NEONATAL ANTECEDENTS OF HEARING LOSS IN VERY PRETERM INFANTS

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Declaration

I, Kathy Chant, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Background

The prevalence of hearing impairment in infants born prematurely or with low birth weight is around 1-2%, up to 10 times higher than babies born at term. The aetiology of which is poorly understood; risk factors are likely to be interrelated. Susceptibility to the audiological toxicity of gentamicin, frequently given to newborn babies, is increased in the presence of m.1555A>G, a mitochondrial mutation.

Objectives

This study aims to investigate the aetiology of hearing loss in infants following neonatal intensive care, and the burden that m.1555A>G represents to deafness in children born at less than 32 weeks gestational age.

Method

This was a case control study of preterm children with hearing loss in Greater London. Controls (up to 5 per case) had normal hearing and were matched for sex, gestational age and hospital in which they received neonatal care. Saliva samples were taken from all children for genetic screening of m.1555A>G. Audiological, pharmacological and clinical data were abstracted from medical notes.

Results

Two hundred and thirty seven children, 57 children with hearing loss, were recruited to the study. Independent risks included low gestational age, low birthweight, patent ductus arteriosus, acquired brain injury, the use of inotrope, steroid, vancomycin, furosemide, elevated bilirubin and creatinine levels. Cumulative doses of gentamicin, vancomycin, and furosemide also increased the risk of impaired hearing. One child with normal hearing had

the m.1555A>G mutation and had been exposed to aminoglycosides. A high frequency hearing loss has since been detected.

Discussion

The prevalence of m.1555A>G was 0.41%, and is unlikely to explain the increased rate of hearing loss in preterm infants. Children with hearing loss were exposed to a greater number of interacting risk factors across a timeline of care. Cumulative ototoxic medication in particular, increased the likelihood of hearing loss, warranting closer monitoring throughout neonatal care.

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1 Chapter 1: Introduction

1.1 Hearing loss in preterm infants

Substantial advances in neonatal care have improved the survival rates of infants born very prematurely. This has primarily led to reduced mortality at extremely low gestations but also to increases in the proportion of survivors without disability, at the cost of a small increase in the numbers of children with moderate or severe impairments in motor, cognitive, visual and hearing domains [1]. In particular there has been no discernible change in the uncommon but important prevalence of hearing loss in this group.

The prevalence of hearing loss in newborn infants in the general population is around 1 in 700-1000 [2], which increases to 2.7 per 1000 children before the age of 5 years [3]. The incidence of hearing loss in children born prematurely is estimated to be up to 10 times higher than the normal paediatric population [4]; hence this thesis will examine potential acquired causes for hearing loss after birth. However, it should be noted that prevalence rates vary greatly between studies, which show significant methodological heterogeneity. In general NICU populations including term and preterm infants, there is a range of 0.07-11% between different studies (Appendix 1), which may increase up to 54% when looking at specific groups of infants within the NICU population (Appendix 2). The range of reported prevalence between studies indicate differences in gestation, severity of illness, and definition and measurement of hearing loss.

Childhood deafness has broad long term implications including social and emotional development, educational achievement, and later vocational opportunities, even in mild cases of unilateral or bilateral hearing loss [5]. Dependent on the age of onset, hearing impairment can be described as prelingual (prior to the development of speech) or postlingual (following speech development) [6]. Early identification is essential and likely to

lead to a reduction in the wider complications of growing up deaf; current policy is for all infants to be screened prior to discharge from hospital (https://www.gov.uk/government/publications/newborn-hearing-screening-care-pathways). Screening failures are then rapidly evaluated to come to a firm diagnosis and commence early interventions.

Following preterm birth, permanent hearing loss is of two main types, sensorineural hearing loss (SNHL) and auditory neuropathy spectrum disorder (ANSD). SNHL is a result of damage to the outer hair cells of the cochlea, but the inner hair cells, auditory nerve and brainstem are intact. These outer hair cells are unable to regenerate and any impairment cannot therefore be reversed. SNHL can be congenital (present at birth) or acquired during the lifetime. Probable causes of hearing impairment include sepsis, hypoxia, hyperbilirubinaemia, noise and drug-induced ototoxicity. Furthermore, risk factors are likely to be interrelated, such as interactions between genetic and environmental risk factors which can be a further cause of hearing loss (discussed in section 1.3.2). However, due to the complexity of these relationships, aetiology remains inconclusive.

In contrast, ANSD is typically "retrocochlear", and can be an impairment of the eighth cranial nerve or the inner hair cells. The outer cochlear hair cells are functional but brainstem responses are abnormal or absent [7]. A reduction in the number of neurons, or a disordered neural signal are thought to underlie the disrupted responses [8]; most cases appear to result from an impaired auditory nerve or inner hair cells. In some individuals with ANSD, genetic abnormalities may predispose to hearing impairment, such as mutations in the otoferlin gene (*OTOF*). The localisation of ANSD may differ depending on the site of the lesion and therefore provides a varying clinical picture between individuals. ANSD emerged in the 1980's as a separate condition from SNHL, but did not become a separate diagnosis until the mid 1990's,

therefore most of the older studies will not have differentiated between the two conditions. Furthermore, there remain studies that still consider ANSD to be a form of SNHL.

The Newborn Hearing Screening Programme (NHSP) was introduced across the UK in 2006. All infants born in the UK are screened for a hearing impairment shortly after birth, and preterm infants prior to being discharged home. Initial screening for all infants assesses the functioning of cochlear outer hair cells using otoacoustic emissions (OAEs). OAEs are the responses of outer cochlear hair cells following an auditory stimulus (either a click or simultaneous tones of differing frequency) which generates a sound detected by a microphone in the ear canal [9]. OAEs are dependent on the functioning of the cochlear outer hair cells and provide no indication of the inner hair cells, auditory nerve or brainstem pathways.

The other commonly used method of hearing assessment is auditory brainstem responses (ABR). This detects abnormalities from the inner hair cells, to the auditory nerve or auditory brainstem pathway by measuring the electric field potentials produced by the brainstem following rapid clicks or tone pips which stimulate the cochlea. Generated field potentials produce a waveform response with the auditory stimulus being decreased until the waves are absent. Waves I and II demonstrate activity of the distal and proximal auditory nerve, with waves III, IV and V indicating activity from the auditory brainstem structures [10]. The number of neurons firing (amplitude) and the speed at which waves are detected (latency) are also monitored.

Hearing function is measured using auditory thresholds, which are the quietest sounds that can be detected. Sound levels are measured in decibels hearing level (dB HL), with a normal threshold at 0 dB HL up to 20 dB HL. The severity of hearing impairment is usually categorised as mild (20-40 dB HL), moderate (41-70 dB HL), severe (71-95 dB HL), or profound (>95 dB HL), ranging from low frequency (250 Hz) to high frequency (8000 Hz).

Whilst early screening is essential for early recognition and timely intervention, it is not always effective at identifying all forms of hearing loss. For example, SNHL may have a delayed onset [11], and could be a progressive loss [12], which may not be detectable from birth. Furthermore, ANSD shows normal OAEs but abnormal or absent ABRs [13], and would not be detectable by routine newborn hearing screening which relies on OAE responses. Some children with ANSD may therefore be missed at initial screening giving false negative results, and there is some debate as to which method should be used for the first test, although for preterm or 'at risk' infants, both tests are now used.

As well as differences in clinical presentation and method of diagnosis, prevalence rates between SNHL and ANSD also vary. Research has reliably shown the rates of ANSD to be lower than SNHL, although inconsistences in population samples and methodology lead to a marked variance in rates between studies. For example, in an American study of 4250 NICU infants, the incidence of ANSD was 5.6/1000; much lower in comparison to SNHL which had a rate of 16.7/1000 [14]. However, in a UK based study of 45 050 infants that looked specifically at severe to profound hearing loss the difference between prevalence rates of SNHL and ANSD was much smaller (0.67/1000 and 0.27/1000, respectively) [15]. Comparisons between prevalence rates vary with selected populations.

Children with prelingual SNHL and ANSD, are at risk of significant speech perception difficulties and speech and language development problems. Both forms of hearing impairment can be irreversible and treatment consists of hearing aids to amplify sound. When hearing aids are insufficient, a cochlear implant (CI) may be considered. Following the detection of sound, CI provides an electrical stimulation directly to the auditory nerve for the transduction of a neural signal. Although hearing aids and CI do not restore normal hearing they enable speech to be perceived; children with hearing loss in receipt of early treatment

will often develop near normal speech and language, especially if there are no additional problems.

There is much uncertainty as to the causal pathway of hearing loss and a range of risk factors have been described, which in turn contribute to current understanding of deafness in relation to neonatal events and treatments.

1.1.1 Risk factors for hearing loss

The Joint Committee on Infant Hearing (JCIH) identified 10 risk factors associated with hearing loss [16]. These were: family history of hearing loss, congenital infections, craniofacial abnormalities, low birth weight (less than 1500g), hyperbilirubinaemia, ototoxic medications, bacterial meningitis, low Apgar scores at 1 or 5 minutes, assisted ventilation for 5 days or more, and syndromes associated with hearing loss. Additional risk factors have also been proposed, including gastrointestinal surgery, cardiac surgery, treatment for hypotension, hyponatremia, the administration of furosemide (including with simultaneous elevated creatinine levels), prolonged oxygen use and noise [12]. Despite continual improvements in neonatal care, many of these risk factors have not decreased in prevalence including necrotising enterocolitis (NEC) and bronchopulmonary dysplasia (BPD) [17]. Interactions between risk factors have also been established [4].

