Transthyretin Gene Mutation Associated with Familial Carpal Tunnel Syndrome in Sample of Iraqi Patients

Rihab H. Al-Mudhafar¹, Ihsan M Ajeena², Iman J Al-Awadi³, Dhefaf H. Al-Mudhafar³, Najah R. Hadi⁴

¹Middle Euphrates Unit for Cancer Researches, Faculty of Medicine, University of Kufa, College of Medicine, Al-Najaf Al-Asraf, Kufa, Iraq
²Clinical Neurophysiology, Department of Physiology, Faculty of Medicine, University of Kufa, Iraq
³AL-Diwaniya Health Directorate, AL-Diwaniya Teaching Hospital, Iraq
⁴Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Kufa, Kufa, Iraq

Corresponding author: Rihab H. Al-Mudhafar, F.I.B.M.S, Pathology, Middle Euphrates Unit for Cancer Researches, Faculty of Medicine, University of Kufa, College of Medicine, Al-Najaf Al-Asraf, Iraq, E-mail: rihab.almdhafar@uokufa.edu.iq E-mail: rihab.almdhafar@uokufa.edu.iq

doi: 10.5455/aim.2021.29.99-103
ACTA INFORM MED. 2021 JUN 29(2): 99-103
Received:  Apr 12, 2021
Accepted:  Jun 17, 2021

ABSTRACT

Background: Carpal tunnel syndrome (CTS) is the most common entrapment neuropathy in upper limbs. It affects women more frequently than men. CTS can be caused by many different systemic diseases and local mechanical conditions and it had signs and symptoms due to compression of median nerve at the carpal tunnel in wrist. Objective: The present study aims to study whether Transthyretin (TTR) gene polymorphisms have association with the development of FCTS. Methods: Sixty-four patients suffering from CTS were enrolled in this controlled cross sectional study. For all, nerve conduction study was performed to prove the diagnosis of CTS and to classify its severity. The mean age of all patients was 44.9±7.8 years, 89.1 % were female, 37.5 % had mild type CTS and 40.7 % had right side CTS. 3 mL of peripheral blood was collected from all patients which is a labeled (EDTA) tube at -20 C° for DNA extraction to identify a particular SNP (rs28933979; 148G/A; V50M) that locates on the exon-2 region which is reported to be associated to the Carpal Tunnel Syndrome. Exon-2 of TTR gene was amplified using the PCR technique and subjected to be tested for presence of SNP by Restriction Fragment Length Polymorphism (RFLP) assay. Results and Conclusion: According to genetic variation, the study cannot discover any TTR gene polymorphism that back to FCTS. Keywords: Familial Carpal Tunnel Syndrome, TTR.

1. BACKGROUND

Carpal Tunnel Syndrome (CTS) is the most frequent type of compressive syndromes. It is defined by compression and/or traction of the median nerve at wrist level (Bickel, 2010). CTS results in a complex set of symptoms due to entrapment of the median nerve by the transverse carpal ligament within the carpal tunnel of the wrist. Initially, symptoms include sensory disorders in the form of paraesthesia, numbness, or tingling of the fingers. Weakness and atrophy of the muscles in the area controlled by median nerve occur in severe conditions (Emril et al., 2019). CTS is believed to be present in 3.8–5.8% of the general population (i). It is particularly affecting individuals between 40 and 60 years of age (2).

1.1. Etiology and types of CTS

The great majority of cases, CTS are idiopathic. Secondary CTS may be related to abnormalities of the container or content. Additionally, dynamic CTS are frequently found under pathological conditions (3).

Idiopathic type: Gender, age, genetic and anthropometric factors (size of the carpal tunnel) is most important predisposing factors for this type of CTS (4).

Secondary type: A/Abnormalities of the container (decreased tunnel size) (5).

B/Abnormalities of content (increase the content size) (6).

C/Dynamic CTS (7).

