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Genomic Analysis Reveals Frequent *TRAF7* Mutations in Intraneural Perineuriomas

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Abstract

Intraneural perineuriomas are benign peripheral nerve sheath tumors that cause progressive debilitating focal extremity weakness. The etiology of perineuriomas is largely unknown. We utilized whole exome sequencing, copy number algorithm evaluation, and high-resolution whole genome microarray to investigate for a genetic causal link to intraneural perineuriomas. Ten of 16 (60%) tumor cases had mutations in the WD40 domain of *TRAF7*, the same location for causal mutations of meningiomas. Two additional perineurioma cases had large chromosomal abnormalities in multiple chromosomes, including chromosome 22q. This study identifies a common cause for intraneural perineuriomas and an unexpected shared pathogenesis with intracranial meningiomas.

Intraneural perineurioma is a hypertrophic peripheral nerve tumor composed of whorls of perineurial cells surrounding axons that does not metastasize outside peripheral nerves.^{1,2} It occurs most commonly in young patients, and presents insidiously with a motor predominant neuropathy or plexopathy affecting individual nerves and/or plexuses.^{3,4} The perineurial cell origin is supported by immunoreactivity to epithelial membrane antigen (EMA). The pathogenic cause is unknown, and currently no therapeutic options exist. Trauma or reactive changes have been considered as causal factors,⁵ but the majority of reported cases have no association with a previous trauma, suggesting a genetic tumorigenic origin.^{3,6} A chromosome 22q11 deletion has been reported in an intraneural perineurioma

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Author Contributions

Study design: C.J.K., M.L.M. Data acquisition and analysis: all authors. Drafting the manuscript and figures: C.J.K., Y.W., G.M., M.L.M.

Potential Conflicts of Interest

Nothing to report.

patient, suggesting a potential clonal expansion neoplasm mechanism.⁶ Deletions of chromosome 22 also occur in other solid tumors, including benign or malignant schwannomas, neurofibromas, and benign meningiomas.^{7,8} In addition, non-neural soft tissue perineuriomas have shown deletions on chromosome 13 and 22q12 identified by fluorescence in situ hybridization.^{9,10} To date, comprehensive, high-resolution genetic studies have not been conducted. For the majority of intraneural perineuriomas, the etiology remains unknown.

We investigated the genetic causal link of intraneural perineuriomas utilizing whole exome sequencing and copy number variation (CNV) analysis. The genetic findings of this study are integrated with our earlier published clinical and histopathological evaluations of the same group of patients.³

Patients and Methods

Patients

The study was approved by our institutional review board. We identified 16 intraneural perineurioma cases with available frozen nerve tissue sample (slurry of isopentane over liquid nitrogen stored at -80°C) within the Mayo Peripheral Nerve Laboratory database from 1985 to December 2012. All identified cases had confirmatory pathologic review by immunostaining with epithelial membrane antibodies. The nerve tissue DNAs of 16 intraneural perineurioma cases were extracted from 25mg of frozen nerve tissue (Qiagen, Valencia, CA). We also obtained 4 blood and 3 saliva samples from 7 of these 16 patients, and extracted their genomic DNA as germline controls.

Immunohistochemistry

Immunohistochemistry on paraffin sections was performed using rabbit polyclonal TRAF7 antibody (Sigma, St Louis, MO) with the Polymer Refine Detection System (Leica Microsystems, Wetzlar, Germany).

Whole Exome Sequencing and Bioinformatics Analysis

Sixteen tumor DNA, 4 blood DNA, and 3 saliva DNA samples were prepared using an Agilent Technologies (Santa Clara, CA) SureSelect Whole Exome V5 capture kit and True-seq library kit (Illumina, San Diego, CA), then sequenced on Hi-Seq4000 (Illumina). Sequencing reads were aligned to a human reference (hg19) using in-house pipeline GenomeGPS as previously described.¹¹ Variant-detections were conducted on exons and flanking 50 base pairs of intronic sequence. Variant data for each case was summarized in variant call format (VCF). VCF files were analyzed using Ingenuity Variant Analysis software (Qiagen). Our recently developed CNV detection algorithm (PatternCNV)¹¹ was applied to the next generation sequencing data to assess CNV.

Whole Genome Comparative Genome Hybridization Microarray

To evaluate and validate the whole genome structural variations including deletions and duplications, we also conducted whole genome Comparative Genome Hybridization (CGH) microarray analysis (Agilent 2×400K SuperPrintG3) using DNA extracted from 16

perineurioma cases. To identify the tumor-specific changes, we used the available matching germline DNA from blood or saliva as controls for 7 cases; for the other 9 cases, we used a purchased CGH control DNA sample (Agilent). This control sample consisted of an equal molar mix of 6 normal control DNA samples, which helps reduce background noise arising from the variation of each individual.