The aetiology of hearing loss in the general population can be categorised as genetic (syndromic or non syndromic) or non genetic, with approximately 50% being attributed to each [3]. However, recent population based studies have found genetic and non genetic causes to be less common than originally estimated; between 24 and 45% of children appear to have hearing loss of unknown aetiology [18-21].

I will now review studies that have evaluated potential causation of hearing loss in babies following neonatal intensive care, starting with non genetic risk factors.

1.2 Non genetic risk factors for hearing loss

Clinical risk factors for hearing loss in infants include gestational age and birthweight, hypoxia, respiratory support, acquired brain injury, hyperbilirubinaemia, infection, noise, and medication, which will be considered in the following sections.

1.2.1 Gestational age and birth weight

Very preterm infants (<32 weeks of gestation), and those with the most immature gestational ages and lowest birth weights in particular, often have a complicated neonatal course. A preterm infant is defined as an infant born before 37 completed weeks of gestation, with subcategories of moderate to late preterm (from 32-37 weeks of gestation), very preterm (between 28 and 32 weeks of gestation) and extremely preterm (born below 28 weeks of gestation). Despite many studies using gestational week as a cut off for inclusion, some, particularly early, studies adopt low birth weight as an alternative inclusion criterion. Low birth weight (LBW) is defined as an infant weighing less than 2500g at birth, very low birth weight (VLBW) is below 1500g and extremely low birth weight (ELBW) is less than 1000g. Additionally, there are infants who are small for gestational age (SGA) who are born with a birthweight below the 10^{th} percentile for gestational age. Subsequently, these babies face additional risk for adverse outcome. Gestational age is preferential over birthweight as the most important predictor of preterm survival [17]. Prematurity and low birth weight are intimately related and have both been associated with hearing loss.

In a large study of 18 564 infants born at less than 32 weeks of gestation, the prevalence of hearing loss was measured according to week of gestation and category of birth weight (250g intervals between 750g and 1500g) [22]. Birth weight and gestational age were both found to be independent predictors of hearing loss, with hearing loss increasing gradually with decreasing week of gestation and decreasing birth weight. Interestingly, the frequency of

hearing loss remained relatively stable above 26 weeks of gestation. An interaction between gestational age and sex was also found, with females at a greater risk at less than 28 weeks, whilst males were at a greater risk of hearing loss when born at a gestational age of 30 weeks or below. This is supported by previous research that has found a male susceptibility to neonatal illness with increased risk of morbidities and poorer neurological outcome [23, 24]. Additionally there was an added risk conferred by being SGA, particularly for babies born below 27 weeks of gestation. This study considered infants born at 24 weeks and above, and it would be valuable to understand how hearing impairment may be more prevalent in survivors of more extremely preterm birth given the increase in survival rates (less than 24 weeks). It would also be of benefit to consider gestational age and birth weight for gestational age in context with illness.

The classification of hearing loss has also been investigated in relation to gestational age and birthweight. In a study of 24 infants with ANSD, 71 with SNHL and 95 normal hearing matched controls, the clinical characteristics of ANSD were examined in comparison to the other groups. The very smallest babies were the most likely to have a diagnosis of ANSD [14]. In the context of additional illness, both groups of infants were more likely to have had prolonged ventilation and BPD in comparison to the control group. It is difficult to disentangle the risks attributable to gestational age, birthweight and being small for dates from other factors which become more common as each decreases. Due to the 10 fold increase in risk of acquired hearing loss in preterm infants in comparison to term born babies, the inclusion criteria for the current study concentrated on babies born at less than 32 weeks of gestation.

1.2.2 Hypoxia

Hearing loss has been associated with hypoxia in both term and preterm infants. A lack of oxygenation and perfusion within the cochlea, specifically the outer hair cells and stria vascularis, results in decreased functioning [9]. Hypoxia limits the amount of energy available

for the sodium-potassium pump to generate the endocochlear potential within the stria vascularis. In the face of a reduced oxygen supply, the positive charge of the endocochlear potential (+80mV) decreases which causes a reduction of potassium moving from the endolymph into the hair cells, therefore lowering the sensitivity of hair cells within the cochlear [25]. Subsequently, audiological sensitivity is reduced leading to an elevation in hearing threshold [26].

The majority of studies focusing on hypoxia consider babies born at term, although several important studies have evaluated preterm infants, with contrasting results.

The clinical condition of the baby around the time of birth is increasingly thought to be a predictor of later outcome [27]. Hille et al (2007) found severe birth asphyxia was associated with hearing loss in 71 children from a nationwide cohort of 2186, born at less than 30 weeks of gestation and/or birth weight <1000g [28]. Although it is unclear as to how birth asphyxia was measured, a study looking at acidosis immediately after birth found similar results [29]. More specifically, a blood pH level below 7.25 in the first two hours following birth or on two or more days during treatment, was thought to have a crucial impact on cochlear perfusion and subsequent hearing impairment. Apgar scores also provide an indicator of clinical condition following birth and while preterm infants with SNHL have a higher incidence of low Apgar scores (at 1 minute) [30], studies using similar populations of preterm infants have not observed the same findings [31-33].

Postnatal hypoxia has also been associated with hearing loss in preterm infants. In a study of preterm (24-34 weeks) and very low birth weight babies (<1500g), hearing loss was associated with a greater number of episodes of low pH and hypoxemia [31]. Level of illness was also taken into account in this study, with infants grouped according to the number of variables representing their neonatal illness. All hearing impaired infants fell exclusively within the group of most unwell babies. Comparisons between infants within this group

showed the babies with SNHL to have a higher gestational age and birthweight but nevertheless, remained the most unwell with the highest illness scores. Although there were only 12 infants with SNHL, this may be suggestive of level of illness and associated treatments influencing hypoxia and therefore hearing loss. Similarly, Abramovich et al (1979) concluded that hearing loss in very low birth weight infants was likely to be a result of perinatal illness inducing hypoxia [34]. Apnoea requiring intubation and ventilation was the most significant predictor of hearing loss. Seven of the ten children with hearing loss had additional developmental deficits indicative of cerebral damage which suggests the hearing impairment could be related to the hypoxia, brain injury or both.

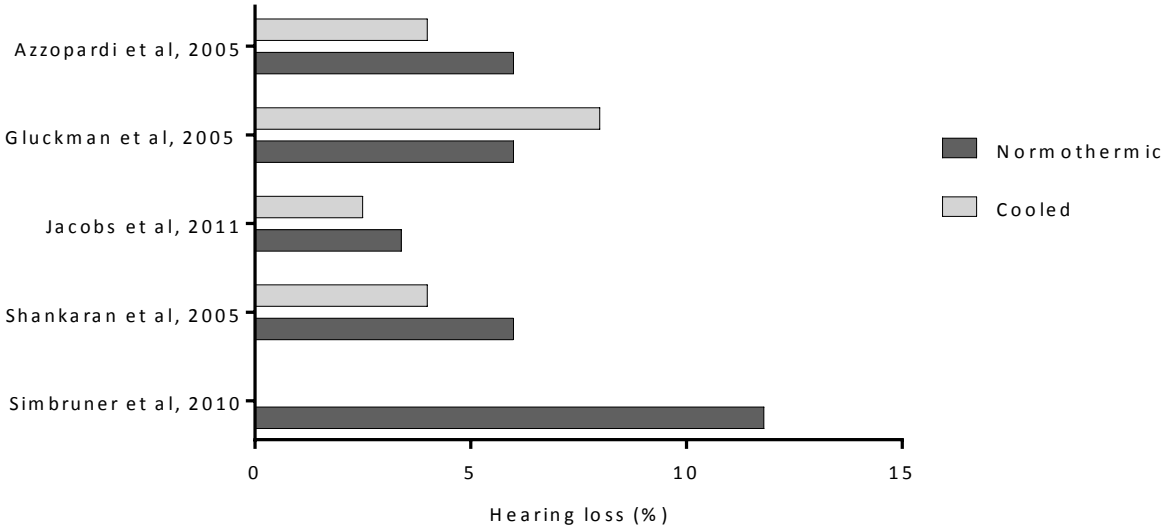
The definition of hypoxia can be somewhat ambiguous between studies with varying measures being used, including Apgar scores (different cut offs at 1, 5, 10 and 20 minutes [18, 33]), acidotic blood levels (with varying cut offs for pH and timing of acidosis [33, 34]), the number or length of apnoeic episodes [31], and apnoea requiring intubation and ventilation [34], causing difficulties in making direct comparisons between studies.

Literature has not identified at which point hypoxia becomes detrimental to hearing. Jiang et al (1995) investigated the effect of perinatal and postnatal hypoxia [26]. Comparisons were made between 3 groups of infants: perinatal asphyxia (based on 1 and 5 min Apgar scores with no neurological deficits), perinatal asphyxia (20 minute Apgar scores or seizures with signs of neurological deficits), and postnatal asphyxia (prolonged asphyxia between 3 and 12 months of age including respiratory failure and aspiration, all with neurodevelopmental deficits). Infants were excluded if they had postnatal complications unrelated to asphyxia that could cause hearing loss, although this was not defined. Prolonged wave V latencies and hearing loss at low intensities were identified, with permanent hearing loss occurring more frequently following asphyxia in the perinatal groups than in the postnatally asphyxiated

group. As hearing loss is markedly less common after 3 months of age it is possible that immaturity of the cochlea could increase susceptibility to damage via hypoxic insult.