Familial type: Familial carpal tunnel syndrome (FCTS) is a rare hereditary disorder characterized by early onset of bilateral disease. When CTS is bilateral and family history is positive, one should rule out hereditary neuropathies of inborn errors of metabolism such as Mucopolysaccharidoses and underlying systemic dis-
orders such as endocrinopathies, renal disease and amyloidosis. Generally, many disease entities have CTS as one of their manifestations, and these must be carefully excluded before a diagnosis of FCTS can be reached (8). The majority of CTS cases with a positive family history are likely due to polygenic factors. Additionally, when the onset of disease occurs at an early age in a patient with a strongly positive family history and/or bilateral symptoms, or in case of absence of more common etiologies should then leads to consider the primary hereditary form of the disease, the FCTS (9). The FCTS is a rare disorder presenting at an average age of 27 years. The symptoms typically begin on one side, but ultimately progress to bilateral involvement in 96% of patients and the clinical features are those of the classic CTS (10). Because median neuropathy is usually bilateral in FCTS, bilateral electro diagnostic testing should be considered when the diagnosis of FCTS is suspected, even if one side is asymptomatic. This is especially important in children, who may not complain of symptoms but have electro diagnostic evidence of median nerve damage.

1.2. Diagnosis of CTS
The diagnosis is bases on:

**The medical history:** The site of such symptoms, whether they occur at night or during the day, if certain positions or repeated movements provoke them and what the patient do to relieve his complaint (Preston and Shapiro, 2021).

**Physical examination:** Physical examination of the patient’s hand and wrist is an important first step towards the diagnosis of CTS and thenar atrophy is seen with severe and chronic CTS (11).

**Provocative tests:** These can be easily done and CTS will be suspected if paresthesia develops and/or increases in the median nerve distribution within one minute or less of practicing any of these tests (12).

**Nerve Conduction Studies (NCS):** NCS is considered to be the gold standard technique for CTS diagnosis, as they quantify and stratify the severity of the syndrome (13).

**Transthyretin (TTR):** It is a homotetrameric plasma transport protein that carries thyroxin and retinol-binding protein. TTR is produced primarily in the liver and in the choroid plexus and retinal pigment epithelium, and is secreted into the blood, cerebrospinal fluid and eyes, respectively (Mashima et al., 2019).

Human Transthyretin is a homo-tetrameric protein characterized by four identical subunits of 14 KDa. TTR is usually stable, exception when a single point mutation occurs and drastically decreases its stability, thus promoting amyloidosis (14). There are over 130 different TTR gene mutations lead to destabilization and dissociation of TTR tetramers into variant TTR monomers (15). Transthyretin-associated hereditary amyloidosis (ATTR) is an inherited disease in which, variant TTR monomers aggregate to form amyloid fibrils that deposit in various tissues that eventually leads to organ dysfunction with several non-disease specific symptoms. Of those mutations, the replacement of valine with methionine at position 30 (ATTR-FAP Val30Met) is the most commonly observed and is the only one found in large number of patients, and is associated primarily with neuropathy (16). Peripheral and autonomic neuropathy, especially CTS, is the most common clinical presentation in TTR amyloidosis (17). The biopsy samples obtained during CTS surgical release revealed deposits of amyloid that stained with anti-human TTR antiserum. Single-strand conformation polymorphism analysis and sequence analysis of polymerase chain reaction (PCR)-amplified exons of the proband’s TTR gene revealed a point mutation resulting in a substitution of histidine for tyrosine at position 114. The mutation was confirmed by PCR-primer-induced restriction analysis.

2. OBJECTIVE
The aim of the paper is to evaluate the relation of Transthyretin gene mutation with familial carpal tunnel syndrome.

3. PATIENTS AND METHOD
This is a case-control study conducted at the Unit of Neurophysiology, Middle Euphrates Neuroscience Center, Al-Najaf Health directorate, between January 2019 and February 2020. The exclusion criteria were pregnancy, obesity, diabetes mellitus, thyroid dysfunction, joint problems or acromegaly. Furthermore, the patient will be excluded if he had a history of local wrist, hand or upper limb trauma. Considering these exclusion criteria, 78 patients with a complaint of hand pain and paresthesia were primarily included in this study. From them, only 64 patients who had positive findings on nerve conduction study (NCS) were enrolled. Only 32 patients suffering Familial carpal tunnel syndrome (FCTS) and same number of controls (those suffering from classical CTS) (a total of 64 participants; 7 males and 57 females) have agreed to participate. For all participants, demographic data were collected, medical history was asked about and the Visual Analog Scale (VAS— a scale use for pain assessment in patients) was calculated. Specific clinical examination as (Phalens test and Tinels test), NCS and some other tests were also done.

