Genotype and clinical course in 2 Chinese Han siblings with Wilson disease presenting with isolated disabling premature osteoarthritis

A case report

Siyuan Ye, MD\textsuperscript{a,b}, Tingjun Dai, MD, PhD\textsuperscript{c}, Bingquan Leng, MD\textsuperscript{d}, Lei Tang, MD\textsuperscript{c}, Liang Jin, MD\textsuperscript{c}, Lili Cao, MD, PhD\textsuperscript{c,*}

Abstract

\textbf{Rationale:} Premature osteoarthritis (POA) is a rare condition in Wilson disease (WD). Particularly, when POA is the only complaint of a WD patient for a long time, there would be misdiagnosis or missed diagnosis and then treatment delay.

\textbf{Patient concerns and diagnosis:} Two Chinese Han siblings were diagnosed as WD by corneal K-F rings, laboratory test, and mutation analysis. They presented with isolated POA during the first 2 decades or more of their disease course, and were of missed diagnosis during that long time. The older affected sib became disabled due to his severe osteoarthritis when he was as young as 38 years old. Two compound heterozygous pathogenic variants c.2790_2792del and c.2621C>T were revealed in the \textit{ATP7B} gene through targeted next-generation sequencing (NGS).

\textbf{Lessons:} Adolescent-onset POA could be the only complaint of WD individual for at least 2 decades. Long delay in the treatment of WD’s POA could lead to disability in early adulthood. Detailed physical examination, special biochemical test, and genotyping through targeted NGS should greatly reduce diagnosis delay in atypical WD patients with isolated POA phenotype.

\textbf{Abbreviations:} ACMG = American College of Medical Genetics, IEM = inborn errors of metabolism, NGS = next-generation sequencing, OA = osteoarthritis, PCR = polymerase chain reaction, POA = premature osteoarthritis, WD = Wilson disease, WES = whole-exome sequencing, WGS = whole-genome sequencing.

\textbf{Keywords:} \textit{ATP7B}, Premature osteoarthritis, Wilson disease

1. Introduction

Wilson disease (WD, OMIM 277900), is an autosomal-recessive genetic disorder of copper metabolism caused by pathogenic variants within the \textit{ATP7B} gene on 13q14.3, leading to massive accumulation of copper in various tissues.\textsuperscript{[1]} The widely cited prevalence of WD is 1 in 30,000, and its onset age is mostly between 5 and 35. The most common clinical manifestations of WD include liver disease and cirrhosis, neurological disorder, and K-F rings at the corneal limbus. WD’s diagnosis is usually established by typical clinical symptoms and signs, serum ceruloplasmin at an abnormally low level, and mutation analysis revealing pathogenic variants in the \textit{ATP7B} gene. Its treatment depends on decoppering therapy such as oral D-penicillamine. Once diagnosed with WD, patients should be treated and monitored all their lifetime. Untreated WD is universally fatal, mainly owing to liver disease.\textsuperscript{[2]}

As a less common presentation of WD, osteoarthritis has been described in some case series with small samples.\textsuperscript{[3–8]} It mainly involves weight-bearing joints such as knees, hip, and spine, and other joints can also be involved. In those previous clinical and radiological studies, most of the osteoarthritis in WD patients began in teenage or early adulthood, which was called as premature osteoarthritis (POA).\textsuperscript{[4–9]} However, it was usually not conspicuous during the early stage of WD. And rarely, when POA presents as the only complaint of a WD patient, misdiagnosis or missed diagnosis and then treatment delay could occur easily.\textsuperscript{[10–12]} Furthermore, although WD’s osteoarthritis was generally considered as mild previously, actually its clinical course and prognosis have never been reported by any longitudinal study. Additionally, the genotype of WD’s isolated POA was also rarely reported.

Herein we describe 2 Chinese Han siblings with adolescent-onset WD. They presented with isolated POA throughout the first 2 decades or more of their disease course, and underwent missed diagnosis during that time. It was the longest diagnosis delay of WD due to isolated POA phenotypes on record. Moreover, the
older affected sib became disabled due to his severe osteoarthritis when he was as young as 38 years old. Finally, molecular diagnosis was achieved through targeted next-generation sequencing (NGS).

2. Clinical reports

2.1. Patient 1

The 39-year-old male proband had been suffering from chronic progressive bilateral polyarthralgia since the age of 15. Initially, his joints pain occurred only in bilateral knees and worsened with activity. Along with the gradual aggravation of his knees pain during the following 2 decades, other joints in bilateral limbs, extremities, low back, and hips were sequentially involved. Furthermore, morning stiffness (<10 minutes), deformities, and decreased mobility occurred in his affected joints, but there was no focal redness or increased warmth. During the initial 2 decades since his disease onset, he had referred to rheumatologists or orthopedists several times, but never received an etiological diagnosis or effective treatment.