Hearing impairment also shows a trend towards being less common following treatment with therapeutic hypothermia (figure 1-1). Therapeutic hypothermia is used to treat near term infants with hypoxic-ischaemic encephalopathy (HIE), by cooling the temperature of the head, the body or both to 33-34^{o}C. In four of five randomised controlled trials there was a 1-13% decrease in the number of children with hearing loss following therapeutic hypothermia [35-38]. Interestingly, selective head cooling was used in the cool-cap trial in comparison to whole body cooling in the other trials and had a higher rate of hearing loss in the cooled group [39]. Differences in technique in this study may have led to a lower temperature within the inner ear, which may explain the discrepancy in the hearing outcome. A meta-analysis of the five studies with two additional studies, one adopting whole body cooling and the other head cooling only ([40, 41] respectively), showed no significant effect of therapeutic hypothermia on SNHL [42]. Nevertheless, the two studies using selective head cooling indicated an increased number of children from the cooled groups, presenting with impaired hearing at neurodevelopmental follow up appointments. Cooling reduces cellular secondary energy failure following the hypoxic insult, potentially decreasing the period of cochlear sensitivity following birth, but selective head cooling may increase the risk of hearing impairment.

Figure 1-1: Sensorineural hearing loss comparison between cooled and non-cooled infants with hypoxic-ischaemic encephalopathy, in 5 randomised controlled trials



In animal models, decreased auditory function has been observed following prolonged episodes of hypoxia [43]. Oxygenation, and circulation within the cochlea were reduced and importantly, systemic blood pressure also dropped producing a local and central response to the hypoxic episode. Hypoxia and changes in cerebral circulation are also related to IVH, which will be discussed in section 1.2.4. Hypoxia, asphyxia and ischaemia are likely to have differing effects which will affect term and preterm infants in varying ways.

1.2.3 Respiratory support

Respiratory support is inextricably associated with hypoxia, sepsis and therefore medication use, and, indeed surgical interventions such as ligation of Patent Ductus Arteriosus (PDA). Respiratory support can include conventional positive pressure ventilation, high frequency oscillation (HFO), continuous positive airway pressure (CPAP), high flow and low flow oxygen. As an individual cause of hearing loss respiratory support has been debated with inconclusive results, mainly due to the differing ways of measuring respiratory support, such as number of days ventilated or more directly related to the categorical clinical condition requiring the

additional support such as bronchopulmonary dysplasia (BPD, defined as an infant being in oxygen at 28 days and in air at 36 weeks corrected gestational age, <30% oxygen at 36 weeks gestational age, or >30% oxygen at 36 weeks gestational age) [44]. Prolonged ventilation can cause persistent inflammation of the lungs which can contribute to BPD.

The risk of hearing loss is higher in infants following persistent pulmonary hypertension (PPHN), for which the need for high levels of respiratory support is crucial [45]. PPHN is associated with a number of causes including meconium aspiration and congenital diaphragmatic hernia (CDH). Treatment in some centres often involves hyperventilation with high levels of oxygen therapy which subsequently can cause cerebral vasoconstriction [46]. Conditions requiring this level of respiratory support can also cause neonatal hypoxia (discussed in section 1.2.2), and the two can be difficult to separate.

Robertson et al (2002) investigated SNHL in 90 survivors of respiratory failure at four years of age in a multicentre study in Canada [47]. An unusually high number of babies were found to have a hearing impairment; all 15 term or near term infants with CDH were found to have SNHL, and 15 (21%) infants with severe respiratory failure but without CDH had SNHL at two years of age which doubled by the age of four (42%). However, there was no difference between the groups in terms of respiratory support, inclusive of high frequency ventilation, the administration of inhaled nitric oxide or extracorporeal membrane oxygenation (ECMO). There is a strong likelihood that these infants had other antecedents for hearing loss such as ototoxic medication and diuretics, yet risk factors for hearing impairment other than respiratory failure were not explored in the study. Despite this, the rate of SNHL remains surprisingly high, which could be related to the selected sample of infants and the severity of illness as these results have not been found by other studies [48]. Rather than respiratory failure or support as a predictor of hearing loss, it could be the simultaneous risks that are incurred as a consequence.

The introduction of antenatal steroids and surfactant replacement therapy have reduced the prevalence and severity of respiratory distress syndrome (RDS) and improved lung function in preterm infants [44]. Subsequently, a reduction in further neonatal morbidities has seen both medications as being protective of hearing loss [49], however, severe respiratory failure continues to be associated with hearing loss in these populations. In a study of 1279 infants born at 28 weeks gestational age or less with a birth weight of 1250g or less, severe or profound hearing loss was found in 40 (3%) babies [12]. Significantly more babies with bilateral hearing loss received oxygen until 36 weeks corrected gestation. Prolonged ventilation is concordant with ligation of PDA and medical or surgical treatment of NEC, both of which were also predictors of hearing loss. Thus it is difficult to isolate which are the independent or combined causes of hearing impairment.

Bergman et al (1985) demonstrated that the maximum number of days requiring respiratory support was a significant predictor of hearing loss in a study of 72 children (36 NICU graduates with SNHL, 36 control) [32]. This study had a relatively small sample size with an equivalent number of controls whereas many studies have an increased number of controls to achieve statistical power. However, similar findings were found in an Italian study of 532 infants, 84 at high risk for hearing loss [50], and a Mexican study of 418 NICU infants, whereby both studies found number of days receiving mechanical ventilation (>9.6 days) was also significantly higher in a group of infants with hearing loss in comparison to controls [49]. Meningitis and intraventricular haemorrhage were other significant characteristics and the duration of ventilation could be related to a lower birthweight and more severe level of illness, both of which were more prevalent in the hearing loss group.

In a comparison of 24 infants with ANSD and 71 infants with SNHL and 95 matched control infants with normal hearing, the number of days requiring mechanical ventilation was specifically associated with ANSD in a comparison to infants with SNHL and normal hearing

[14]. In contrast, SNHL was more common in survivors of high frequency oscillation. BPD was associated with hearing loss in both groups but was also significantly greater in the babies with ANSD in comparison to SNHL. In a cohort of preterm infants with a birthweight of <750g, BPD was found in 5 of 6 of the infants with SNHL [51]. However, mechanical ventilation and the use of furosemide were also evident within the clinical history, both of which are additional antecedents to hearing loss. Although respiratory support is unlikely to be toxic to the auditory system, potential causation may be via ventilator noise, frequent hypoxic episodes or concomitant treatments.

Thus several studies have found increased risk of hearing loss among babies with neonatal lung disease but few have attempted to tease out factors which may drive this association.

1.2.4 Acquired brain injury

Although intraventricular haemorrhage (IVH) is not listed as a risk factor for hearing loss, it has been considered as such in many studies. Cranial ultrasound is used to diagnosis and monitor IVH and grades of IVH severity have been classified as follows. A grade I IVH is a subependymal haemorrhage confined to the germinal matrix. The germinal matrix in preterm infants is highly vascular but has a poor capillary support network, increasing vulnerability to brain injury. In particular, germinal matrix IVH causes damage to the glial precursor cells during their migration to cortical layers, impacting cortical development and therefore resulting in a greater likelihood of neurodevelopmental deficits [52]. Grade II is a germinal matrix haemorrhage that has ruptured through the ependymal lining to the lateral ventricles but has not caused ventricular distension, grade III involves ventricular dilatation as a result of blood filling the ventricular space, and a grade IV means there is an associated haemorrhagic parenchymal infarction [53]. Babies born below 32 weeks of gestation are the most at risk of IVH along with very low birth weight babies. An IVH will typically occur within the first 72 hours after birth [54]. Possible risk factors aside from prematurity, include a

vulnerability to changes in cerebral blood volume, cerebral vascular immaturity resulting in a propensity to bleed, and an immature respiratory system increasing the risk of acidosis and hypoxia.

Periventricular leukomalacia (PVL) is a further form of acquired brain injury, and previous studies have also included it as a risk factor for hearing loss. PVL is characterised by damage to the cerebral white matter surrounding the ventricles resulting in poor neurodevelopmental outcome. Aetiology of PVL is complex but is likely to be impacted by hypoxic ischaemic injury and may take longer to diagnose than an IVH.

Research into the relationship between acquired brain injury and hearing loss has provided mixed results. Studies vary in approach from including the smallest gestations and birthweights to the nature of hearing impairment and the severity of the bleed. Meyer et al (1999) found the most severe intracranial haemorrhages, at grade III or IV, or PVL, were not a significant risk factor in a comparison of 777 infants, 41 with hearing loss and the remainder with normal hearing [55]. All children enrolled to the study had at least one of the ten risk factors for hearing loss as determined by the JCIH, with the addition of severe IVH, maternal substance abuse and PPHN. None of these factors were associated with hearing loss, however the number of infants with a severe intracranial haemorrhage was small and the population included term babies who are less likely to have this complication. Nevertheless, similar results were also found by Salamy et al (1989), in a study of 224 premature infants (born between 24-34 weeks of gestation), with a low birthweight (below 1500g), 12 with hearing loss [31]. This study considered a number of influences on hearing impairment, but the number of affected children was low, and both studies are underpowered.

The pathogenesis of hearing loss following a cerebral bleed was investigated by Slack et al (1989) in a post-mortem study [56]. It was speculated that there might be direct injury to the cochlea from blood tracking into the middle ear in association with IVH. Of 3 preterm babies,

two had IVH and in one blood had tracked into the inner ear and was associated with a markedly reduced number of cochlear hair cells. They speculate that, had this child survived, there may have been SNHL. Also noteworthy, is the exposure to additional risk factors for hearing loss during treatment, including cardiac arrest and pneumothorax which could indicate acidosis/hypoxia, and sepsis and necrotising entercolitis treated with aminoglycoside antibiotics. This emphasises the difficultly in extracting specific predictors of hearing impairment in infants who are likely to have experienced multiple illnesses simultaneously.