The diagnosis of CTS was based on clinical and electrophysiological grounds and its severity was classified into mild, moderate and severe according to the modified Padua Criteria (1):

a) Mild CTS: Prolongation of median distal sensory latency > 3.5 ms.

b) Moderate CTS: in addition to point (i), reduced median SNAP amplitude (< 50% compared to unaffected side or < 10 μV are considered abnormal) or prolonged median motor distal > 4.4 ms.

c) Severe CTS: in addition to points (i) and (2), reduced median CMAP amplitude (< 50% compared to unaffected side or < 4 mV) and denervation of median innervated muscles on needle EMG.

DNA analysis for detecting TTR mutations
The Transthyretin gene (TTR) of Homo sapiens consists of four exons (1-4). In this study, Approximately 3 mL of peripheral blood was collected from all patients which is labeled Ethylenediaminetetraacetic acid (EDTA) tube at -20°C for DNA extraction, we tried to identify a particular SNP (rs28933979; 148G/A; V50M) that locates at position chr18:31592974 on the exon-2 region which is reported to be associated to the Carpal Tunnel Syndrome.
Transthyretin Gene Mutation Associated with Familial Carpal Tunnel Syndrome in Sample of Iraqi Patients

TTR gene was amplified using the PCR technique and subjected to be tested for presence of SNP by Restriction Fragment Length Polymorphism (RFLP) assay in the Middle Euphrates unit for cancer research, Faculty of Medicine, University of Kufa. All statistical analyses were performed by using statistical package of social science software (SPSS) computer program (Version 22, SPSS Inc., Chicago, IL, USA). Categorical variables were expressed as absolute number and percentage (%) and were analyzed by Chi-square test to assess the significance. Continuous variables were expressed as means ± SD and were analyzed by t-test when comparison between two groups. Otherwise, ANOVA test was used to test the level of significance for continuous variables when more than two groups were assessed. A p-value equal to or less than 0.05 and 0.001 were considered to be significant and highly significant, respectively.

4. RESULTS

The mean age of the 64 patients with CTS was 44.9±7.8 and they were divided into 3 age groups. Also, the patients are divided according to the severity into another 3 groups. Females had constituted 89.1% of patients enrolled in this study and which side is affected were also listed (Table 1).

**Table 1. Patients demographic data**

| Variant            | Groups | No. | Percentage |
|--------------------|--------|-----|------------|
| Age group          |        |     |            |
| 20-34 years        | 33     | 51.5% |
| 35-49 years        | 21     | 32.8% |
| 50-65 years        | 10     | 15.6% |
| Total              | 64     | 100% |
| Gender             |        |     |            |
| Male               | 7      | 10.9% |
| Female             | 57     | 89.1% |
| Total              | 64     | 100% |
| Affected limb      |        |     |            |
| Left hand          | 13     | 20.3% |
| Right hand         | 26     | 40.7% |
| Bilateral          | 25     | 39%  |
| Total              | 64     | 100% |
| Severity of CTS    |        |     |            |
| Mild               | 24     | 37.5% |
| Moderate           | 18     | 28.1% |
| Severe             | 22     | 34.4% |
| Total              | 64     | 100% |

TTR gene was amplified using the PCR technique and subjected to be tested for presence of SNP by Restriction Fragment Length Polymorphism (RFLP) assay in the Middle Euphrates unit for cancer research, Faculty of Medicine, University of Kufa. All statistical analyses were performed by using statistical package of social science software (SPSS) computer program (Version 22, SPSS Inc., Chicago, IL, USA). Categorical variables were expressed as absolute number and percentage (%) and were analyzed by Chi-square test to assess the significance. Continuous variables were expressed as means ± SD and were analyzed by t-test when comparison between two groups. Otherwise, ANOVA test was used to test the level of significance for continuous variables when more than two groups were assessed. A p-value equal to or less than 0.05 and 0.001 were considered to be significant and highly significant, respectively.

