 of 144 at age 62. There was no ataxia or parkinsonism. Sluggish neuropathic features were shown by unsteadiness on the Romberg’s test, and diminished vibration sense, however halluces position sense and deep tendon reflexes were preserved. The patient was not disturbed by the tremor in her daily activities; therefore, the propranolol was not prescribed.

In summary, the kindred includes 22 members affected by hearing loss, 11 of which also presented with imbalance complaints. Furthermore, nine family members developed an action tremor, co-occurring with both hearing loss and imbalance in seven subjects. Each symptom typically arose at 40-50 years of age (Table 1). Hearing loss was the first symptom in ten patients, while tremor preceded the other symptoms in five patients and the imbalance complaint was the first symptom in four patients. The tremor was an action tremor (postural and/or kinetic) involving the head/neck and/or the arms/hands, sometimes including the voice. The hearing loss was frequently profound enough to cause deafness or the need for a cochlear implant. The imbalance complaint was not associated with features of cerebellar ataxia or primary sensory dysfunction (its vestibular origin was confirmed in all four patients assessed with detailed vestibular testing).

3.2. Genetic findings

Blood samples from ten family members were available for genetic study. Their relatedness was verified using NeuroX data and PLINK v1.07 (Purcell, et al., 2007). None of the NeuroX rare coding variations co-segregated with disease in the family. Next, we conducted WGS of 6 family members (Fig. 1A), including an unaffected individual (III:6; age 64) and 5 affected subjects from two generations (III:1, III:3, III:4, III:8, IV:9), which were selected based on their similar phenotype comprising all three symptoms (hearing loss, complaint of imbalance and action tremor). We searched for rare variants shared by all five affected subjects of first/second-degree relatives but absent in the unaffected individual.

We did not detect any rare short tandem repeats, coding CNVs or insertions or deletions co-segregating with disease in the family. Among SNVs, we identified 5 rare heterozygous missense variants present in all five affected subjects but absent in the unaffected family member (Table 2). Four of these variants (except for RPL11 p.R433H) have CADD scores >20, representing the top 1% of deleterious variants in the human genome (Rentzsch, et al., 2019). Based on the Clinvar database, only the COCH p.P51S substitution (rs28938175) is a known pathogenic mutation causing Deafness, autosomal dominant 9 (OMIM: 601389), which is also characterized by vestibular dysfunction with incomplete penetrance (Fransen, et al., 2001). Sanger sequencing (Fig. 1B) revealed that the p.P51S mutation co-segregates with both hearing loss and balance issues in all eight affected family members but was absent in two unaffected subjects.

Notably, the p.P51S mutation did not co-segregate with action tremor in patients III:5, III:9 and IV:15. In contrast, the p.R247W in MCM9 (rs761922861) is of interest for the presence of tremor (Fig. 1B) because all five family members affected by tremor were p.R247W-carriers, while family members without tremor (III:2, III:5, III:6, III:9, IV:15) were free from this variant. Results of Sanger sequencing for the substitutions in CTNS (p.K220R), ITGAM (p.I87ITT) and RPL11 (p.R433H) did not suggest a link with disease phenotype, as each of these three variants was present in two individuals without tremor, including 1 individual who was over age 60 (Fig. 1A).

4. Discussion

We conducted a WGS evaluation of a unique multigenerational Dutch-Canadian family affected by a combination of neurological symptoms, including hearing loss, balance issues or action tremor. The hearing loss (progressing to profound impairment) together with the balance problems is explained by a single pathogenic p.P51S substitution in COCH, which is a founder mutation reported in Dutch and Belgium families with adult-onset autosomal dominant non-syndromic progressive sensorineural hearing loss, often accompanied by vestibular dysfunction (Fransen, et al., 2001). Similar to the p.P51S-carriers in our family, the onset of hearing loss in the reported p.P51S-carriers ranged from 30 to 60 years; while vestibular involvement varied from lack of symptoms to the presence of vertigo (JanssensdeVarebeke, et al., 2019).

COCH encodes the cochlin protein playing an important role in the inner ear. Post-mortem analyses of the inner ear tissue of p.P51S-carriers revealed abundant extracellular cochlin deposits in the cochlear and vestibular labyrinths (Robertson, et al., 2006) resembling the protein aggregation in neurodegenerative disorders (e.g., aggregates of α-synuclein in Parkinson’s Disease). The severe atrophy of fibrocytes in the areas with cochlin inclusions points toward the primary sites of pathological changes, however the mechanism leading to the misfolding and aggregation of cochlin is unknown (Robertson, et al., 2006). In brain, COCH expression is abundant only in the basal ganglia - a part of the brain essential to balance (https://www.proteinatlas.org/). In Parkinson’s disease, the basal ganglia is characterized by a loss of dopaminergic innervation leading to complex motor and non-motor symptoms, including difficulties with balance (Saeed, et al., 2020). Currently, there are no autopsy reports on the brain pathology of COCH mutation carriers, however it would be important to know if cochlin deposits are present in basal ganglia, which could contribute to the balance issues.