Lower grades of haemorrhage have also been associated with SNHL [30]. As the most commonly diagnosed neurological abnormality in extremely low birthweight infants, the effects of grade I-II IVH was investigated in a neurodevelopmental follow up study of babies born <1000g [52]. Of the 362 infants in the study, 104 had a grade I-II IVH, these infants were significantly more likely to have either a unilateral or bilateral hearing impairment at their 20 month neurodevelopmental assessment in comparison to the children with no cranial ultrasound abnormalities. Interestingly, due to lower use of antenatal steroids, there was also a higher rate of BPD than babies with a normal cranial ultrasound, suggestive of a relationship between low birth weight, respiratory support and IVH, demonstrating the challenges of isolating potential aetiological factors for hearing loss.

The protective effect of antenatal steroids and pulmonary surfactant was also found in a study of 418 NICU babies with and without hearing loss (OR 0.38, 95% CI 0.19-0.73 and OR 0.33, 95% CI 0.18-0.58 respectively) [49]. Hypoxia is a known antecedent for IVH, as is hypotension. Treatment for hypotension with bolus fluids, inotropes or steroid may also increase the risk of both higher severities of IVH, and hearing loss in extremely low birthweight babies [57]. Subsequently, this raises questions in relation to whether the causes

of hearing impairment in a population with IVH are much more subtle and in a multifaceted nature.

The role of IVH in the outcome of hearing loss remains inconclusive, although given that the rate of hearing loss in very preterm infants remains relatively unchanged over the last few decades whilst the frequency of IVH have decreased, it appears unlikely that IVH is an independent risk factor for hearing impairment. It is possible that the localisation of the bleed and the contributing factors involved in causing the IVH, rather than the presence or severity of a bleed play a role in the loss of hearing.

1.2.5 Hyperbilirubinaemia

Hearing loss is the most common symptom of kernicterus or severe bilirubin encephalopathy [58], and although kernicterus is now far more uncommon than it once was due to improved monitoring and early intervention, it can still occur [59]. The prevalence of severe hyperbilrubinaemia in the UK and Republic of Ireland was 7.1/100 000 live births over a two year period (95% CI 5.8-8.6) [60]. Kernicterus is the yellow staining of the brain tissue caused by unbound unconjugated bilirubin crossing the blood-brain barrier and reflects areas of brain injury [61]. The clinical tetrad for kernicterus involves athetoid cerebral palsy, impaired hearing, failure of upward gaze and hypoplasia of the dental enamel. Hyperbilirubinaemia is thought to cause an imbalance in neuronal homeostasis damaging the brainstem auditory nuclei and possibly the cells of the spiral ganglion and auditory nerve [62], raising the sensitivity of the auditory system to bilirubin induced neurotoxicity. Furthermore, auditory dysfunction has been noted at levels below the exchange criterion [63]. Hyperbilirubinaemia has also been emphasised as a cause of auditory neuropathy [64] whereby the inner hair cells may function but there is an abnormal response (prolonged latencies and threshold increases) between the cochlea and the brain stem.

Attempts to assess the contribution of hyperbilirubinaemia to hearing loss has produced inconclusive results. In one of the largest neurodevelopmental outcome studies reporting hyperbilirubinaemia, high bilirubin levels were not associated with the prevalence of hearing loss [65]. However, the study was not designed specifically to consider hearing impairment with term and preterm infants being grouped together; preterm infants are thought to be more susceptible to the effects of bilirubin encephalopathy. Studies show marked methodological variance in terms of population sample, the age at which hearing loss is measured, the cut off for severe hyperbilirubinaemia, and how conclusions are drawn as to what is considered a risk, inclusive of the use of prevalence rates and statistical differences between groups.

An example of this, is the use of total serum bilirubin (TSB). TSB is used as a marker for the commencement of phototherapy, although the definitive intervention for hyperbilirubinaemia is exchange transfusion. The levels at which treatment begins varies depending on the gestational age of the baby, and also by protocol which varies across neonatal units. Exchange transfusions have been associated both as being a risk factor for hearing loss [49], and as being protective of hearing loss [32]. It was postulated that an early exchange transfusion may prevent the level of damage caused to the auditory system in very preterm infants as there are a number of variables that prevent bilirubin binding to albumin, which early treatment would avoid. Studies are inconclusive in their findings in terms of this being a risk factor or protective for hearing impairment, probably due to the varying clinical requirements to trigger an exchange transfusion between different neonatal units. Variation between neonatal protocol for phototherapy and exchange transfusion was highlighted by a UK study of 263 hospitals, finding a wide disparity of treatment commencement for week of gestation, sickness criteria, and the use of conjugated and unconjugated bilirubin [66].

In Rhee et al's (1999) study, hyperbilirubinaemia requiring exchange transfusion was associated with hearing loss in infants born at less than 31 weeks gestation [67]. Despite sample size being small (11 infants, 10 of which were male, with severe hyperbilirubinaemia) and co existing risk factors not being considered, this study raises interesting points. Firstly, all infants had peak serum bilirubin levels which were > 26mg/dL, which is above the suggested level for considering kernicterus (20mg/dL) [61], yet there were only 4 infants of 11 with hearing loss and 2 displayed improvement at follow up. Some patients with signs of the auditory damage caused by elevated bilirubin levels have been observed to resolve with age [62], although this is not always the case, leading to questions surrounding why only 2 of the children showed evidence of hearing impairment at follow up. Secondly, the timing of the treatment may be influential. Infants in the hearing loss group had exchange transfusions slightly later than the normal hearing group (6.8 and 5.3 mean days, respectively), suggesting that earlier transfusions might be protective which would coincide with previous research [32]. Thirdly, the displacement of bilirubin from albumin causing toxicity can be affected by several other risk factors for hearing loss, such as aminoglycoside antibiotics and acidosis. Two of the infants had septicaemia and would have received antibiotics, therefore the maximum level of bilirubin may not be independently indicative of hearing loss. Coexisting risk factors at the time of the maximum total bilirubin may have revealed other confounding factors that cause a marked reduction in bilirubin binding capacity, and therefore the permanent loss of hearing. Lastly, the measurement of bilirubin itself, in particular peak serum bilirubin levels. As a biochemical marker, total serum bilirubin is thought to lack sensitivity both as a predictor for neural toxicity and as a predictor of ANSD in infants with high levels [68]. As only unbound bilirubin is able to cross the blood brain barrier reflecting potential toxicity. Free bilirubin may be a more reliable predictor of ANSD in late preterm and term babies: unbound levels has been shown to be higher in patients with ANSD than normal hearing patients, but there were no differences in peak bilirubin.

Dowley et al (2009) found hyperbilirubinaemia was the most significant risk factor in 12 preterm infants with ANSD out of 30 infants with hearing loss, followed by sepsis and exposure to gentamicin [15]. Infants with ANSD were more likely to have received neonatal care and been more unwell. These groups included term and preterm infants, and comparisons were made between the ANSD and non-ANSD groups per variable. A multivariable analysis would have been useful in establishing whether elevated bilirubin levels were independently predictive of ANSD or whether this was related to other risk factors involved in neonatal treatment that could affect binding affinity. In a study that also grouped and compared the most unwell infants with and without hearing loss (level of illness was based upon life support variables, days of antibiotics and number of blood transfusions), elevated peak bilirubin levels were significantly different between groups [31]. Although the significance of hyperbilirubineamia was ascribed to an outlier with liver disease, these infants were more likely to have received longer durations of diuretics and received these concomitantly with aminoglycosides or vancomycin. As the groups were matched for level of illness, and hyperbilirubinaemia could only be attributed to one infant as a precursor for hearing impairment, these variables could be playing an influential role.

The combinations of risk factors in conjunction with hyperbilirubinaemia have been associated with hearing loss. In 15 children with SNHL born at less than 33 weeks gestation, high bilirubin levels were more likely to cause hearing loss when in combination with acidosis or aminoglycoside treatment [4]. In a further study, acidosis and raised bilirubin levels were also found to be influential in the cause of hearing loss in jaundiced infants with low birth weight (<1500g in comparison to a control group >1500g) [69]. The duration of jaundice was longer in those who had impaired hearing, in whom there were a greater number of episodes of acidosis. Aminoglycosides, many other drugs and acidosis are known to displace or impair binding between bilirubin and albumin, emphasising the importance of considering a congruence of risk factors on hearing loss.

As audiological changes have been detected below the exchange level, attempts have been made to reduce neurotoxicity as a consequence of unconjugated hyperbilirubinaemia. Lower treatment thresholds have been trialled in preterm infants to reduce hearing impairment in infants born below 32 weeks gestational age [63], or with very low birthweight [70]. The aggressive treatment of elevated bilirubin produced inconclusive results in both studies in terms of the effect on hearing impairment and longer term neurodevelopmental outcome. A lack of improvement in outcome may be suggestive of hyperbilirubinaemia being less toxic to the auditory system than previously estimated, or that these populations had other risk factors for hearing impairment.

The relationship between hyperbilirubinaemia and hearing impairment in preterm infants is complex and to some extent, explains the inconclusive results from previous research. Whilst early exchange transfusion may prevent the direct impact of jaundice on hearing impairment, the accepted level at which exchange occurs differs across neonatal units. Late exchange transfusions may not reverse damage that has already occurred. The indirect effects of hyperbilirubinaemia most likely involve the interaction with other aspects of serious neonatal illness.