Structural Model of TRAF7 WD40 Domain

The structure of TRAF7 WD40 domain was modeled by homology with the structure of WRD5 WD40 domain (Protein Data Bank code 2H14) using the software Modeller. PyMOL (Schrödinger, New York, NY) was used to generate the structure representations.

Results

Clinical Characteristics

Summary of clinical characteristics is in the Table. The median age at the time of our evaluation was 30 years (range = 2–57 years). The median length of presentation was 54 months (range = 6–360 months), all with slowly progressive motor weakness. Median Neuropathy Impairment Score was 10.25 points (range = 3.25–49 points).^{3,12} The nerves affected in order of frequency were sciatic (n = 6), tibial (n = 2), brachial plexus (n = 2), radial (n = 2), peroneal (n = 1), ulnar (n = 1), lumbosacral plexus (n = 1), and median (n = 1). Characteristic diagnostic intraneural perineurioma pathology was available in all patients (Fig 1A).

Exome Sequencing Identifies Somatic Novel Mutations Predicated to Alter Protein Function

Whole exome sequencing obtained an average coverage depth of $\times 306$ for all samples. Because intraneural perineurioma is a rare disorder, we applied stringent selection criteria for mutation filtering as follows: (1) variants absent from all 4 blood and 3 saliva DNA samples, (2) absent from our in-house ~ 250 exome sequencing data (processed using the same analysis pipeline) from cases without perineurioma; (3) minor allele frequency < 0.0001 in ExAC databases, dbSNP147, 1000 Genomes, ESP6500, and Ingenuity-community collected exome database totaling $\sim 100,000$ samples, of which $\sim 86,000$ are exomes and 14,000 are whole genome sequencing; (4) Polyphen2 and SIFT evaluation as damaging for nonsynonymous variants; and (5) exist in at least 3 of 16 tumor cases. These criteria returned only 1 gene, tumor necrosis factor receptor-associated factor 7 (*TRAF7*). Specifically, 10 of 16 cases share 3 novel, heterozygous, damaging mutations in *TRAF7*: p.L519P (n = 3), p.H521R (n = 3), and p.S561R (n = 4), and all 3 mutations of *TRAF7* are within its WD40 domain, with p.L519P and p.H521R within exon 17 and p.S561R within exon 18 (see Fig 1D). These 3 mutated sites map to a limited region of TRAF7 by our protein structure modeling (see Fig 1E).

Copy Number Variation Analysis Identified Somatic Chromosome Abnormalities

We applied CNV analysis algorithm on our exome sequencing data and found 2 cases (not overlapping with 10 cases with *TRAF7* mutation) demonstrated macro duplication/deletions on multiple chromosomes (Fig 2). Specifically, Cases 2 and 10 showed large copy number

abnormalities expanding sizable regions on multiple chromosomes. Case 2 demonstrated large deletions (Chr10, 11, 19, 22) and duplications (Chr16, 17, 19). Case 10 demonstrated large deletions (Chr2, 7, 10, 11, 22) and entire chromosome 13. The data from the CGH microarray analysis of 16 cases matched our CNV results obtained from exome data analysis, confirming all identified large chromosome duplication and deletions. Note both Cases 2 and 10 had chromosome 22 deletions, consistent with earlier reports.⁶

Discussion

Our comprehensive and high-resolution genomic analysis of intraneural perineuriomas identifies 3 recurrent *TRAF7* somatic mutations, providing strong evidence for a specific tumor gene driver in intraneural perineurioma. The 3 novel mutations (p.L519P, p.H521R, and p.S561R) were shared among 10 cases. No clinical or histologic differences were found between patients having *TRAF7* mutations (62.5%), those having large genomic deletions/duplications (12.5%), and those with no discovered mutations (25%).

