Clinical and molecular characterization of Kelch-like Protein 11 paraneoplastic neurological syndrome

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Funding: not required

Project Summary

The aim of this study is to provide a clinical and molecular characterization of Kelch-like Protein 11 paraneoplastic neurological syndromes through a multicenter retro-prospective collaborative study. In particular we aim:

- to identify cases of paraneoplastic neurological syndrome (PNS) associated with anti Kelch-like Protein 11 (KLHL-11) and to collect relevant data, to expand clinical and oncological accompaniments, and to evaluate potential prognostic factors,
- to further validate the proposed MATCH score, for the identification of suspected KLHL-11 cases,
- to evaluate the sensitivity and specificity of different laboratory assays for the detection of KLHL-11 antibodies,
- to collect biological samples including PBMCs, DNA, tumor and/or CNS tissue (if available) to provide insights into the pathogenesis of the disease.

Background and Rationale

Among the recently described paraneoplastic neurological syndromes (PNS), antibodies against Kelch-like protein 11 (KLHL-11) initially defined a treatment-refractory brainstem and cerebellar syndromes associated with testicular neoplasms (especially “burned-out” tumors) [1]. In the years following its first description, the spectrum of phenotypes and oncological accompaniments has progressively expanded also including clinical features of limbic encephalitis, myeloneuropathy, opsoclonus-myoclonus and seizures in association with ovarian teratoma, lung adenocarcinoma and chronic lymphocytic leukemia [1–4]. In a minority of cases, there is no evidence of underlying tumors, in patients with a compatible clinical phenotype, despite a prolonged screening [5,6].
Response to immunotherapy and to cancer treatment may be unsatisfactory in many cases [1–4] as seen in other disorders associated with antibodies to intracellular antigens, even though a delayed recognition of this disease may play a pivotal role in the prognosis.
Consistently, evidence regarding the pathophysiology of disorders associated with anti-KLHL-11 antibodies suggests that these antibodies are not pathogenic and that T cells are the main drivers of the immune response. Furthermore, HLA studies demonstrated that HLA-DQB1*02:01 and HLA-DRB1*03:0 are associated with this condition [2].
Epidemiological data suggest that KLHL-11 may be relatively frequently observed in patients with suspected PNS, however the diagnostic is performed only in few expert laboratories worldwide. To help clinicians in suspecting and testing patients with suspected KLHL-11 antibodies, a clinical score (MATCH score) including specific items (i.e. male sex, ataxia, testicular cancer, hearing loss) with a cut off ≥4 was developed [3] and showed high specificity and good sensitivity. The score was successfully validated in an independent cohort [7], even though some reports underlie that this score can miss atypical phenotypes [5].
Lastly, the laboratory diagnosis of KLHL-11 relies on tissue-based assays and cell-based assays employing cells expressing KLHL-11. However, while cell-based assays seem to be sensitive and specific for the detection of these antibodies, the role of tissue-based assays have been questioned [4]. This discrepancy may be explained by the different methods used (as tissue-based

immunohistochemistry versus immunofluorescence) to detect antibody binding to brain tissue and warrant further analyses.

References

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Preliminary Data

Antibody testing for Kelch-like protein 11 (KLHL-11) is performed in few expert laboratories worldwide since no commercial test is available. At the Neuropathology Laboratory of the University of Verona we developed and tested two in-house assays: a cell-based assay employing HEK293T cells transfected to express Kelch-11 protein and a modified immunofluorescence assay on rat brain slices, optimized for the detection of KLHL-11 antibodies. Attached images demonstrates the peculiar “starry sky” pattern in rat cerebellum and hippocampus and the peculiar punctate positivity seen in assays using KLHL-11 transfected cells (commercial antibody).

Methods and Sample Size

The present study has a retro-prospective design.

Stored serum and CSF samples of patients with suspected paraneoplastic neurological syndrome referred for intracellular antibodies or tissue-based assays testing will be retrospectively tested for KHLH-11 antibodies. Relevant demographic, clinical, radiological, paraclinical and prognostic, as well as oncological accompaniments will be retrospectively collected in an electronic anonymized spreadsheet.

Other biological samples including DNA, PBMCs, tumor and neuropathological retrospectively available samples will be stored for future uses including HLA analysis, evaluation of T-cell receptor, evaluation and molecular characterization of KHLH-11 protein expression in cancer tissue, and characterization of neuropathological findings of patients with anti-KLHL-11 antibodies.

Essential clinical data (demographics, clinical syndrome, other autoantibodies specificities, associated tumors) of negative samples will be collected for the calculation of PNS-CARE score and for the validation of MATCH score, as controls.

The prospective cohort will include patients referred for testing of KHLH-11 antibodies and similar clinical data and biological samples will be collected.

Regarding the diagnostic assays, the antibody testing will be performed in serum and CSF with in-house cell-based assays, immunohistochemistry, and immunofluorescence on rat brain slides, adapted from previously described protocols [3,4].

A descriptive statistic for relevant demographic, clinical, radiological, paraclinical, and prognostic data will be performed.

For the identification of prognostic factor, linear regressions will be performed to identify potential predictors (including demographics, clinical, treatment, paraclinical, oncological and radiological features) of favourable outcome (defined as a mRS ≤ 2). Then, a binary logistic regression model will be calculated to evaluate potential predictors of the primary outcome, including those predictors that resulted statistically significant in the linear regressions. All tests will be 2-tailed, and p-values<0.05 will be considered as statistically significant. Statistical analyses will be performed with SPSS 26 (IBM, Armonk, New York, United States) and with R Statistical Software (v4.1.2; R Core Team 2021).
We will evaluate the sensitivity and specificity of the MATCH score including all seropositive KLHL-11 patients and patients with probable or definite paraneoplastic neurological syndromes that tested negative for KLHL-11.
Lastly, a sensitivity and specificity analysis will be performed to evaluate the performance of different tissue-based assays (immunohistochemistry vs immunofluorescence) using patients tested positive on cell-based assays and with consistent clinical phenotype as true positive results, and patients with other paraneoplastic neurological syndromes (probable or definite) tested negative on cell-based assays as true negative results.

Ethics and Patient Consent

Submitted to the local ethics committee (Verona).

Support Request

- Samples of patients with suspected paraneoplastic neurological syndrome (i.e. referred for intracellular antibodies or tissue-based assays testing);
- Collection of clinical data;
- Collection of biological samples (PBMCs, DNA, tumor tissue from biopsy/tumor removal, CNS/PNS tissue from biopsies).

Rules for Authorship

Physicians providing clinical data or biological samples beyond serum/CSF of patients that will result positive for KLHL-11 antibodies will be included as authors. Neuroimmunology laboratories that will refer a significant number of samples of suspected paraneoplastic neurological syndrome will be also granted authorship. The remaining subjects will be included as collaborators of the study group.