1.2.6 Infection

Infection is a common occurrence within the neonatal population due to an immature immune system. There are two main congenital causes of hearing loss, rubella and cytomegalovirus (CMV). Meningitis is the most common postnatal infection that causes hearing loss. Korver et al (2011) identified 185 children with permanent hearing loss to investigate the causes of impairment. The prevalence of CMV was 8.6% of which the majority had a profound impairment, meningitis in 3.2% and rubella in 1.1%, although there was no control group for comparison [18].

Cytomegalovirus (CMV) can be congenital or postnatally acquired. Congenital CMV infections can have a devastating effect in the developing fetus with primary infections being more severe than a reactivated infection. CMV is also known to cause late onset progressive hearing loss which can be unilateral or bilateral [71], by altering white matter in the central nervous system which affects the functioning of the cochlea [9]. The mechanism by which this occurs remains unknown.

Infants with congenital CMV can be symptomatic or asymptomatic; symptoms can include low birth weight, jaundice, seizures, microcephaly, pneumonia and a rash. Dahle et al (2000) conducted a longitudinal study of 860 newborn infants with congenital CMV [72]. Hearing loss was evident in 180 of which 7% had been asymptomatic and 40% symptomatic, indicative of a greater risk of hearing loss following a symptomatic presentation. However, some of the signs of CMV have been independently associated with hearing loss in infants. Perinatal risks were compared in a study of 504 infants with asymptomatic congenital CMV; of which only gestational age and lower birth weight were associated with hearing loss [73]. Boppana et al (2005) investigated the differences in viral load of congenital CMV in children with SNHL [74]. This was a cohort study of 76 infants with congenital CMV, 12 were found to have SNHL, of which 8 children had clinical symptoms of the virus, and 4 without. Children were identified and enrolled following positive saliva samples taken during the first week of life, urine samples were collected within the first month and follow-up hearing tests were completed at the age of one year or older. Of those who were asymptomatic for CMV but had hearing loss, there were higher viral loads of CMV detected in urine samples than those who had normal hearing. In addition, infants that were premature (<37 weeks), or had a low weight for gestational age, were more likely to have SNHL than normal hearing but only prematurity reached significance. Although numbers in this study are small, the risk of CMV and low birth weight correspond with the findings from Fowler's (2003) study [73]. Screening for viral load might identify those at the greatest risk for hearing impairment.

Up to 90% of congenitally infected infants will display no symptoms of the virus at birth [75]; asymptomatic infants are not routinely tested for CMV in the UK as screening is deemed unjustifiable. Passing the initial hearing screen means that these children will not be monitored in subsequent assessments, and as a late onset progressive hearing loss this further increases the potential impact of hearing loss. The role of CMV in childhood hearing loss is likely to be underestimated for several reasons; the variation in the age of onset and the progression of the hearing impairment, and the methodology of studies which will often adopt retrospective data collection from medical notes, all of which could mask the true numbers of infection within samples of children with a hearing deficit.

Bacterial meningitis tends to cause a bilateral hearing impairment although unilateral may occur. Neonatal meningitis can be caused by infections including Group B Strep (GBS), gram negative infections, listeria and fungi. Meningitis is thought to impair the functioning of the peripheral spiral ganglion cells and dendrites [76]. In a study of 41 ex-NICU infants, from a sample of 777, bacterial sepsis and/or meningitis was found to be a significant independent risk factor for hearing loss [55]. Infants were eligible for the study based upon exposure to at least one risk factor for hearing loss, and multivariate comparisons were used in this study. This finding was supported by a smaller study of 416 preterm infants, whereby meningitis was more prevalent in the hearing loss group than the normal hearing controls [49]. Number of days of ventilation, IVH and exchange transfusion were also associated with hearing loss. However, it is not clear whether a multivariate analysis was used to consider confounding risk factors, questioning whether meningitis would be an independent predictor of SNHL given the impact of other variables which were also significantly greater than the control group.

Congenital rubella is an infection that can cause multiple abnormalities inclusive of sensorineural hearing loss [77]. The earlier in pregnancy it occurs the more likely the infant

is to be affected due to an increased susceptibility to the teratogenic effects of infection. The virus replicates within the inner ear causing a progressive loss which is likely to be bilateral and ultimately profound. Postnatal rubella is unlikely to have the same impact. Rubella is now much more uncommon due to the uptake of the rubella vaccine, although remains prevalent in countries without a vaccination programme for rubella.

Neonatal necrotising enterocolitis (NEC) has also been implicated as a cause of hearing impairment. NEC is a disorder of the gastrointestinal tract which can result in inflammation leading to a perforation of the gut, associated infection and surgical treatment for the perforation [78]. Extremely premature and low birthweight babies are at the greatest risk for developing NEC. Jiang et al (2014) compared infants born between 30-36 weeks gestation that had been diagnosed with NEC to a healthy preterm group and a healthy term group of infants [79]. Brainstem auditory evoked responses (BAER) were used to test hearing; to reduce confounding effects, infants with other major perinatal complications aside from NEC, that could affect the auditory brainstem pathway were excluded. In comparison to the healthy controls, the preterm infants that had previously been diagnosed with NEC, showed signs of a central rather than peripheral auditory brainstem abnormality. Specifically a delayed neural conduction was considered to reflect impaired myelination or synapse dysfunction. Gastrointestinal surgery was also a predictor of hearing loss in a study of infants born with a lower gestational age (less than 28 weeks) [12], although this is likely to be indicative of the level of illness requiring surgery. NEC is associated with a range of physiological factors including circulatory difficulties, acidosis and the use of ototoxic medication. NEC is unlikely to be a direct cause of SNHL but rather greatly increases the risk with the treatment involved.

Neonatal sepsis is estimated to affect up to 20% of preterm infants [80]. Late onset sepsis in very low birthweight babies (<1000g) can occur in up to 50% [81]. Manifestation of neonatal

sepsis is often unspecific and can progress rapidly; therefore treatment is often commenced when infection is suspected. Aminoglycoside antibiotics are widely used in the treatment of gram negative sepsis and are frequently the first line of treatment for suspected infection as well. Neonatal sepsis was found to be an independent predictor of hearing loss in the presence of other risk factors for hearing loss in 777 infants [55]. Similarly, infection increased the risk of poor neurodevelopmental outcome, inclusive of hearing loss in extremely low birthweight infants (<1000g) in comparison to those who had not had sepsis [82]. Current clinical practice is to provide pre-emptive treatment for presumed sepsis following preterm birth until confirmed otherwise. This however, causes several difficulties with studies on neonatal sepsis. Methodological differences in the determination of neonatal sepsis are apparent, including using early and late onset sepsis, the use of positive blood cultures, raised inflammatory markers, and the number of days of antibiotics which may include treatment for suspected sepsis, therefore hearing loss may be more likely related to the treatment rather than the unconfirmed infection.

Infection in infants can have lasting adverse neurodevelopmental outcomes, and has been consistently associated with hearing loss in multiple types of infection. It is less clear as to whether the sepsis or the subsequent physiological or medicinal risk factors are the cause.

1.2.7 Noise

Exposure to noise within the neonatal intensive care unit is continual and potentially damaging to the immature audiological system of infants. The sudden and unpredictable sounds of ventilators, monitor alarms, conversations and babies crying can all exceed recommended noise levels for NICU [83]. Immediate effects can include startling, agitation, fluctuations in heart rate and oxygen levels, and sleep disturbance [84]. Longer term effects have associated noise with hearing loss. In animal models, noise primarily affects the outer hair cells which are unable to regenerate [85]. High frequency noise caused small areas of

loss from the outer hair cells to begin with, which progressed to damaging the organ of Corti and myelinated nerve fibres as noise exposure continued. Low frequency noise affected a broad area of outer hair cells, with prolonged noise exposure resulting in similar damage to that of prolonged high frequency noise.

Noise intensity, particularly from respiratory support is thought to exceed levels of ambient noise within the ear, although advances in technology seek to reduce this. Rastogi et al (2012) compared mechanical ventilation and bubble CPAP in 344 premature infants with a birth weight <1500g [86]. CPAP produces a greater amount of noise than mechanical ventilation which was expected to identify a notable difference between the groups. Infants requiring any respiratory support were more likely to have hearing loss than an infant who had not needed either. However, there was no difference in risk between those treated with mechanical ventilation in comparison to those treated with CPAP when the hearing loss group were seen 2 years after treatment. Therefore, the increased noise exposure encountered as a result of prolonged CPAP did not increase the risk of hearing loss. Interestingly, in multivariate analysis, only ventilation, apnoea and NEC were independently associated with a failed hearing screen, each of which could indicate the necessity of a prolonged period of respiratory support. An alternative explanation is that noise is likely to exacerbate the concurrent complications of apnoea and NEC, including physiological instability and risks associated with ototoxic medication.

In a review of ototoxicity and noise, it was suggested that noise potentiates the effects of aminoglycoside ototoxicity by increasing the stimulation of hair cells, and opening the channels by which aminoglycosides enter the cells [87]. Threshold shifts are evident even when insults from noise and aminoglycosides are not simultaneous [88]. Furthermore, there are additional ototoxins that can increase damage to the cochlea by aminoglycosides, such

as loop diuretics. Whilst this process is not well understood, safe doses of each medication in the presence of noise, could result in interactions that increase the risk of hearing loss.

The synergistic interaction between environmental and pharmacological effects requires further investigation. Studies are limited by experimental technique, specifically a lack of randomisation, and controlled environments. Noise has been implicated as a risk factor for hearing loss but there is currently little substantial evidence to support this.

