

# The diagnostic utility of clinical exome sequencing in 60 patients with hearing loss disorders: A single-institution experience

## 1 | INTRODUCTION

Congenital ear anomalies and hearing impairment are often present in patients with underlying genetic disorders. Germline genomic variants are responsible for at least 50% of congenital and/or childhood-onset sensorineural hearing loss (SNHL). Furthermore, 20%–60% of patients with bilateral microtia and congenital aural atresia may have an identifiable genetic syndrome.<sup>1</sup>

Standard diagnostic approaches involving single gene testing and chromosomal microarrays have limited utility in diagnosing monogenic conditions with high degrees of genetic and phenotypic heterogeneity.<sup>2</sup> Delay in confirming a genetic diagnosis can lead to incorrect diagnostic workup, uncertain prognosis, inadequate treatment, delayed referral to relevant medical subspecialties and lack of anticipation of potential additional comorbidities.

The development of next-generation DNA sequencing (NGS) has contributed significantly to the diagnosis, study and care of Mendelian monogenic disorders. The capacity to perform simultaneous sequencing of multiple genomic regions makes NGS particularly appropriate for the investigation of genetically and clinically heterogeneous conditions, such as hereditary hearing loss. NGS-based-targeted gene panels and exome sequencing have become increasingly available for monogenic disorders. Reported diagnostic rates range between 20% and 50%, depending upon the patient cohort and the chosen testing platform.<sup>3</sup>

Next-generation sequencing approaches can lead to molecular diagnoses which can inform clinical decision-making. Clinical exome sequencing (CES) is therefore increasingly being used for testing patients with congenital ear anomalies and hearing disorders.<sup>4–6</sup> As the clinical availability of NGS increases, there is a need to evaluate its impact in routine practice. This study reports experience with CES in 60 consecutive patients with congenital ear or hearing disorders with a suspected genetic aetiology, to determine diagnostic yield and document the clinical implications.

## 2 | PATIENTS AND METHODS

We conducted a retrospective survey of clinical and molecular results from 60 consecutive patients with ear and hearing disorders, referred for proband-only clinical exome sequencing (CES) to the NHS North West Genomic Laboratory Hub. CES was performed for diagnostic purposes, with prior consent by the patients and/or their guardian(s). All patients were evaluated and referred for CES by a consultant clinical geneticist. CES experiments were conducted using a custom-designed Agilent SureSelect XT Focused Exome capture library and the NextSeq 500 sequencer (Illumina, Inc). A phenotype-driven virtual gene panel was generated per patient, customised based on their clinical features, as described previously.<sup>7</sup> Following bioinformatics analyses (supplemental material), the clinical significance of candidate variants was interpreted independently by two registered Clinical Scientists as per the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) guidelines.<sup>8</sup> These guidelines assign genomic variants identified into a five-tier system based on different types of evidence (population data, computational and predictive analysis, functional criteria and segregation data). As per these criteria, genomic variants are classified as pathogenic, likely pathogenic, variant of unknown significance, likely benign and benign.<sup>8</sup> Where needed, cases were further reviewed at internal Multidisciplinary Team meetings or through internal communication between the consultant clinical geneticist and clinical scientists from the Rare Disease Clinical Exome team.

## 3 | EVALUATION OF CES DIAGNOSTIC UTILITY

A search was conducted in the internal referral database identifying patients with phenotypes matching query terms (Table S1, supplemental material) that indicated the presence of hearing loss and/

or abnormalities of the external, middle or inner ear, either isolated or as part of their phenotype. Gender, age at referral, clinical features, previous genetic testing, CES results and actions prompted after CES were obtained from available medical records, the laboratory's internal database. Finally, to determine the diagnostic rate, CES results were categorised as follows: (1) confirmed, (2) possibly confirmed and (3) not confirmed (Table S2).

## 4 | RESULTS

### 4.1 | Cohort clinical characteristics

Seventy-eight per cent (47/60) of patients were under age 18 (median 6, range 0–73 years, 95% CI 7.51–14.73). A 40:60 female: male ratio was observed. Twenty patients (33%) had apparently isolated sensorineural hearing loss (19 bilateral and 1 unilateral SNHL). Twenty-seven patients (45%) had SNHL as part of a complex phenotype (25 bilateral and 2 unilateral SNHL). Ten (17%) had a degree of microtia and/or atresia accompanied by other clinical features. Three (5%) were referred with mixed hearing loss with additional clinical features, most commonly neurological or ophthalmic disorders (Table 1).