The TRAF7 protein belongs to a family of signal-transducers, the tumor necrosis factor (TNF) receptor superfamily.¹³ In addition to having a RING finger domain and a zinc finger domain, TRAF7 is unique in this superfamily of proteins by having 7 WD40 repeats at its C-terminus (see Fig 1D).¹⁴ TRAF7 specifically interacts with MEKK3 and regulates the TNF- α /NF- κ B signal transduction pathway.¹⁵ TRAF7 is a proapoptotic E3 ubiquitin-ligase, and p53 has been shown as a target of TRAF7 for ubiquitination.¹⁶ Interestingly, germline mutation in the WD40 domain of *DCAF8*, also an E3 ubiquitin-ligase, has been linked with giant axon neuropathy.¹⁷ Those patients have axonal swellings with dense inclusions, possibly resulting from the failure of intermediate filament breakdown. Two recent series of >300 meningioma cases showed *TRAF7* mutations also frequently occur as a genetic causal factor, linked with more benign course.^{18,19} Thus, *TRAF7* is considered as the main genetic driver for the most common primary brain tumor, and now one of the most common benign nerve tumors of young persons. All mutations identified in meningioma also occurred in the WD40 domain. Two residues, H521 and S561, mutated in both meningioma and perineurioma, but with different amino acid change.^{18,19} Protein modeling shows the 3 identified *TRAF7* mutation sites map to a limited region of the protein (see Fig 1B). These mutations are predicted to inactivate the interaction of TRAF7 with other proteins or destabilize the WD40 domain. In grade 1 meningiomas, aside from *TRAF7*, 4 other genes, *AKT1*, *SMO*, *NF2*, and *KLF4*, also had mutations. We did not find any rare potential causal variants in these 4 genes.

Similar to meningiomas,^{18,19} chromosomal instability was found in our patients. Both cases with macro deletion/duplications had chromosome 22q deletions. We did not observe any chromosome instability in cases with *TRAF7* mutation, and *NF2* abnormalities were limited to the 2 patients with macro deletions of chromosome 22q. *NF2* mutations tend to show greater genomic instability and malignancy potential.^{18,19} Of note, 1 of our cases with macro chromosomal deletion/duplication (Case 10) also has CHEK2 p.Y390C, a variant associated with lung cancer.²⁰ That patient is without malignancy and is the oldest onset in our cohort. No patients had malignant transformation.

It will be important to investigate *TRAF7* mutations in the extraneural varieties of perineuriomas, including soft tissue, sclerosing, and reticular forms. There is a greater risk of malignant transformation among them, and *TRAF7* mutations may not be seen.⁴ Currently, local resection of intraneural perineurioma is problematic, given their endoneurial intertwined nature. Because perineurial-based tumors are now recognized to share a similar pathogenic mechanism with meningeal benign tumors, a better understanding of tumorigenic pathogenesis will be critical in discovering novel therapies.

Data Deposit

The sequencing data will be accessible through National Center for Biotechnology Information data submission SUB2189278.

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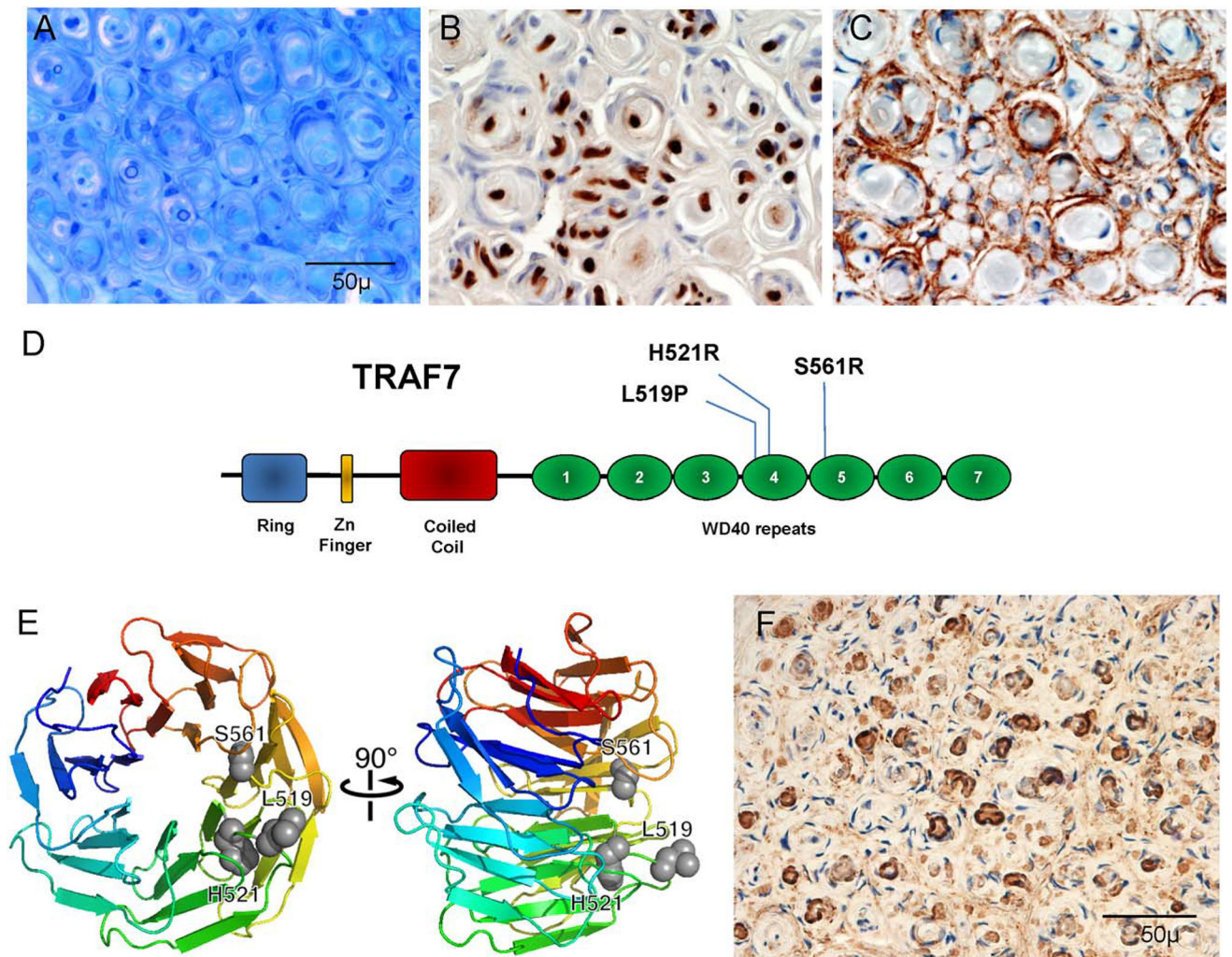


