Author's response to reviews

Title: Application of targeted multi-gene panel testing for the diagnosis of inherited peripheral neuropathy provides a high diagnostic yield with unexpected phenotype-genotype variability

Authors:

Thalia Antoniadi (thalia.antoniadi@nhs.net)
Chris Buxton (chris.buxton@nhs.net)
Gemma Dennis (gemma.dennis@nbt.nhs.uk)
Natalie Forrester (natalie.forrester@nbt.nhs.uk)
Debbie Smith (debbie.smith@nbt.nhs.uk)
Peter Lunt (peter.lunt@bristol.ac.uk)
Sarah Burton-Jones (sarah.burton-jones@nbt.nhs.uk)

Version: 2  Date: 26 June 2015

Author's response to reviews: see over
Dear Dr Tim Sands,

Thank you very much for the opportunity to review our manuscript and resubmit, following the Reviewers’ comments. We very much appreciate the consideration and constructive recommendations that the Reviewers have given, and we have made every effort to address these.

Below we describe our responses to each comment.

Reviewer Heather McLaughlin

Major compulsory revisions

1. The validation of the assay was presented in the Gene Dossier that was submitted to the UK Genetic Testing Network and was approved in January 2013; this is available on the UKGTN website and for that reason we did not include any details in the initial manuscript. We have now addressed this omission by referring to the key points in page 5, paragraph 2 and paragraph 4.

2. We have made our classification strategy clear with the inclusion of a new table, Table 2: Criteria applied in the classification of variants. We have also updated the supplementary tables to include frequency, conservation, in silico pathogenicity predictions and results of segregation studies where available. No functional studies were undertaken.

We have added a comment to the supplementary table 1 (listing the clearly pathogenic variants), to confirm that they have been reported in the literature with confirmed pathogenicity; however there are in excess of 130 associated references and we felt that this would make the list of references extremely long without necessarily adding value. If however this is additionally required, we are happy to address.

Minor essential revisions

1. The phrase has been altered to read: "In our experience NGS is efficient and removes the need for serial gene sequencing in most cases".

Discretionary revision

2. The detail requested is presented in Table 1, where the phenotypes associated with each genes are listed with their OMIM disease and gene numbers.

Reviewer Byung-Ok Choi

Major points

1. We have addressed this point by presenting our criteria in Table 2, Criteria applied in the classification of variants. We have also updated the supplementary tables to include frequency, conservation, in silico pathogenicity predictions and results of segregation studies where available.
2. We are in agreement that this panel is not an efficient way to detect PMP22 CNV and we have now clarified that the reason we wanted this to be possible was to ensure that a genetic diagnosis will not be missed (see paragraph 1 page 5).

3. We agree that detailed clinical information would be useful for novel variants and complex cases. As a diagnostic laboratory we are provided with a limited and variable level of detail regarding the clinical symptoms and history of patients referred to us for testing. We continue to work in correspondence with clinicians to gather further information. It may be that such cases could be presented comprehensively with a clinical focus in the future, as this level of detail is not achievable in the immediate time frame. At this time, we wish to make public the gene variants that we have found in order that other interested parties can access this information and contact us should they wish to discuss further. This could lead to potential collaborations for functional analysis of unclassified variants, which may assist in their classification and contribute to our overall understanding of the disease.

Additional revisions/comments

Please note, in the process of revision we identified two SCN9A variants (in the same single patient) in Supplementary table 2 which had been reclassified as benign since the time of drafting the initial manuscript. These have therefore been removed from that table. Other numbers and statistics throughout the text have been updated accordingly.

We have added a statement regarding consent in the Methods section, under Patients.

Bristol Genetics Laboratory is an accredited (CPA/UKAS) UK NHS Diagnostic Laboratory. Here we present anonymous results from our routine diagnostic cohort; this work was not a research study and therefore was not subject to ethical approval. The decision to request the test was made by each requesting clinician according to their clinical judgement, in the interest of the patient, and according to their local ethical guidelines.

We very much look forward to hearing from you again in the near future.

Yours sincerely

Sarah Burton-Jones

Acting Principal Clinical Scientist; Neuromuscular Division