At the age of 34, our proband suffered from drooling and bilateral hand tremors. His serum ceruloplasmin tested at an abnormally low level (Table 1), and K-F rings were demonstrated by a slit-lamp examination. Thus, a diagnosis of WD was considered. Nevertheless, the biochemical examination revealed no hepatic dysfunction, and no remarkable morphological change of liver was found by ultrasonography. After 1-year administration of D-penicillamine (0.125–0.375 g/d, low dose) and benzhexol (2 mg/d), his tremors and drooling disappeared, whereas the articular disorders were still progressive. Radiographs of knees, hips, and hands showed severe changes according to osteoarthritis (Fig. 1). Various inflammation-related biomarkers were negative (Table 1).

After 5-year administration of D-penicillamine at that low dose, the 39-year-old proband referred to our neurogenetic clinic for further care. The neurological examination was normal, but K-F rings were still obvious. Orthopedic examination confirmed deformities, mild tenderness, and limited mobility in affected joints. Computed tomography scan and magnetic resonance imaging of his brain showed no abnormality. Further biochemical studies displayed no clue indicating any other endocrine disease or metabolic disorder. One of his older brothers also presents with the similar isolated POA phenotype, and their parents are not of consanguinity. Any other special history was denied.

Table 1

The epidemiological, clinical, and serological features of these 2 WD sibs.

| Clinical course | The proband | The older sib |
|-----------------|-------------|---------------|
| Onset age of POA, years | 15 | 13 |
| Age when K-F rings were found, years | 34 | 38 |
| Age when neurological disorder occurred, years | 34 | Null |
| Age of disability by POA, years | Null | 38 |
| Age when D-penicillamine was initially administrated, years | 34 | 41 |
| Age at present, years | 40 | 42 |
| Age when D-penicillamine was initially administrated, years | 34 | 41 |
| Age of disability by POA, years | Null | 38 |
| Age when K-F rings were found, years | 34 | 38 |
| Age when neurological disorder occurred, years | 34 | Null |
| Age when K-F rings were found, years | 34 | 38 |
| Age when neurological disorder occurred, years | 34 | Null |
| Age of disability by POA, years | Null | 38 |
| Age when D-penicillamine was initially administrated, years | 34 | 41 |
| Age at present, years | 40 | 42 |

| Index of copper metabolism | The proband | The older sib |
|-----------------------------|-------------|---------------|
| Serum copper (mg/dL) (normal range 11.0–12.0) | 3.4 | 122.6 |
| Serum ceruloplasmin (mg/dL) (normal range 22.0–58.00) | 2.79 | 97.69 |
| Serum ANA titer (1:1000) (normal < 1:160) | (-) | (-) |
| Various serum autoantibodies related to autoimmune diseases | (-) or Normal | (-) or Normal |
| Marked orthopedic signs at present | (++) | (++) |
| Deformation of affected joints | (++) | (++) |
| Limited mobility of affected joints | (++) | (++) |
| Bilateral Patrick’s sign | (++) | (++) |
| Increased warmth or erythema of affected joints | (++) | (++) |
| Past and personal history | Normal | Normal |
| Systemic symptoms | Normal | Normal |
| Acute episode of arthritis | Normal | Normal |
| Exposure to alcohol or cigarette | Normal | Normal |
| Physical load in occupation | Normal | Normal |
| Exposure to trauma | Normal | Normal |
| Exposure to special eating | Normal | Normal |
| Initial development of skeletal system | Normal | Normal |
| Other special history | Normal | Normal |

‡‡ Elderly affected sib became disabled due to his severe osteoarthritis when he was as young as 38 years old. Finally, molecular diagnosis was achieved through targeted next-generation sequencing (NGS).
2.2. Patient 2

The proband’s 41-year-old affected male sibling (sib) came to our clinic at the same time. This sib’s phenotype was very similar to our proband’s, with even more severe adolescent-onset POA, K-F rings in both corneas, serum ceruloplasmin at abnormally low level, and no indication of liver disease. However, neurological disorder was absent in this patient (Table 1). The sib’s knees pain beginning at the age of 13 marked the onset of his progressive bilateral polyarticular osteoarthritis, which had been the only complaint for 28 years. His POA had never been treated in the past. He became limited to wheelchairs when he was 38, because of severely limited mobility of the affected joints.

3. Materials and methods

This study was approved by the Ethics Committee of the Qilu Hospital of Shandong University. Informed consent for the publication of this case report was obtained from the patients and related family members.