4. RESULTS

The mean age of the 64 patients with CTS was 44.9±7.8 and they were divided into 3 age groups. Also, the patients are divided according to the severity into another 3 groups. Females had constituted 89.1% of patients enrolled in this study and which side is affected were also listed (Table 1).

**Table 2. Genotype distribution of Transthyretin (TTR) gene polymorphisms in FCTS and classical CTS**

| SNP | Sample | U gene ID | GG | GC | CC |
|-----|--------|-----------|----|----|----|
| rs28933979G/C | 1 | EW01 | GG | | |
| rs28933979G/C | 2 | EW02 | GG | | |
| rs28933979G/C | 3 | EW03 | GG | | |
| rs28933979G/C | 4 | EW04 | GG | | |
| rs28933979G/C | 5 | EW05 | GG | | |
| rs28933979G/C | 6 | EW06 | GG | | |
| rs28933979G/C | 7 | EW07 | GG | | |
| rs28933979G/C | 8 | EW08 | GG | | |
| rs28933979G/C | 9 | EW09 | GG | | |
| rs28933979G/C | 10 | EW10 | GG | | |
| rs28933979G/C | 11 | EW11 | GG | | |
| rs28933979G/C | 12 | EW12 | GG | | |
| rs28933979G/C | 13 | EW13 | GG | | |
| rs28933979G/C | 14 | EW14 | GG | | |
| rs28933979G/C | 15 | EW15 | GG | | |
| rs28933979G/C | 16 | EW16 | GG | | |
| rs28933979G/C | 17 | EW17 | GG | | |
| rs28933979G/C | 18 | EW18 | GG | | |
| rs28933979G/C | 19 | EW19 | GG | | |
| rs28933979G/C | 20 | EW20 | GG | | |
| rs28933979G/C | 21 | EW21 | GG | | |
| rs28933979G/C | 22 | EW22 | GG | | |
| rs28933979G/C | 23 | EW23 | GG | | |
| rs28933979G/C | 24 | EW24 | GG | | |
| rs28933979G/C | 25 | EW25 | GG | | |
| rs28933979G/C | 26 | EW26 | GG | | |
| rs28933979G/C | 27 | EW27 | GG | | |
| rs28933979G/C | 28 | EW28 | GG | | |
| rs28933979G/C | 29 | EW29 | GG | | |
| rs28933979G/C | 30 | EW30 | GG | | |
| rs28933979G/C | 31 | EW31 | GG | | |
| rs28933979G/C | 32 | EW32 | GG | | |

Table 2. Genotype distribution of Transthyretin (TTR) gene polymorphisms in FCTS and classical CTS GG: Wild-type genotype (the mutation is not existed in exon 2 of the TTR gene). GC: The two alleles G and C are existed in the exon 2 of the TTR gene. CC: The SNP rs28933979 (G/C, V>M) is detected in the exon 2 of the TTR gene.
5. DISCUSSION

Most of our patients were middle aged (Table 1) and this seems reasonable because more of consultant clinic visitor were middle age worker. The working age population has raised the chance of getting CTS than the general population and this may be due to the degenerative changes events from repetitive activity in the hand (18). Such finding was also recorded by other study (Al-Shami, 2019). Females had constituted 89.1% of patients enrolled in this study and this high percentage may be due to the fact that carpal tunnel is much smaller in females than males. In addition, female’s daily activity at home may aggravate the disease. Moreover, female hormonal changes like that during the menstrual cycle and pregnancy had been proven to play a role (19). Many previous studies had recorded same results (20). According to affected hand most of our participants had demonstrated right hand affection, while those with bilateral hand affection represent only 39% (Table 1). This can be explained by the usual unilateral hand activity load by most of the people until complaint started, possibly due to the increased liability to trauma, then the patient starts to use the other hand ending with bilateral disease (Taylor et al., 2017).