Notably, COCH p.P51S did not co-segregate with action tremor in our family, and phenotype studies of multiple p.P51S-carriers did not report an action tremor among their symptoms (JanssensdeVarebeke, et al., 2019). Hence, the tremor is likely the result of a separate genetic factor, which is supported by the presence of an isolated tremor in the proband’s mother (I:1). Our stringent WGS filtering approach resulted in a single candidate variant (MCM9 p.R247W) linked to the presence of tremor in the family. All five family members with tremor were carriers of the p.R247W, which affects a codon highly conserved in evolution (Fig. 1C) and is an extremely rare variant with a minor allele frequency of 0.00003 (observed in only one of 31,406 individuals in GnomAD). Remarkably, the p.R247W substitution has a very high CADD score of 34, representing the top 0.1% of deleterious variants in the human genome (Rentzsch, et al., 2019).

The functional consequences of MCM9 p.R247W in connection to tremor remains to be clarified in future cell-biology and disease-
model studies. Based on prediction by InterPro (https://www.ebi.ac.uk/interpro), the p.R247W variant is mapped to the OB-fold domain used for nucleic acid recognition (11–266 residues of MCM9). The OB-fold family of proteins are critical for DNA replication, recombination and repair (Theobald, et al., 2003). Indeed, MCM9 encodes the minichromosome maintenance 9 homologous recombination repair factor that is rapidly recruited to DNA damage sites (Park, et al., 2013), and the MCM9 complex has a helicase activity needed for DNA mismatch repair (Traver, et al., 2015). MCM9 is ubiquitously expressed in human tissues, including brain (https://www.proteinatlas.org). Of note, homozygous loss-of-function mutations in MCM9 are responsible for ovarian dysgenesis-4 (OMIM: 610098) (Wood-Trageser, et al., 2014). Also, three sporadic patients with premature ovarian insufficiency were reported to be the carriers of heterozygous p.L475F, p.L974S or p.A1130T substitutions in MCM9, however the disease relevance of these variations remains to be replicated (Guo, et al., 2020). Importantly, carriers of the p.R247W in our study have multiple children and hence are unlikely affected by ovarian insufficiency.

The MCM9 locus was not previously associated with action tremor (Sikas, et al., 2020) and our study limitation is the absence of a large dataset to assess the frequency of the rare p.R247W substitution in cases with tremor vs. controls. Of note, a recent exome sequencing study of eight Spanish families with essential tremor did not reveal any rare variants co-segregating with disease in more than two families (Diez-Fairen, et al., 2021), and it is possible that the p.R247W variation in MCM9 is family-specific as well. Indeed, there is accumulating evidence that the entity referred to as “essential tremor” may be a family of diseases (Louis, 2014, Zhang, et al., 2021).

5. Conclusion

The hearing loss and balance issues in the Dutch-Canadian family are explained by the pathogenic p.P515S mutation in COCH, while the action tremor could be linked to the p.R247W variant in MCM9, which deserves further investigation in functional and genetic studies of action tremor. Our report underscores the value of detailed family-history taking to identify patterns of inheritance and disease segregation. It also highlights the notion that a single person/family can be affected by more than one genetic condition. As we learn more about the genetic contribution to disease and the co-occurrence of genetic conditions in the same person or family, it might be argued that whole-genome assessment is a more efficient approach to clinical genetic testing, rather than testing ‘best fit’ single gene or panels of genes.

Disclosure Statement

None of the authors have conflict of interest, including financial interests in the results described.

Acknowledgements

The authors are grateful to the family members who made this study possible, as well as to Dr. Brett Trost and Dr. Stephen W. Scherer for their guidance in the analysis of structural variants. Full financial disclosure for each author for the past year are: JFB: consultancy fees and a medical congress paid by Abbvie (2 thirds of the registration fee); MZ: none; ED: none; CS: none; JR: none; AEL: has received consultancy fees from Abbvie, Acorda, AFFiRiS, Bio-gen, Denali, Janssen, Lilly, Lundbeck, Maplight, Paladin, Retrophin, Roche, Sunovion, Sun Pharma, Theravance, and Corticobasal Degeneration Solutions; ER: none. This study in part was funded by the Canadian Consortium on Neurodegeneration in Aging (E.R.), the Shanghai Pujiang Program 19PJ1410300, the National Natural Science Foundation of China (82071430), and the Fundamental Research Funds for the Central Universities (M.Z.).

Supplemental materials

Supplemental material associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2021.12.004.

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