3.1. Genomic DNA extraction

Five subjects included 2 WD-affected sibs, their parents, and one of their non-WD older brothers. The nuclear genomic DNA samples were extracted from their ethylenediaminetetraacetic acid anticoagulated peripheral blood using a TIANamp Blood DNA Kit (TIANGEN).

3.2. Target-sequence capture combined with NGS

For the proband’s DNA sample, whole genome library was prepared using KAPA Library Preparation Kits for Illumina sequencing platforms, and all the coding sequences together with exon flanking sequences of 439 known pathogenic genes involving inborn errors of metabolism (IEM) were captured using an Agilent SureSelect Target Enrichment System. Then paired-end sequencing for the proband’s target sequence was performed using an Illumina HiSeq2500 next-generation sequencer.

Clean reads were mapped to the reference human genome hg19/GRCh37 using software Burrows-Wheeler Aligner. Genome Analysis ToolKit was used to identify single-nucleotide variations and insertions/deletions. All of the identified variants were annotated using software ANNOVAR, and significant variants were obtained using the single-nucleotide polymorphism database (NCBI dbSNP), Exome Aggregation Consortium (ExAC), 1000 Genomes Project, and Exome Sequencing Project (ESP6500).

3.3. Sanger sequencing

For all subjects’ DNA samples, exons 11 and 12 of ATP7B including target variants were amplified by polymerase chain reaction (PCR) according to previously published primers. Sanger sequencing was then performed in the 2 WD sibs’ DNA samples to verify the target variants, and in the other 3 subjects’ DNA samples to determine target variants status.

3.4. Sequence variants interpretation

The pathogenicity assessment of the target variants was performed strictly according to the guidelines published by the American College of Medical Genetics (ACMG) for the interpretation of sequence variants.

The tools or web resources used for variants interpretation are as follows: Human Gene Mutation Database Professional (HGMDpro), 1000 Genomes, ExAC Browser, ESP6500, ClinVar, Variant Effect Predictor, NCBI-Gene, Human BLAT Search, InterPro protein sequence analysis and classification, MutationTaster, SIFT, PolyPhen-2, PROVEAN Protein, and so on.

Figure 1. Radiographs of the WD proband’s osteoarticular changes at his age of 34–35. (A) The posteroanterior plain film of the right hand showing joint space narrowing, subchondral sclerosis, subchondral cysts, marginal osteophyte formation, and intra-articular loose bodies. (B) The posteroanterior plain film of the left knee joint showing genu valgum deformity, marked demineralization, and square deformity of the lower end of the femur. (C) The posteroanterior plan film of pelvis showing the blurred interface of joints, marginal irregularity, and abnormal shape of bilateral greater trochanters, intra-articular loose bodies near the neck of the left femur. (D) Pelvis CT scan showing osteophytes forming bridge on the anterior margins of bilateral sacroiliac joints. CT = computed tomography, WT = wild type.
4. Results

4.1. Genotype and variants interpretation

Two compound heterozygous variants were found in the ATP7B gene of our proband, which are c.2790_2792del in exon 12 and c.2621C>T in exon 11 (NM_000053). These 2 variants at DNA level could lead to variants p.I930del and p.A874V in ATP7B protein, respectively. Core family screening confirmed cosegregation of these 2 variants with disease. In detail, the older WD sib also carried these 2 same variants, their father carried only c.2790_2792del, their mother carried only c.2621C>T, and their available non-WD sib was just a carrier of variant c.2790_2792del (Fig. 2).

According to the ACMG guidelines, variant c.2790_2792del was classified as pathogenic based on evidence of pathogenicity containing PS4, PM1, PM2, PM3, PP1, PP3, PP4, and PP5; variant c.2621C>T was also classified as pathogenic based on evidence of pathogenicity containing PS3, PS4, PM1, PM2, PM3, PP1, PP2, PP3, PP4, and PP5. Details regarding the pathogenicity of these 2 variants can be obtained in supplementary online material (http://links.lww.com/MD/B980).

4.2. Clinical summarization

The epidemiological characteristics, and clinical and serological features of these 2 WD sibs are summarized in Table 1. These 2 WD sibs were finally diagnosed at DNA level and were prescribed D-penicillamine 0.75–1.0g/d, pyridoxine 30mg/d, and vitamin E 100–300mg/d according to the recommendations of guidelines. A 9-month follow-up of the above-mentioned treatment showed alleviation of these 2 WD sibs’ arthralgia, while there was neither remarkable improvement nor deterioration of their articular deformity and dysfunction.