The authors in (19) had suggested the presence of inheritable variations in the carpal tunnel size or contents, which would usually manifest themselves bilaterally. This was consistent with the findings of other researcher (19). Our study revealed that the majority of the patients had mild disease. This may be due to good patients’ awareness to the symptoms resulting in early seeking of medical consultation, in addition to the availability and feasibility of the electro diagnostic tests that can detect the disease at early stages. According to genotype distribution of Transthyretin (TTR) gene polymorphisms many studies have been conducted to estimate the association between different gene polymorphisms and the development of FCTS (3). To our knowledge, the present study might be the first study concerned with the role of TTR gene polymorphism in the development of FCTS among Iraqi patients. According to genetic variation, the study cannot discover any TTR gene polymorphism that back to disease. Burger and his coworkers at 2016 showed the results of genetic studies on a self-reported Colored South African population group of women with clinically and electro physiologically confirmed CTS and healthy volunteers without any history of CTS symptoms were genotyped for 4 MMP gene variants: MMP10 rs486055 (C/T), MMP1 rs17995750 (G/GG), MMP3 rs679620 (A/G), and MMP12 rs2276109 (A/G). The results of this genotyping showed no association between these gene variants and FCTS (negative outcome). The direct causes of idiopathic carpal tunnel syndrome remain obscure. Three mechanisms are suspected to be involved in genetic predisposition to carpal tunnel syndrome: collagen synthesis, collagen degradation, and protection against oxidative stress affected in connective tissue (3). Several gene groups are involved in the regulation and modulation of these mechanisms, and results from the presented studies have shown their possible effect on the development of carpal tunnel syndrome. Variants within the COL1A1, COL5A1 and COL11A1 genes encoding synthesis of minor collagen subtypes may be potentially involved, as they alter the mechanical properties of tendons and other connective tissue structures within the carpal tunnel. The collagen within connective tissue structures is also remodeled by matrix metalloproteinase (MMPs) and variants of these genes have also been investigated for their possible role in the risk of CTS development. Next, the variants of genes encoding glutathione S-transferase synthesis were found to be involved in CTS etiology. These mechanisms may play a potential role as genetic risk factors in carpal tunnel syndrome and in its familial occurrence. A recent genome-wide association study (GWAS) of CTS identified a couple of CTS-associated variants within genes implicated in growth and extracellular matrix architecture, although the causative link has not been established. Sporadic and idiopathic CTS, particularly with early onset and/or bilateral symptoms, may result from combined effects of genetic susceptibility and environmental factors. Interestingly, a quantitative analysis of published scientific evidence regarding the etiology of CTS found that the major risk factors for CTS are more likely to be genetic and biological than environmental or occupational.

6. CONCLUSION

The study cannot discover any TTR gene polymorphism that back to FCTS. To study TTR gene polymorphisms associated with familial carpal tunnel syndrome on the other exons; b) To study larger sample size of patients with FCTS.

REFERENCES

1. Lee J, Lim S, Kim S. Validation of known risk factors associated with carpal tunnel syndrome: A retrospective nationwide 11-year population-based cohort study in South Korea. bioRxiv. 2018;1-25. doi: 10.1101/253666
2. Petrovera D. and Richettec P. Treatment of carpal tunnel syndrome: from ultrasonography to ultrasound guided carpal tunnel release. Joint Bone Spine. 2018 Oct; 85(5): 545-552. doi: 10.1016/j.jbspin.2017.11.005.
3. Zyluk A. The role of genetic factor in carpal tunnel syndrome etiology: A review. Adv Clin Exp Med. 2020 May; 29(5): 623-628. doi: 10.17219/acem/118846.
4. Lozano-Calderon S, Anthony S, Ring D. The quality and strength of evidence for etiology: example of carpal tunnel syndrome. J Hand Surg Am. 2008; 33(4): 525–538. doi: 10.1016/j.jhsa.2008.01.004
5. Nusrat A. Diagnosis of carpal tunnel syndrome in perspective of clinical features, neuropsychologistic studies and high resolution ultrasound. World Journal of Advanced Research and Reviews. 2020; 6(3): 86-96. doi: 10.30574/wjarr.2020.6.3.0182.
6. Samões R, Taipa R, Valdrez K, Gonçalves I, Melo M, da Silva AM, et al. Amyloid detection in the transverse carpal liga-
Transthyretin Gene Mutation Associated with Familial Carpal Tunnel Syndrome in Sample of Iraqi Patients

1. Transthyretin Gene Mutation Associated with Familial Carpal Tunnel Syndrome in Sample of Iraqi Patients. Amyloid. 2017 Jun; 24(2): 73-77. doi: 10.1080/13506129.2017.1313222.