1.2.8 Medication

A range of medications have long been associated with preterm hearing loss. These include aminoglycoside antibiotics, indometacin, dexamethasone, and loop diuretics. Some of the effects of ototoxicity may be through potentiation of drug induced ototoxicity by undercurrent physiological events that lead to high drug levels. Examples of this are displacement from albumin binding, liver dysfunction and renal impairment.

Aminoglycosides are widely used as a first line antibiotic for suspected or proven bacterial infection. The ototoxic side effects of this group of medication are well known, hence the strict monitoring of peak blood level concentrations. Aminoglycoside induced renal impairment is usually reversible, however audiological impairment is often permanent. Aminoglycosides are cleared slowly from the inner ear, and can affect hearing after the cessation of ototoxic medication. Ototoxic medication was the most commonly presented risk factor in a study of 4478 infants with high risk for hearing loss who had been cared for in NICU [89], although gentamicin in particular has been associated with causing permanent SNHL. Aminoglycosides accumulate within the basal hair cells of the inner ear causing irreversible damage. The basal outer hair cells are affected initially resulting in a high frequency loss [90], which subsequently progresses to the lower frequencies as the apical cells also become affected, all of which are unable to regenerate [91].

Gentamicin

Despite the known ototoxic properties of gentamicin, this has been debated by a number of studies with mixed findings. A recent study of 1582 infants born at less than 32 weeks gestation (from which there were 25 with hearing loss and 50 matched controls) observed no difference in cumulative doses or trough serum levels of gentamicin between infants with and without hearing loss that were matched for sex, gestational age and very low birthweight [92]. Vella-Brincat et al (2011) also found gentamicin alone did not increase the risk of hearing loss, and suggested gentamicin was in fact protective against hearing loss. This study compared 2347 term and preterm infants following a NICU admission >48 hours, separating babies into groups dependent on which antibiotics (gentamicin and vancomycin) or combinations of which they had received. A significantly lower number of children failed their OAE screen in the gentamicin group in comparison to the no antibiotic control group (OR 0.66, 95% CI 0.45-0.97) [93]. It was therefore suggested that gentamicin may protect against hearing loss. Nevertheless, both studies claiming the low risk of gentamicin were limited by a small number of children with confirmed SNHL which weakens the impact of the results. In particular, Vella-Brincat et al (2011) found only 30 children had confirmed hearing loss at follow up, half of which had received no antibiotics and almost 20% of those referred for further testing had been lost to follow up [93].

Vancomycin

The ototoxic effects of vancomycin are unclear, although ototoxicity is thought to be a consequence of high serum concentration levels or from prolonged or repeated courses. Vella-Brincat et al (2011) as discussed above, implicated vancomycin as a risk to hearing in infants following NICU [93]. However these results were not repeated in a study of 625 infants (45 with hearing loss) admitted to NICU with at least one risk factor for hearing impairment [94]. There was no increased risk of hearing loss in infants exposed to peak or

trough levels above the recommended levels, or exposed to prolonged durations of vancomycin. A limitation of this study was the use of failed neonatal hearing screening, repeating the assessment would enable delayed onset hearing loss to be included. Nevertheless, the case studies of the children that exceeded therapeutic concentration levels or had longer durations of treatment with vancomycin, also had additional risk factors for hearing loss. Whilst there is limited evidence that vancomycin is an independent risk for hearing loss, exposure to concomitant risks may exacerbate the threat, which will be discussed.

Furosemide

Furosemide is a loop diuretic frequently used in neonatal care for fluid balance regulation, including treatment of oedema, to increase urine output and during blood transfusion. Furosemide is thought to change the composition of the endolymph within the inner ear causing decreases in endocochlear potential [95]. Nevertheless, the association between furosemide and hearing loss has yielded contrasting results. In a study of 1360 preterm infants (born <32 weeks of gestation or with a birthweight of <1500g), furosemide was found to be an independent risk factor for hearing loss (OR 4.6 95% CI 4.8-25.3) [96]. The analysis for this study was multivariate and included a range of neonatal risk factors including diagnoses such as BPD, and ototoxic medication such as aminoglycosides. Whilst the confidence intervals were wide for the analysis of furosemide this is representative of the low prevalence rate of infants in the study that were found to have hearing loss (19 babies). These results were not found in a retrospective review by Rais-Bahrami et al (2004) whereby an association between furosemide and hearing loss was not found [97]. This study did not compare the potential additive effect of other ototoxic medications.

Combinations of ototoxic medication

Interactions between ototoxic medications have also been associated with hearing loss. In the study by Vella-Brincat et al (2011) the group of infants who received gentamicin plus vancomycin had the highest risk of hearing loss after follow up assessments [93]. A total of 30% of the gentamicin plus vancomycin group were hearing impaired in comparison to the groups of infants that received gentamicin alone, vancomycin alone, or the control group that received no antibiotics, suggestive of an increased ototoxicity in the presence of both antibiotics.

Robertson et al (2006) found a relationship between the use of individual aminoglycosides and hearing loss when treatment coincided with the use of loop diuretics, in a population of term or near term infants [71]. An overlap between vancomycin and diuretics was also more likely in the hearing loss group. Aminoglycosides in the presence of renal dysfunction increase the amount of medication accumulating in the inner ear. Borradori et al (1997) found infants with hearing impairment born at less than 35 weeks were more likely to have had longer durations and higher doses of furosemide along with aminoglycosides [98]. Cumulative doses of aminoglycosides and furosemide were also both higher for cases than controls, and although the paper speculated as to the interaction between aminoglycosides and diuretics and the potential effect of both, this study was not able to analyse this. Both studies were limited by a lack of multivariate analysis in which independent risk factors could be ascertained in the presence of other markers of illness.

De Hoog et al (2003) also considered the importance of cumulative ototoxic medication on hearing [94]. This study included 625 NICU infants with at least one risk factor for hearing loss. A total of 45 infants failed their hearing screen and the remaining 580 comprised the control group. This study looked at individual peak and trough concentration levels, duration of therapy, total exposure (mg/kg) of vancomycin, tobramycin and furosemide, with the

addition of combinations of these three medications. Findings revealed no association between hearing screen failure with any of the variables in multivariate analysis. As the effects of ototoxic medications can continue beyond the end of treatment, the total number of children with a permanent hearing loss could have been higher at follow up assessments, and therefore results could be underestimated. A further limitation was not testing the high frequencies (>4kHz), since hearing loss as a consequence of ototoxic medication is likely to present initially in the higher ranges.

Aminoglycosides not only present an ototoxic risk but also a nephrotoxic risk, which can increase the circulating volume of aminoglycosides and also the need for diuretics. In laboratory studies, accumulation of aminoglycosides within the inner ear enabled an increased permeability of loop diuretics to penetrate the inner ear cells in a higher concentration than when aminoglycosides have not been given [90]. Furosemide administration when serum creatinine levels are raised may also lead to an accumulation of ototoxic drug which has been associated with SNHL [4]. The elimination of ototoxic medication is dependent on adequate renal function which is often impaired in very preterm infants, and for which loop diuretics are prescribed. This highlights the complex interaction not only between ototoxic medications but also the physiological condition of the baby at the time.

The use of diuretics alongside neuromuscular blockers (NMBs) such as pancuronium bromide was also found to be associated with hearing loss [97]. This relationship was also found by Robertson et al (2006) [71]. As NMBs can cause oedema which is then treated with diuretics, the relationship between the two is complex and difficult to separate.

Interestingly, and in contrast to previous research, ototoxic medication has been linked specifically with ANSD. Xionis et al (2007) found exposure to furosemide, vancomycin and aminoglycosides was associated with ANSD in comparison to a matched normal hearing

control group [14]. There were longer durations of aminoglycoside exposure in the ANSD group in comparison to the children with SNHL and the control group. Furthermore, furosemide was independently associated with ANSD and SNHL in a multivariate analysis in which aminoglycoside antibiotics and vancomycin were entered together with dexamethasone. Amikacin similarly to furosemide, has also been associated with ANSD, in which increased latencies in waves I-III were present in newborn infants [93]. Higher serum levels that remained within the therapeutic range were positively correlated with an increased brainstem conduction time. In previous studies, ototoxic medication has tended to be associated with cochlear hair cell loss, rather than abnormal or absent brainstem responses.

Indometacin

The association between the administration of indometacin and hearing loss may be indirect. Indometacin is used to treat PDA or prophylactically to prevent IVH, but can decrease renal function which might enable the accumulation of circulating ototoxic medication. Indometacin was given to infants with hearing loss more frequently than infants with normal hearing matched for gestational age and birthweight, although this did not reach significance and exposure rates were low [98]. A randomised controlled trial of 547 extremely low birth weight infants compared the neurodevelopmental outcome following treatment with prophylactic indometacin aimed to reduce the risk of both PDA and IVH [99]. Indometacin was administered at 24 hour intervals within the first 3 days of life and outcome was compared to a placebo group of 569 babies. Infants had no greater risk of hearing loss than the control group who received placebo. Indometacin is used less frequently due to the increased risk of developing NEC, and multivariable analyses have mostly supported a lack of correlation between hearing loss and indometacin.