5. Discussion

Osteoarthritis is a less common manifestation of WD. There has yet been no convincing evidence of osteoarthritis’s prevalence in WD patients, due to the limit of previous scattered case series studies. The tissue damage secondary to copper excess in joints may serve as a mechanism for WD’s osteoarthritis. It has been previously reported that POA could be the onset complaint of WD patients, and even be the only symptom for several years leading to misdiagnosis or missed diagnosis. Particularly, there was a WD individual presenting with isolated POA lasting for the longest 15 years on record. However, the prognosis and natural course of those WD patients with isolated POA were not described by any previous study, and the genotype of WD’s isolated POA was also rarely reported.

In our report, it is revealed that adolescent-onset POA could be the only complaint of WD individual for at least 2 decades. Additionally, in contrast to the previous opinion that the joint involvement in WD was generally mild, it is demonstrated in the case of the older affected sib that osteoarthritis in WD could be disabling in the third decade of life. There may be some factors explaining the much more severe POA phenotype of the proband’s older affected sib than the proband. First, unlike the proband, this affected sib had always been addicted to alcohol.
and cigarette, had fallen and suffered trauma for several times, and never received decoppering therapy during the disease course. Moreover, compared with the proband, this sib had an earlier disease onset and experienced more manual labor load during disease course. Since there has been no study describing the natural course and prognosis of WD’s POA, and a prospective natural history study must not be performed by reason of ethic, our report recording these 2 WD sibs’ natural course of POA would be precious.

Compound heterozygous variants c.2790_2792del and c.2621C>T within the ATP7B gene of our 2 WD sibs are extremely rare in populations according to 1000 Genomes, ESP6500, ExAC, and our in-house population genetics database (supplementary online material, http://links.lww.com/MD/B980). Furthermore, neither of these 2 pathogenic variants is common in Chinese WD. Variant c.2790_2792del was just firstly reported in a large Chinese WD cohort most recently (c.2621C>T) mutation is located within a large cytoplasmic loop named A-domain containing the phosphatase activity, whereas the c.2621C>T mutation is located within an α-helix transmembrane domain TMS (Fig. 2D). According to in vitro studies, A874V-ATP7B protein mutant showed apparent destabilization and endoplasmic reticulum (ER) retention, and lost copper transport activity, thus likely causing WD phenotype.[19,20]

Mainly as a part of a syndrome, POA mostly develops secondary to some rheumatic diseases, infection, trauma, or overuse of joints, or biomechanical defects such as osteochondrodysplasias, which can be identified easily by related specialists. By contrast, primary POA which often presents as nonsyndromic is very rare and commonly hard to be related etiologically. Actually, it usually indicates an underlying Mendelian disorder, of which some IEM like WD, ochronosis, or hemochromatosis take up an overwhelming proportion, especially when the family history is positive.[9,21] Among those >800 reported mutations of the ATP7B gene (HGMDpro, access time: 4 January, 2017), missense/nonsense mutations comprise almost 550, small deletions and insertions contain a little >200, splicing variants include no >100, large-scale variant is very rare, and there is no dynamic mutation. Therefore, NGS would be effective for the rapid molecular diagnosis of WD.[22] Additionally, since the clinical and biochemical phenotypes of our affected sibs had indicated the specific disorder WD, a relative limited targeted NGS was performed with an economic consideration, instead of whole-exome sequencing (WES) or whole-genome sequencing (WGS). However, if there is no sign or biochemical phenotype indicating some specific genetic disorder for a patient with isolated POA, WES or WGS would be more helpful to reveal the pathogenic sequence variants.[21]

In terms of the treatment for WD’s osteoarthrits, there has not been any special therapy besides decoppering agent yet. Actually, there also has yet been no evidence regarding decoppering therapy’s effectiveness for WD’s osteoarthrits. This could be attributed to the intrinsic deficiency of previous case series studies in a cross-sectional manner, to the heterogeneous baseline small sample, and selection bias in the only follow-up study.[23]

Although the follow-up of our patients indicated that D-penicillamine did not remarkably improve their advanced osteoarthrits, it had probably slowed the progression of OA. If our patients had been diagnosed at the disease onset and their treatments had been started then, their POA phenotype might have been not so severe.

Further clinical research should firstly focus on establishing a multiregional prospective WD cohort of high quality, in which there would be elaborate description of WD’s POA covering its clinical epidemiological features, radiological characteristics, genotype-phenotype correlation, long-term follow-up picture, reaction to therapies, and so on.

6. Conclusions

When a clinician encounters unexplained isolated POA which even has lasted for a very long term as 2 decades or more, the possibility of WD as a Mendelian disorder should be ruled out. Early diagnosis, treatment as well as protection of joints would significantly improve the prognosis of WD’s POA; on the contrary, long delay in its treatment could lead to disability in early adulthood. Detailed physical examination, special biochemical test, and genotyping through targeted NGS should greatly reduce diagnosis delay in atypical WD patients with isolated POA phenotype.

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