### 4.2 | Genetic investigations prior to CES

Thirty-six patients (60%) had some genetic testing prior to undergoing CES (Table 2). No variants of clinical significance were reported

**TABLE 1** Additional phenotypes in patients with syndromic SNHL and syndromic microtia-atresia ( $n = 41$ )

| Category                                   | No. of patients with reported abnormality (%) |
|--------------------------------------------|-----------------------------------------------|
| Neurodevelopmental delay                   | 16 (39%)                                      |
| Ophthalmic disorders                       | 14 (34%)                                      |
| Central nervous system malformation        | 7 (17%)                                       |
| Other neurological abnormalities           | 6 (15%)                                       |
| Oral cleft                                 | 6 (15%)                                       |
| Congenital cardiovascular defect           | 5 (12%)                                       |
| Limb abnormalities                         | 4 (10%)                                       |
| Facial dysmorphology                       | 3 (7%)                                        |
| Skin disorder                              | 3 (7%)                                        |
| Renal abnormalities                        | 3 (7%)                                        |
| Abnormal thyroid physiology/<br>morphology | 2 (5%)                                        |
| Retrognathia/micrognathia                  | 2 (5%)                                        |
| Upper aerodigestive tract abnormality      | 2 (5%)                                        |
| Gastrointestinal abnormality               | 2 (5%)                                        |
| Early-onset obesity                        | 1 (2%)                                        |
| Growth abnormality                         | 1 (2%)                                        |

Abbreviation: SNHL, sensorineural hearing loss.

### Key points

- Genetic aetiologies are an important cause of congenital ear anomalies and hearing impairment.
- Next-generation sequencing (NGS) strategies, such as targeted gene panels or clinical exome sequencing (CES), are effective tools in the diagnosis of patients with inherited hearing impairment, with clear advantages over previous genetic testing approaches.
- Clinical exome sequencing in our cohort shows the genetic heterogeneity of syndromic and non-syndromic congenital ear and hearing disorders, highlighting the clinical utility of undergoing genomic investigations.
- Multidisciplinary decision-making for diagnostic workup and management, including close collaboration between genetics, otolaryngology, audiology and other allied specialties, is key in the investigation of congenital ear and hearing disorders.
- Timely molecular genetic diagnosis can streamline patient care and potentially improve clinical outcomes.

in 32 patients (88%), and inconclusive findings were reported in the remaining four: two had chromosomal microarray variants of uncertain significance (VUS), namely a balanced rearrangement of chromosome 10 and a deletion on the X chromosome. Two patients had single heterozygous variants in *GJB2*; c.306G>C, p.(Lys102Asn) and c.101T>C, p.(Met34Thr), but no second *GJB2* variant was found *in trans* in either patient, rendering these findings unlikely to account for their auditory phenotype.

### 4.3 | Variant spectrum and diagnostic yield

Forty distinct variants were identified in 24 genes (Table S3). Genes most frequently harbouring variants were *SLC26A4*, *LOXHD1*, *CDH23* and *CDH7*. Eleven variants (27%) were novel at time of analysis. Predicted loss-of-function and missense variants were reported in equal proportions ( $n = 13$ , respectively). Twenty-six variants (65%) were found as pathogenic or likely pathogenic and fourteen variants were classified as variants of uncertain significance (VUS) (35%) according to ACMG guidelines.<sup>8</sup>

Clinical exome sequencing resulted in an overall diagnostic yield of 31% (19/60). This was higher in patients with sensorineural hearing loss: 60% (12/20) in the non-syndromic 22% (6/27) in the syndromic SNHL groups (Figure 1). Only one case with syndromic microtia-atresia, was categorised as “possibly confirmed”, with a homozygous class 3 variant in *ORC6* (Meier-Gorlin syndrome type 3). Autosomal recessive inheritance underpins 85% of diagnoses in this cohort without a family history. Inconclusive findings were reported in 11 patients (18%): Seven presented with complex phenotypes

