

SUMMARY OF FINAL REPORT

Genetic and functional analysis of the gene causing SCA13 in UK patients with ataxia

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Background and aims:

The dominantly inherited spinocerebellar ataxias (SCAs) are a genetically and clinically complex group of hereditary neurodegenerative disorders. Despite the identification of many of the underlying genetic defects, the common pathological pathways in SCA remain poorly understood. The discovery of the mutated gene for SCA13 as the voltage-sensitive potassium channel *KCNC3* points to an important role for potassium channels in the pathogenesis of cerebellar ataxia and opens the interesting possibility that modulation of potassium channel function may be a therapeutic strategy in ataxia. The main goal of the proposed research was to define the frequency of *KCNC3* mutations in the UK ataxia population and to functionally characterize newly identified mutations, if any.

Results:

We screened a mixed panel of 119 patients with or without a positive family history of cerebellar ataxia for abnormalities in the *KCNC3* gene. DNA samples were amplified using oligonucleotide primers spanning each exon and associated splice junction of the *KCNC3* gene. Amplified products were screened for changes by denaturing high-performance liquid chromatography (dHPLC) on a WAVE 4500HT platform (Transgenomics). All samples that were showing aberrant WAVE forms were submitted to direct sequencing. We did not find any obvious deleterious functional changes in either the coding sequence or the splice junctions in the *KCNC3* gene. A number of intronic mutations were identified as well as a novel SNP in exon 2 of the *KCNC3* gene (silent mutation GGC (Gly) to GGT (Gly)). Taken together, our data suggest that mutations in *KCNC3* are not likely to contribute significantly to spinocerebellar ataxia in the UK.

In parallel, primary Purkinje neuron cultures were established in the laboratory. We planned to use these for studying the functional effects of mutations in genes associated with cerebellar ataxia, however as we did not identify any novel *KCNC3* mutations in our patient panel, we did not proceed with the originally proposed functional assays. However, any mutations identified in the future will be analyzed in our primary culture system.

Given our extensive collection of ataxia patient DNAs, we took the opportunity to screen this patient databank for mutations in the *PRKCG* gene, which is mutated in SCA14. Our screens in the past have led to the characterisation of the first UK family harbouring a novel mutation in protein kinase C (PKC) gamma leading to SCA14 (manuscript in preparation). Subsequent functional studies of this PKC gamma mutation have identified a new cellular mechanism that might provide an explanation for the neurodegeneration occurring in SCA14 (manuscript in preparation). To identify further mutations in the *PRKCG* gene, we screened our panel of ataxia patients for *PRKCG* gene alterations as described above. We have identified a number of aberrant WAVE forms and the respective DNA samples are currently analyzed by direct sequencing. Our preliminary data as well as human *PRKCG* mutations published in the literature suggest that SCA14 is a cause of ataxia in the UK population but at a low level.

Based on our findings, we also screened the Harwell ENU mutagenesis mouse DNA bank for mutations in *PRKCG*. There is currently no SCA14 mouse model available and conventional methods including transgenic and knock-in approaches have failed so far in producing one. We have screened more than 5000 F1 progeny of mutagenised mice by dHPLC using the WAVE platform as described above. We have identified a functional mutation in exon 4 of the mouse *PRKCG* gene, i.e., a single A-to-G base pair change resulting in a dramatic aspartate-to-glycine amino acid change. This finding is very exciting as exon 4 is a hot-spot for *PRKCG* mutations in humans. The mutant mice have been rederived from the frozen sperm archive at Harwell and are currently backcrossed and aged in our animal facility. We will soon start to analyse these mice for any defects in motor function.

Lay summary:

The gene *KCNC3* codes for a protein called KCNC3 which is a potassium channel. This gene is mutated in people with SCA13. The aims of this project were to determine the frequency of *KCNC3* mutations in UK patients with ataxia and to examine the effects of the mutations on the normal function of the protein.

We screened 119 patients with or without a family history of cerebellar ataxia for abnormalities in the *KCNC3* gene. No obvious deleterious functional changes were found in the *KCNC3* gene. Although some areas of the *KCNC3* gene were found to be different to normal, the alterations were either not the correct type or were not in the correct regions of the gene to alter the formation of the protein. Our data therefore suggest that mutations in *KCNC3* are not likely to contribute significantly to spinocerebellar ataxia in the UK.

At the same time as the screening, we also developed primary Purkinje neuron cultures. These cultures were intended to be used to test the function of the mutated KCNC3 protein. However, as we did not identify any novel *KCNC3* mutations in our patient panel, we did not proceed with the originally proposed functional assays. These primary cultures will, however, be a useful system for analysing the functional effects of any mutations identified in the future as causing cerebellar ataxia.

As the DNA of UK ataxia patients was already available to us from the first part of the study, we took the opportunity to screen the samples for mutations in a different

gene; the *PRKCG* gene. The *PRKCG* gene is mutated in SCA14. A number of abnormal forms of the gene were discovered and our preliminary data suggest that SCA14 is a cause of ataxia in the UK population but at a low level.

There is currently no SCA14 mouse model available to researchers. Conventional methods of developing such a model, including techniques whereby a mutated form of a gene is inserted into an animal's DNA, have not been successful. Therefore a different approach, using mice with randomly-created mutations in their DNA, was tried. The DNA of these mice was analysed to investigate whether any of them carried mutations in the *PRKCG* gene. We identified a mutation in the mouse *PRKCG* gene that alters the way that the PRKCG protein is formed. This discovery is particularly exciting as the mutation lies in a region of the *PRKCG* gene that is a hot spot for *PRKCG* mutations in humans. The mutant mice are currently being bred in our animal facility and we will soon start to analyse them for any defects in motor function.

Benefits to people with ataxia arisen/likely to arise from this research:

Our data suggest that mutations in *KCNC3* are a very rare cause, if any at all, of spinocerebellar ataxia in the UK. In contrast, we have already identified the first family with mutations in *PRKCG* in the UK and might spot further SCA14 patients through our continued screening efforts. These findings are important for the diagnosis and genetic counselling of ataxia patients in the UK. In addition, our SCA14 mouse model will be of great value in understanding the molecular mechanisms underlying spinocerebellar ataxia as well as for drug testing.

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