Dexamethasone

Intravenous dexamethasone may be used to help wean infants who require prolonged mechanical ventilation to prevent complications such as BPD [100]. A rapid improvement in lung function is triggered by the anti-inflammatory effects of systemic steroids. However, exposure to dexamethasone has also been implicated as a cause of hearing loss. In a comparison between infants with ANSD, SNHL and normal hearing controls, exposure to dexamethasone was found to be associated with hearing loss in both of the impaired hearing groups [14]. Although the infants with ANSD had a higher exposure frequency, it was not a predictor of this type of hearing loss over SNHL. Furthermore, the association between dexamethasone and hearing loss was not independent of a diagnosis of BPD, indicating the complexities in establishing independent risk factors. Due to a correlation with long term adverse neurological complications dexamethasone is used far less frequently [101].

The ototoxic effects of individual medications remain debated in literature, differences in results are likely to stem from a variation in study methodology and sample population. Risks are likely to correlate with the concurrent exposure of ototoxic medication including aminoglycosides, loop diuretics, and vancomycin, with physiological risk factors such as raised creatinine levels. Further risk factors involve a genetic predisposition to deafness which will be explored in the next section.

1.3 Genetic causes of hearing loss

The aetiological contribution of genetics to hearing impairment is thought to be around 50% [3], although this is likely to differ between developed and developing countries. Patterns of inheritance can be from one parent carrying a dominant gene (autosomal dominant), from parents who both carry a recessive gene (autosomal recessive), through the maternal line (mitochondrial inheritance), or can affect predominantly males only (X linked, which can be a dominant or recessive inheritance). Hereditary hearing loss is estimated to be 18% autosomal dominant, 80% autosomal recessive and the remaining 2% X linked and mitochondrial inheritance [102].

Autosomal inheritance

Autosomal inheritance is determined by the nuclear genome, in which 22 pairs of linear chromosomes are located within the nucleus of each cell. Each chromosome is made up of deoxyribose nucleic acid (DNA) wound around proteins called histones. DNA contains the genetic information as a code made up of chemical bases: adenine (A), guanine (G), cytosine (C) and thymine (T). The order of the bases influences the traits or conditions that are expressed in an individual. Each base has a sugar molecule and a phosphate molecule backbone, and these 3 components make up a nucleotide. Nucleotides join to form a strand of DNA, with the bases matching to form pairs, A with T, and C with G which forms the double stranded helix. When cells divide, the DNA strands will be replicated, and the functioning of a cell is dependent on an exact replica of the previous cell being copied.

An individual has two copies of each gene, one copy inherited from each parent. A dominant mutation in a gene from either parent will result in a 50% chance of their child inheriting that mutation. Whereas, there is a 25% chance of a child inheriting and exhibiting a recessive gene mutation as two copies are required, one from each parent.

X linked inheritance

X linked inheritance is determined by the X chromosome, which is larger and contains a greater number of genes than the Y chromosome. A female carrier of an X linked condition has a 50% chance of passing this on to her daughters who would be healthy carriers as they have an unaffected gene on the other X chromosome. 50% of sons would be affected as they only have one X chromosome. Affected males would not pass it on to their sons but all daughters would be carriers.

Mitochondrial inheritance

The mitochondrial genome differs from the nuclear genome in a number of ways. Firstly, mitochondrial DNA is located within the mitochondria, outside of the nucleus of a eukaryote cell, and a cell will contain several thousand copies of mitochondrial DNA molecules in comparison to the 46 in a diploid nuclear cell. The mitochondrial genome is much smaller; it is comprised of approximately 16 000 DNA base pairs in comparison to over 3 billion base pairs in the nuclear genome. DNA within the mitochondria is responsible for providing instructions for the production of cellular energy in the form of adenosine triphosphate (ATP), and for the synthesis of proteins needed by and used within mitochondria in this process. Mitochondrial DNA has a circular shaped chromosome similar to bacteria, rather than the linear chromosomes within the nucleus. Mitochondrial DNA also has an increased susceptibility to mutagenesis. Patterns of inheritance also differ from the inheritance of nuclear chromosomes, whereby mitochondrial DNA can only be passed on from the mother. Paternal mitochondrial DNA located in the tail of the sperm detaches and is discarded following fertilisation, resulting in maternal uniparental transmission. Hearing loss as a result of mutations in mitochondrial chromosomes would be inherited by all children, but only female offspring would pass this on to their children. Nuclear DNA is inherited equally from both parents.

The genetic causes of childhood hearing impairment can be split into syndromic and non syndromic hearing loss. Approximately 70% of genetic hearing loss will be non syndromic, with the remaining 30% accounting for syndromic hearing losses [103].

1.3.1 Syndromic hearing loss

A syndrome is characterised by a group of symptoms that occur together and include features in other body systems. Syndromes can be classified by the pattern of inheritance, autosomal dominant, autosomal recessive, X linked, chromosomal and some syndromes have an unknown inheritance (table 1-1). Therefore, investigations into the aetiology of hearing impairment may involve clinical tests additional to audiology screening such as ophthalmic tests, cardiac or renal investigation.

Typically the most common types of autosomal dominant, and autosomal recessive hearing loss, are Waardenburg syndrome and Usher syndrome respectively [104], but this may differ between populations. In a population based European study of children with permanent childhood hearing loss, from moderate to profound, hearing impairment was attributed to hereditary causes in 38% of all children [18]. From the children with a genetic aetiology, syndromes comprised 15% whilst the remainder were classified as non syndromic or non specified with a positive family history. The most frequent syndromic cause of hearing loss in this population was Pendred syndrome (4%).

Hearing loss is a recognised clinical feature of around 400 syndromes, the most common syndromic causes of hearing loss are presented in table 1-1 along with type of hearing loss and clinical features.

Table 1-1: Common syndromic causes of hearing impairment

Syndrome		Type of hearing loss	Clinical features
Autosomal dominant	Branchiootorenal syndrome (BOR)	Conductive, sensorineural, mixed	Preauricular pits or tags, malformation of inner, middle or outer ear, hypoplastic/dysplastic kidneys, branchial fistulae
	CHARGE syndrome	Conductive/sensorineural	Coloboma, heart defects, choanal atresia, restricted growth, genital anomalies, ear abnormalities, learning difficulties
	Stickler syndrome	Progressive, conductive/ sensorineural	Myopia, joint anomalies, micrognathia, cleft palate
	Townes Brocks	Sensorineural/conductive, can be progressive	Anomalies of the hand, foot and ear, imperforate anus
	Treacher Collins	Mixed	Downslanting palpebral fissures, malar and zygomatic hypoplasia, macrostomia, small, malformed external ear
	Waardenburg syndrome (types I-IV)	Non progressive, unilateral or bilateral, sensorineural	Hypopigmentation of hair/eyes/skin, I - dystopia canthorum, joining eyebrows II - without dystopia

			III - limb deformities IV - some of above with intestinal obstruction
Autosomal recessive	Jervell and Lange-Nielsen syndrome	Profound bilateral sensorineural	Prolongation of QT interval, with syncope and sudden death
	Pendred syndrome	Severe to profound, bilateral, progressive sensorineural	Enlarged vestibular aqueducts and goitre
	Usher syndrome	Mild to profound, sensorineural	Retinitis pigmentosa
	Zellweger syndrome	Profound, bilateral sensorineural	Low birth weight, jaundice, hypotonia, ear eye and nose anomalies, short digits, developmental delay
X-Linked	Alport syndrome	Progressive sensorineural (varying severity)	Hematuria, impaired renal function, ear and eye anomalies
	Mohr-Tranebjaerg syndrome	Progressive sensorineural	Progressive movement disorder (dystonia), visual deficits, behavioural problems

Chromosomal	Down syndrome	Usually conductive, can be bilateral sensorineural	Hypotonia, facial anomalies, palmar crease, cardiac anomalies, delayed development
	Turner syndrome	Conductive/sensorineural	Short stature, webbed neck, eye anomalies, low set ears
Unknown inheritance	Goldenhar syndrome	Bilateral sensorineural	Facial hypoplasia, ear anomalies, cardiac defects, macrosomia, cleft palate/lip, coloboma

1.3.2 Non syndromic hearing loss

Genetic predisposition to hearing loss is investigated when there is no other cause identified or there is a family history of deafness. Heredity plays a major role in congenital hearing impairment, however mutations in genes have also been identified as influential in progressive hearing loss [105]. Non syndromic hearing loss is categorised by inheritance, with approximately two thirds being autosomal recessive, one third being autosomal dominant, and more rarely 1% are X linked and there is approximately the same proportion for mitochondrial inheritance [105].

Autosomal dominant non syndromic hearing loss is caused by mutations in over 60 loci with around 30 of those genes identified to date [106]. Mutations can affect the production of proteins which encode all sorts of components of the hearing pathway: potassium channels [107], tectorial membranes [108] and gap junctions [109], and structural molecules, which reduces the transmission of signals between the inner ear and the auditory pathway.

Non syndromic hearing loss is more likely to be inherited in an autosomal recessive pattern (80% of cases). This means that the majority of children with genetic hearing loss are born to parents with normal hearing. Connexin 26 is a protein encoded by the *GJB2* gene which is the most frequent autosomal recessive cause of sensorineural deafness [110]. Both parents need to be carriers of the gene mutation to pass it on to their offspring. Connexin 26 related hearing loss is usually a congenital, non progressive hearing impairment, ranging from mild to profound and tends to be symmetrical [111]. Connexin 26 is a component of intercellular gap junction channels in the cochlea [112]. The mutation disrupts potassium ion recirculation pathways resulting in a loss of endolymphatic potential and consequently hearing loss [113]. In a Norwegian study of children referred for cochlear implant, 21.5% of children had *GJB2* mutations which was the most frequently found genetic cause of non-syndromic hearing loss

[21]. This prevalence was higher still in a study of neonates referred with hearing impairment, whereby *GJB2* accounted for 37.9% of cases [19].