**TABLE 2** Genetic testing prior to CES

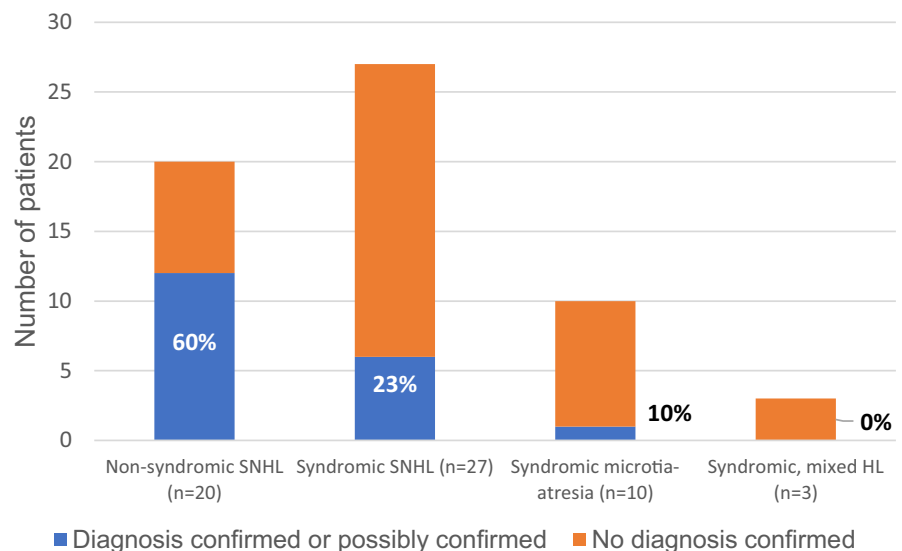
| Genetic investigation                     | No. of cases | Clinical category                                                                                           |
|-------------------------------------------|--------------|-------------------------------------------------------------------------------------------------------------|
| <i>GJB2/GJB6</i> testing                  | 19           | Non-syndromic SNHL ( <i>n</i> = 14)<br>Syndromic SNHL ( <i>n</i> = 4)<br>Syndromic mixed HL ( <i>n</i> = 1) |
| Chromosomal microarray                    | 12           | Syndromic SNHL ( <i>n</i> = 9)<br>Syndromic microtia-atresia ( <i>n</i> = 3)                                |
| Fragile X syndrome                        | 3            | Syndromic SNHL ( <i>n</i> = 2)<br>Syndromic microtia-atresia ( <i>n</i> = 1)                                |
| m.1555A>G                                 | 2            | Syndromic SNHL ( <i>n</i> = 1)<br>Non-syndromic SNHL ( <i>n</i> = 1)                                        |
| NGS gene panel <sup>a</sup>               | 2            | Syndromic SNHL ( <i>n</i> = 2)                                                                              |
| Specific single gene testing <sup>b</sup> | 1            | Syndromic SNHL ( <i>n</i> = 1)                                                                              |

Abbreviations: NGS, next-generation sequencing; SNHL, sensorineural hearing loss.

<sup>a</sup>Leeds leukodystrophy and mitochondrial leukodystrophy panels; Newcastle panel of genes for complex I deficiency.

<sup>b</sup>SCA17 gene.

**FIGURE 1** Diagnostic rate of clinical exome sequencing (CES) per clinical hearing loss category. Percentages indicate proportion of cases with confirmed and/or possibly confirmed genetic diagnosis. HL, hearing loss; SNHL, sensorineural hearing loss



including developmental delay, learning disability, myopathy and/or dual sensory impairment (Table S3). No plausibly pathogenic variants were identified in 30 patients (50%).

#### 4.4 | CES directs clinical care

Clinical exome sequencing results directly informed clinical decision-making in 15 patients (25%). Five were referred for assessment or reassessment by other specialists. One patient referred with non-syndromic SNHL, had a homozygous likely pathogenic variant in *CDH23*, prompting reassessment by ophthalmology to assess for any evidence of Usher syndrome. Two patients were referred to specialist multidisciplinary clinics for inherited cardiac anomalies and CHARGE syndrome respectively. Two patients were referred back to audiology for further detailed phenotypic evaluation. In five cases, molecular findings

prompted genetic investigations in similarly affected family members. Results in a patient with bilateral SNHL and myopathy with a possible dual diagnosis involving *CEP78* and *ETFDH* enabled testing and early diagnosis in a similarly affected daughter. Another patient was found to be a carrier for Becker muscular dystrophy, which enabled cascade testing and clinical investigations for family members. Finally, nine patients without confirmed diagnosis underwent whole genome sequencing as part of the 100 000 Genomes Project.<sup>9</sup>

## 5 | DISCUSSION

The implementation of genomic sequencing approaches has demonstrated diagnostic utility in the context of SNHL.<sup>10</sup> Here, we report our experience with CES in a cohort of patients presenting with a variety of ear and hearing loss phenotypes. Our diagnostic rate of

31% is broadly comparable to the available literature for this patient group<sup>4,5</sup> and shows an increased diagnostic utility in comparison with previously available genetic testing. In settings where patients present with a diverse range of genetically and clinically heterogeneous conditions, CES can offer effective diagnostic rates and represent a long-term, more cost-effective and suitable choice compared with targeted NGS approaches (i.e., targeted diagnostic gene panels).<sup>2,11</sup> While a few technical limitations still exist,<sup>5</sup> CES clearly can increase genetic diagnostic rates in comparison with previously available standard genetic testing for hearing loss.