FIGURE 1.

Typical histology and gene discovery in intraneural perineurioma. (A–C) Illustrative case (A) epoxy section at similar level and magnification as shown in paraffin preparations with pseudo-onion bulb formations around occasionally seen thin myelinated fibers with (B) Schwann cell preparation (S-100) demonstrating sparse reactivity of the Schwann cells at the center and (C) prominent epithelial membrane antigen (EMA)-reactive staining of the leaflets of the pseudo-onion bulbs. (D) Functional domains and location of recurrent TRAF7 mutations. The WD40 domain is the region with identified mutations in meningiomas. (E) Rainbow cartoon representation of a model of TRAF7 WD40 domain structure. The 3 residues mutated in intraneural perineuriomas are highlighted as gray spheres and cluster together on the protein predicted to interfere with TRAF7 interaction with other proteins and/or to cause instability of the WD40 domain. (F) TRAF7 immunoreactivity in perineurioma occurs in the same areas that reacted to EMA.

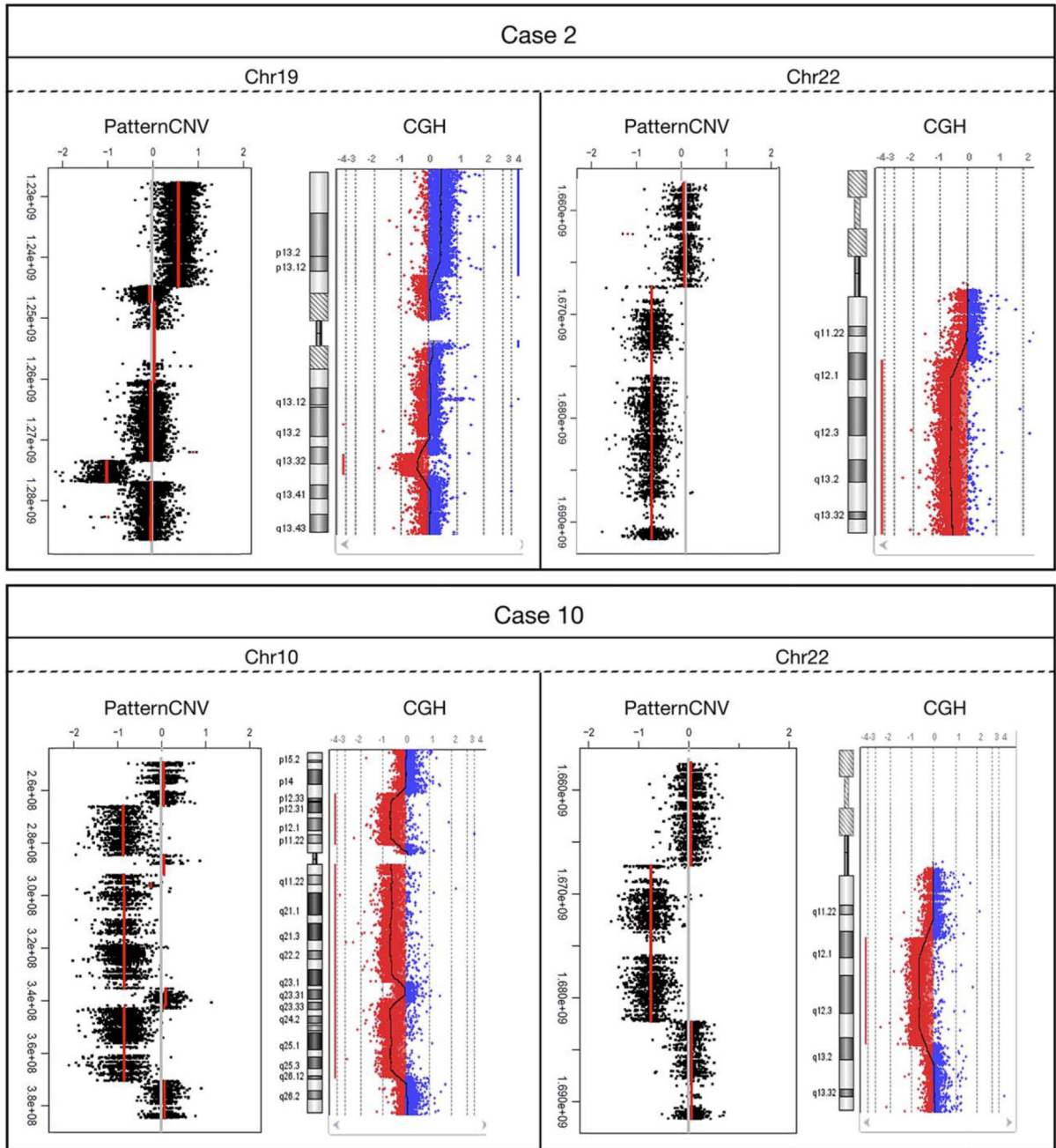


FIGURE 2. Cases 2 and 10 showed large copy number abnormalities expanding sizable regions on multiple chromosomes. Shown are examples of deletions and duplications found on chromosomes 10, 19, and 22. Note both cases have large deletions on chromosome 22q. The Comparative Genome Hybridization (CGH) microarray analysis of 16 cases matched all copy number variation (CNV) results obtained from exome data analysis, providing confirmation for all deletions and duplications.

