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This document is the Accepted Version [AM]

**Citation:**

WEI, X., ZHANG, S., YANG, W., LUO, Z., SUN, P., DALTON, Caroline, FACHIM, H.A. and REYNOLDS, G.P. (2019). Neuromyelitis optica spectrum disorder in three generations of a Chinese family. *Multiple Sclerosis and Related Disorders*, 32, 94-96. [Article]

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## Neuromyelitis optica spectrum disorder in three generations of a Chinese family

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### Keywords

Demyelinating disease, aquaporin-4, familial transmission, anticipation, NECL2

### Abstract

Neuromyelitis optica spectrum disorder is an inflammatory demyelinating disease that is largely sporadic. Familial disease has been reported in one or two generations, although its basis remains unknown. We report here three subjects meeting diagnostic criteria for NMOSD in one family: a father and son, and the maternal aunt of the father. Anticipation, of 27 years, was apparent in transmission from father to son. Aquaporin-4 antibodies were observed in the aunt but not the father and son, nor in other family members. A putative pathogenic mutation in the NECL2 gene was not found in this pedigree. This first report of NMOSD in three generations of one family underlines the heterogeneity of familial NMOSD.

### Introduction

Neuromyelitis optica spectrum disorder (NMOSD) describes inflammatory demyelinating disease of the nervous system characterized by optic neuritis and long segmental spinal cord injury. The disease may demonstrate a recurring-remitting course but with residual spinal cord demyelination and, in the majority of cases, serum aquaporin-4 (AQP4) antibodies which are considered to be pathogenic. Patients often have limb dysfunction, numbness and visual impairment. As there is variability in the presentation, imaging and laboratory findings indicating possible disease heterogeneity, consensus diagnostic criteria have recently been formulated for NMOSD (Wingerchuk et al., 2015).

Occurrence of NMOSD is largely sporadic, although there have been reports of familial cases over one or two generations (Matiello et al., 2010). The genetic basis of familial inheritance of NMOSD is unknown; case-control studies have reported associations with polymorphisms in a variety of genes including interleukin-17, HLA antigens and CD proteins as well as AQP4. Apart from AQP4 however, these have not been studied in familial cases, and AQP4

polymorphisms have not yielded consistent findings of linkage with NMOSD from the few familial cases studied (Matiello et al., 2011). However, a recent paper (Xu et al., 2018) has described a functional deletion variant of a candidate gene, the cellular adhesion molecule NECL2, in three members of one NMOSD family and in a proportion of sporadic cases but not in control subjects.

We report here three cases of NMOSD over three generations of a Chinese family and investigate whether linkage with the previously-reported and potentially pathogenic NECL2 mutation extends to this family.

## Methods

All subjects had given written informed consent for this study, which was approved by Puer People's Hospital Ethics Committee.

Following the identification of two patients, father and son, with suspected NMOSD, who were related to a patient previously diagnosed with NMO, 11 genetically-related asymptomatic family members also had their serum AQP4 antibody status determined initially by ELISA and subsequently confirmed by cell-based assay.

We developed a pyrosequencing method for determination of a nine-base deletion mutation in the NECL2 gene (rs770344177, c.1052\_1060delCCACCACCA) (Xu et al., 2018). Following DNA extraction from blood samples, the sequence was amplified by PCR using primers, including a biotinylated reverse primer, as follows: Forward 5'GTTGTGTTTCAGCATTGTGGGT3', Reverse [Biotin]5'CTGGGATGTTTCTGAGGTCCTT3' (Eurofins). PCR reactions were carried out with 20 ng of DNA using the PyroMark PCR kit in a final volume of 25 µl containing 12.5 µl 1x PyroMark PCR Master Mix, 2.5 µl 1x CoralLoad Concentrate, 1 µl of each primer in a final concentration of 0.05 µM, 8 µl RNase-free water. Amplification conditions were as follows: 95°C for 15 min, 45 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 30 s, finally, 72°C for 10 min.

The InDel analysis was determined with a PyroMark Q24 pyrosequencer (Qiagen UK) using 15–20 µl PCR product and a sequencing primer 5'CACAACCTATCCCTCCTCCCAAC3' (Eurofins). Pyrosequencing setup and data reading were conducted by PyroMark Q24 2.0.6.20 software (Qiagen UK). Samples underwent PCR and pyrosequencing in duplicate; inconsistencies between samples were resolved following further repetition.