7. Mackinnon E. Pathophysiology of nerve compression. Hand Clin. 2002 May; 18(2): 251-241. doi: 10.1016/s0749-0712(01)00012-9.

8. Cantwell SR, Batdorf NJ, Adams JE, Moran SL. Primary Familial Carpal Tunnel Syndrome with Long-term Follow-up: A Study of Two Families and Review of the Literature. Journal of Aesthetic & Reconstructive Surgery. 2016; 2: 1-6.

9. Albert V, Subramanian A, Agrawal D, Bhoi SK, Pallavi P, Mukhopadhyay AK. RANTES levels in peripheral blood, CSF and congugated brain tissue as a marker for outcome in traumatic brain injury (TBI) patients. BMC Res Notes. 2017 Mar 24; 10(1):139. doi: 10.1186/s13104-017-2459-2.

10. Li C, Wang N, Schaffer A, Liu X, Zhao Z, Elliott G. et al. Mutation in COMP cause familial carpal tunnel syndrome. Nature Communications. 2020; 11: 1-16.

11. Wipperman J, Goerl K. Carpal tunnel syndrome: diagnosis and management. American Family Physician. 2016; 94(12): 993-999.

12. Almasi-Doghaee M, Boostani R, Saedi M, Ebrahimzadeh S, Moghadam-Ahmadi A, Saedi-Borujeni MJ. Carpal compression, Phalen’s and Tinel’s test: Which one is more suitable for carpal tunnel syndrome? Iran J Neurol. 2016; 15(3), 173-174.

13. Preston C, Shapiro E. Electromyography and Neuromuscular Disorders. In: Clinical–Electrophysiologic Correlations; 4thed, eBook ISBN: 9780323758291, 2021.

14. Ciccone L, Shi C, Lorenzo D, Baelen A, Tonali N. The positive side of the Alzheimer’s disease amyloid cross-interactions: the case of the AB1-42 peptide with Tau, TTR, CysC, and ApoA1. Molecules. 2020; 25(10), 2439; doi:10.3390/molecules25102439.

15. Benson M. The metabolic and molecular bases of inherited disease, 8thed. New York: McGraw-Hill 4159–91; 2000.

16. Sekijima Y, Ueda M, Koike H, Misawa S, Ishii Y, Ishii T, Ando Y, et al. Diagnosis and management of Transthyretin familial amyloid polyneuropathy in Japan: red-flag symptom clusters and treatment algorithm. Orphanet J Rare Dis. 2018 Jan 17; 13(1): 6. doi: 10.1186/s13023-017-0726-x.

17. Youngstein T, Gilbertson A, Hutt F, Coyne E, Rezk T, Manwani R, et al. Carpal Tunnel Biopsy Identifying Transthyretin Amyloidosis. Arthritis Rheumatol. 2017 Oct; 69(10): 2051. doi: 10.1002/art.40162.

18. Zyluk A. Is carpal tunnel syndrome an occupational disease? A review. Pol Orthop Traumatol. 2013 May; 78: 121-126.

19. Mohammad A, Nasiri M, Namazi H, Ashraf J, Ashraf A. Correlation between Female Sex Hormones and Electro diagnostic Parameters and Clinical Function in Post-menopausal Women with Idiopathic Carpal Tunnel Syndrome. J Menopausal Med. 2016; 22(2): 80-86. doi: 10.618/jmmn.2016.22.2.80.

20. Mahima K, Alexander M, Matilde L, Mary M. Clinical presentation, diagnosis, and treatment of TTR amyloidosis. Journal of Neuromuscular Disease. 2019; 6(2): 189-199. doi: 10.3233/JND-180371