TABLE

Perineurioma Mutation and Clinical Summary of Patients Studied

Case	Mutation Found	Affected Nerve(s)	Age at Evaluation, yr	Gender	Symptom Duration, mo	Presenting Symptom	Pain	Sensory Loss	NIS
C1	TRAF7p.L519P	Sciatic	14	M	18	Weakness and atrophy	N	Y	7
C2	Abnormal CGH ^a	Tibial	35	M	180	Weakness	N	Y	9.5
C3	TRAF7p.S561R	Sciatic	12	F	6	Weakness	Y	Y	7
C4	TRAF7p.S561R	Ulnar	13	F	24	Weakness	Y	Y	3.25
C5	None	Sciatic	30	M	78	Weakness	Y	N	22
C6	TRAF7p.H521R	Brachial plexus	35	M	360	Weakness	N	Y	49
C7	TRAF7p.S561R	Lumbosacral plexus	2	F	24	Weakness	N	Y	30
C8	TRAF7p.L519P	Tibial	37	M	77	Weakness	N	Y	9.5
C9	TRAF7p.L519P	Radial	30	F	48	Pain	Y	Y	6
C10	Abnormal CGH ^a	Radial	57	F	108	Weakness	N	Y	11
C11	TRAF7p.H521R	Brachial plexus	12	F	48	Weakness	N	N	19
C12	TRAF7p.S561R	Sciatic	7	M	60	Weakness	N	N	14
C13	None	Sciatic	34	M	48	Weakness	Y	Y	11.5
C14	TRAF7p.H521R	Median	40	M	108	Weakness	Y	Y	9
C15	None	Peroneal	18	M	18	Weakness	N	Y	7
C16	None	Sciatic	31	M	156	Weakness and atrophy	Y	Y	12.25

^aSee Figure 2.CGH = chromosomal genome hybridization; F = female; M = male; N = no; NIS = Neuropathy Impairment Score, which sums total motor, sensory, and reflex abnormalities^{1,2}; Y = yes.