Timely identification of a genetic diagnosis can have strong implications in the context of ear and hearing disorders. Firstly, the early confirmation or exclusion of syndromic conditions can inform future diagnostic workup and promote more cost-effective use of healthcare resources.<sup>12</sup> Compared with standard of care, CES can reduce unnecessary diagnostic interventions through the increased identification of molecular diagnoses. It has been shown that, while CES incurs additional costs, it can be cost-effective in hearing loss due to the increase in diagnostic yield.<sup>13</sup> Results can streamline diagnostic interventions and treatment options. In a hearing loss context, timing of testing can be key in assessing the clinical utility and consequent economic impact of genomic sequencing. It is possible that redefining the hierarchy of diagnostic testing battery is required to balance the number of interventions needed to obtain an adequate amount of clinical data to be used for clinical interpretation of genomic sequencing variants. Further health-economics research should be conducted to validate this across different payer healthcare systems.

Secondly, the increasing integration of genomic, clinical and laboratory data, including outcomes, can be used to develop prognostic models that inform management decision-making. Although it is highly unlikely that genomic diagnosis would preclude cochlear implantation or any other type of hearing habilitation or rehabilitation, molecular diagnosis can facilitate the identification of patients in need of targeted rehabilitation due to a predicted risk of poor performance. There is a growing body of evidence on the use of genetic diagnosis for prediction of cochlear implantation outcomes,<sup>14,15</sup> and in SNHL associated with enlarged vestibular aqueducts, the presence or absence of key genomic variants may also be of prognostic value for hearing loss severity and/or progression.<sup>16</sup> Early genetic diagnosis can inform clinical care teams of an increased risk of hearing loss progression, warranting closer surveillance that prompts early consideration of cochlear implantation. Finally, genomic findings are clinically relevant to family members, permitting confirmatory testing and accurate counselling about reproductive risks and choices.

While this study is limited by a relatively small sample size and its retrospective design, it confirms the current diagnostic capability of CES and offers some insights into real-life, clinical use of genomic sequencing. It highlights the heterogeneity in patients currently referred to NHS Genomic Medicine services. It is also important to note that the diagnostic capability of CES is enriched by good phenotyping. Analysis of CES data is clinically driven and thus requires

detailed phenotyping. The need for phenotype reassessment in some patients denotes the importance of pursuing further integration between clinical and genomic services. Multidisciplinary team evaluation by ENT surgeons, audiologists and clinical geneticists can enhance the quality of phenotype data and reduce referral delays between specialities. Consequently, this can facilitate interpretation of CES data, shorten turnaround times and expedite molecular diagnosis, allowing for a timely optimisation of individualised hearing and disease surveillance.

In summary, CES is a powerful tool in the diagnostic investigation of patients with ear and hearing disorders, with direct implications for patient care. Due to the prevalence and diversity of genetic aetiologies for congenital ear anomalies and hearing impairment, incorporating early molecular diagnosis into existing comprehensive multidisciplinary care has the potential to improve both patient counselling (regarding recurrence risks and disease prognostication) and, in turn, clinical outcomes.

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#### CONFLICT OF INTEREST

We also hereby declare that none of the authors have any disclosures to share, nor any conflict of interest.

#### AUTHOR CONTRIBUTIONS

LPMR, EMMBW, IAB and GCMB designed and coordinated the study. LPMR, EMMBW, CK and AT collected the data. LPMR, EMMBW, HS, IAB and GCMB analysed and interpreted the data. SSB contributed to bioinformatics and maintenance of bioinformatics pipeline. AT contributed to virtual gene panel algorithm and development of clinical exome web referral system. LD contributed to exome sequencing experiments. CK, RW, EMMBW, SSB, CC, SB, AS, CF, JHMcD, TAB, JCS, SD, KC, WGN and GCMB contributed to genetic and/or phenotypic data. LPMR, EMMBW, HS, IAB and GCMB wrote the manuscript. All the authors revised the manuscript for important intellectual content and approved the final version.

#### ETHICAL APPROVAL

This study was conducted as an audit of results and performance following changes to the standard procedures for the referral and analysis of the existing Clinical Exome service. Through the clinical exome-informed consent process, patients consented to having their

sample, genomic data and clinical information shared internally and with other researchers through scientific publications, controlled-access databases and open-access databases.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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