## Results

*Index case.* A 34-year-old Chinese Han woman (Patient 1) in Yunnan, China, developed vision loss in her right eye in May 1999, subsequently with some deterioration in the left eye, having an expanded disability status scale (EDSS) score of 5. Five months later she had developed left limb weakness but could walk with assistance (EDSS: 7). Visual evoked potential and magnetic resonance imaging (MRI) of the brain and spinal cord were examined and she received a diagnosis of NMO. CSF analysis was normal other than slightly raised protein (0.5g/L). After dexamethasone treatment her symptoms improved but she had residual blurred vision in her right eye. Subsequently the patient had more than 10 episodes of decreased visual acuity and limb weakness. Her binocular vision decreased gradually with the number of episodes. Further CSF analysis showed oligoclonal banding to

be negative. MRI in 2011 showed no apparent brain abnormality but the spinal cord lesions exceeded 3 segments (Figure 1), and optic nerve injury was indicated in the visual evoked potential examination. The patient was prescribed methylprednisolone which alleviated the symptoms. There was no symptom exacerbation after prescription of azathioprine (100mg bid) in 2011. In 2009 she tested negative for anti-Sjögren's-syndrome-related antigen A (anti-SSA) and Anti-SSB. In 2018 she tested positive for serum AQP4 antibodies and negative for MOG antibodies.

*Patient 2*, the son of an older sister of patient 1, first developed symptoms of lower extremity weakness and urinary incontinence in November 2008 at the age of 38. Symptoms gradually worsened over 25 days resulting in complete paralysis of the lower limbs (EDSS: 8.5). MRI showed a normal brain while the spinal cord exhibited a lesion over 5 segments and he was diagnosed with acute myelitis. CSF analysis showed increased white cell count and raised protein (0.86g/L). He was prescribed methylprednisolone; in the following 3 years there were six episodes of disease recurrence, mainly with bilateral lower limb weakness (EDSS: 3-6). after initiating treatment with azathioprine (100mg bid) in 2011 there were only two further episodes. CSF oligoclonal banding was negative. He was diagnosed as having NMOSD and, in 2018, symptoms of vision loss began to appear. He tested positive for anti-SSA in 2008 and 2017. Serum AQP4 antibody tested negative in 2018 when MRI was also reviewed (shown in Figure 2) and serum MOG antibodies tested negative in 2018.

*Patient 3*, the son of patient 2, developed numbness in both lower extremities in May 2014 at the age of 11. MRI examination identified a cervical spinal cord lesion which extended over 3 segments (Figure 3) with normal brain imaging; routine CSF analysis was normal and oligoclonal banding was negative. He was diagnosed with NMOSD and received treatment with gamma globulin. In 2017 he was free of symptoms and tested negative for anti-SSA and Anti-SSB; in 2018 he tested negative for serum AQP4 antibodies and in 2019 serum MOG antibodies tested negative.

Eleven other members of the family tested negative for serum AQP4 antibody (Figure 4). The three patients and these family members (excepting the children of patient 2's eldest brother) underwent genotyping for the NECL2 polymorphism rs770344177 and were all unequivocally identified as homozygous wild-type, i.e. free of the deletion mutation.

## **Discussion**

The occurrence of familial NMO is estimated to be approximately 3% of cases (Matiello et al., 2010; Papais-Alvarenga et al., 2015) and that for NMOSD is likely to be similar. Reported familial cases occur primarily in first-degree relatives and in almost all reports only two patients are identified in each pedigree. Genetic anticipation is apparent in several of these families with parent-child age differences at onset varying from 23-42 years (Kavoussi et al., 2015).

As far as we are aware, this is the first report of a family with NMOSD represented in each of three generations, although it is notable that the oldest case is a second-degree relative of the patient in the following generation. We cannot rule out the occurrence of NMOSD in the aunt of patient 2 being a sporadic case rather than a genetic relationship, particularly given the difference in AQP4 immunostatus, although this seems unlikely. It is notable in this

respect that Papais-Alvarenga et al. (2015) identified two sisters with NMOSD who were discordant for anti-AQP4, while five of the 12 families reported by Matiello et al. (2010) were also heterogeneous in AQP4 status.

There is genetic anticipation in transmission from father to son with a difference in age of onset of 27 years. The relative rarity of reports of NMOSD over three generations may reflect this genetic anticipation (i.e. death may precede disease onset in the first generation) and indicates that the reported proportion of hereditary disease may be an underestimate. Variability of disease onset age in siblings is generally small although 16 years difference between two sisters has been observed previously (Matiello et al., 2010). A large difference in onset age is implied between patient 1 and her unaffected older sister, the presumed obligate carrier.

A genetic mutation with strong evidence for association with familial NMOSD, a coding region deletion in NECL2, has been observed in two daughters with NMOSD and an asymptomatic mother, and in 5.7% of sporadic cases, but is absent in 212 control subjects (Xu et al., 2018). However, the patients and their extended family studied here did not exhibit this potentially pathogenic deletion mutation. We can conclude that this specific deletion is not present in all familial NMOSD cases, underlining the genetic heterogeneity of familial NMOSD (Matiello et al., 2011), although we cannot rule out association of familial disease with other mutations of the NECL2 gene or elsewhere in the genome.

### **Acknowledgements**

We greatly appreciate the help of Dr Siân Price, Royal Hallamshire Hospital, Sheffield for her constructive criticism of an earlier version of this report. We are most grateful for the cooperation of the extended family studied here.

No specific funding was received for this research.

Declarations of interest: none.

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