Mitochondrial mutations are much rarer than both autosomal dominant and autosomal recessive inheritance. Nevertheless, mutations in the mitochondrial DNA may interact with environmental factors which results in deafness. Mitochondrial 12S ribosomal RNA has been associated with mutations that idiosyncratically exacerbate aminoglycoside induced hearing loss [114], and is of particular relevance to a preterm population. The frequency of aminoglycoside administration in neonatal care was discussed in section 1.2.8. Mutations within the 12S rRNA increase structural similarity to bacterial rRNA which is the primary target of aminoglycoside antibiotics. One of the most common mutations within this gene is m.1555A>G. At position 1555 a point mutation of guanine in place of adenine occurs. The mutation is thought to enable the aminoglycosides to bind more readily to mitochondrial ribosomes, decreasing the rate of protein synthesis to below the required rate for a functional cell, subsequently causing damage to cochlear hair cells [115]. Degeneration of the hair cells which are dense with mitochondria, can lead to irreversible cell hair death [116].

Individuals carrying m.1555A>G therefore have a predetermined susceptibility to the ototoxic effects of aminoglycosides. Bilateral, profound and progressive hearing loss is a consequence of receiving aminoglycosides even when blood levels are maintained within clinical recommendations. In this population, the penetrance of deafness has been reported to be 100% following exposure to even a single dose of aminoglycosides [117]. Importantly, in this study there was reported deafness in some family members prior to the introduction of aminoglycosides in clinical practice, suggestive of a risk of impairment in the absence of aminoglycosides. However, the median age of onset of hearing loss was much lower in those that had received aminoglycosides, than those that had not been exposed (5 and 20 years respectively), although the time taken to deafness following ototoxic exposure had not been

ascertained. Interestingly, age of onset and aminoglycoside exposure were self-reported, confirmation from medical records would enable greater reliability. In a study of four Arab-Israeli families with maternally-inherited aminoglycoside induced deafness within the families, the only mutation common to all of them was m.1555A>G [118]. Similarly, case studies of three children with leukaemia who initially presented with normal hearing, experienced audiological deterioration over the course of their treatment [119]. Each child received multiple courses of aminoglycosides for neutropenia and all were retrospectively found to carry m.1555A>G. Measures have since been taken to screen for m.1555A>G prior to the treatment of aminoglycosides in paediatric oncology.

Following the reporting of an absolute penetrance, a number of studies have investigated the interaction between the mutation and aminoglycosides in both family and population studies, few of which were found to have the same results. Al-Malky et al (2014) investigated the penetrance of m.1555A>G in 59 children with cystic fibrosis [120]. This was a selected sample based on the likelihood of children having been exposed to aminoglycoside antibiotics or likely to have future exposure to these antibiotics as part of their treatment. The mutation was found in 2 children who had both previously received repeated courses of aminoglycosides; one child had a severe high frequency hearing loss, and the other had normal hearing. The penetrance in this study was below the previously reported 100%. This study raised important questions regarding non-penetrance and the possible recruitment bias of previous research with regard to the use of familial studies, or solely hearing impaired samples. Mixed penetrance across studies could also possibly be explained by the type of aminoglycoside an individual was exposed to, or by the number of doses or courses of ototoxic medication. Streptomycin was more commonly associated with deafness in individuals with the mutation than kanamycin [116]. Both of these antibiotics are less frequently used in the UK, yet the mutation is prevalent across the UK. A population cohort

study of 9371 European children unselected for hearing ability, revealed a prevalence of the mutation to be 0.19% with no known aminoglycoside use [121].

Aminoglycoside antibiotics are widely used in many countries owing to their effectiveness and low cost. In a Chinese study of 2417 deaf-mute students with severe to profound hearing loss, 126 were found to have m.1555A>G giving a prevalence rate of 5.21% [122]. A history of aminoglycosides was reported in 52 of those with the mutation (41.27%); one control was a carrier of the mutation and had normal hearing. However, no aminoglycoside exposure was reported for the control and exposure was self-reported in the impaired group. Based upon the higher prevalence of the mutation in the deaf population in Guo et al's (2010) study, combined with the widespread use of aminoglycosides in China, it is important to consider the implications for a neonatal population where hearing loss is more likely than term born infants, and aminoglycoside exposure is frequent. Preterm infants are more likely to have concomitant disease and therefore a predetermined susceptibility could be overlooked when retrospectively considering the cause of hearing loss. Children are not routinely screened for this mutation unless there is a clinical reason, and so aminoglycoside-induced hearing loss would be expected to be higher than the general population if there is a 100% penetrance of deafness.

Prevalence rates for m.1555A>G in neonatal populations vary between 0 and 0.69% with differences in population sample, for example gestational age and birth weight (Appendix 3). In a cohort of 703 infants from neonatal intensive care, m.1555A>G had a prevalence of 0.28% however there was no related hearing loss despite these infants having had a minimum of 9 days gentamicin exposure [123]. This indicates a lower frequency of aminoglycoside induced hearing loss than expected and further emphasises a penetrance below 100%. In a recent European study, 3 out 10 preterm infants with m.1555A>G who had received gentamicin failed their newborn hearing screen [124]. Aminoglycosides combined

with the mutation were a significant predictor for failing the hearing screen (95% CI 1.07-1.49). However, data from both studies are slightly misleading as hearing outcome is primary data based solely upon the newborn hearing screen. Follow up studies would confirm hearing loss, and specify the type of hearing impairment. As this is usually a progressive hearing impairment with a possibility of a late onset presentation, numbers could increase over the two years following birth, and thus the sole use of neonatal hearing screening potentially underestimates the full impact of aminoglycoside induced deafness in susceptible individuals. Johnson et al (2010) in a study of 436 NICU graduates (with a birthweight <2500g), found 4 infants with m.1555A>G mutations [125]. All 4 had received gentamicin but only one demonstrated abnormal hearing. Each of the infants with normal hearing had received 2 days of gentamicin, in comparison to 4 days of exposure in the child with hearing loss, concluding that there could be a threshold effect for aminoglycoside induced hearing loss in carriers of the mutation. Additionally, studies with an inclusion criteria of individuals with only severe to profound deafness [126], are likely to miss many affected children with early stages of a progressive hearing loss, therefore further underestimating the impact of the mutation.

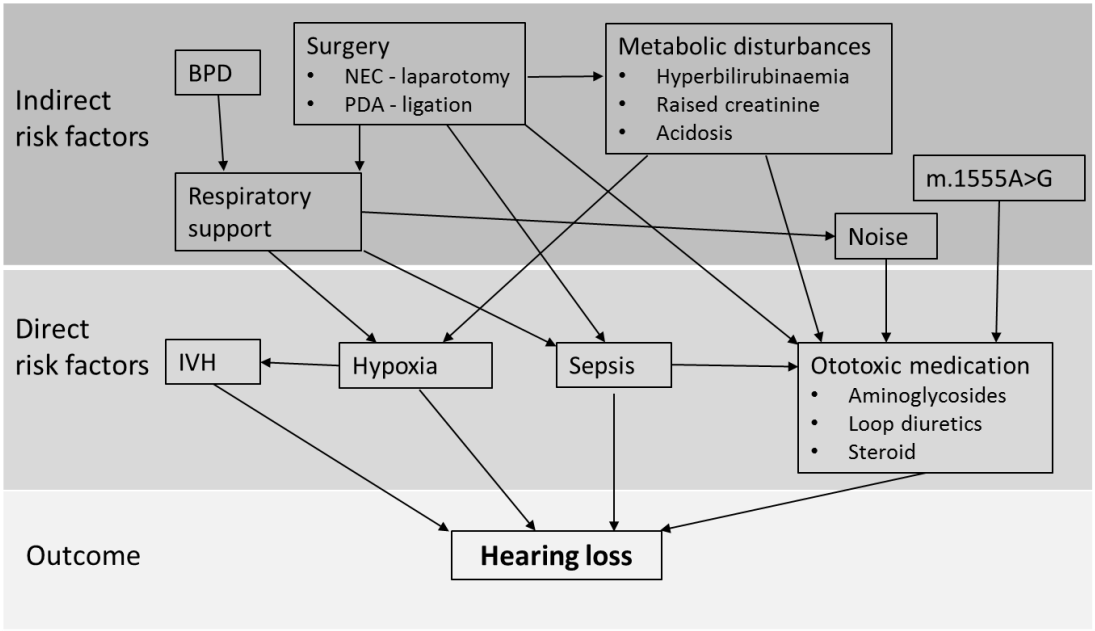
The interference of mitochondrial function is indisputably critical to hearing loss. However the risk of aminoglycosides to individuals with the m.1555A>G mutation, is somewhat unclear. Inconsistency has been observed within a single family of carriers of the mutation in terms of deafness presenting in individuals with and without aminoglycoside exposure [117] and within patient groups [120]. Aminoglycosides also present a risk to hearing loss independently, but susceptibility appears to be increased in the presence of both m.1555A>G and ototoxic antibiotics. Aminoglycoside induced hearing loss in carriers of m.1555A>G has not only been found to lower the age of onset [117], but also increase the severity of impairment [127]. It is not clear what causes individual inconsistency within a family of carriers, whether deafness is due to the type of aminoglycoside used, dependent on the dose

administered or whether there are additional confounding factors. The prevalence and penetrance of m.1555A>G remains undetermined in previous literature owing to mixed populations and methodology including ascertainment bias. Due to the high rates of aminoglycoside use in the care of preterm infants, the differences in prevalence of m.1555A>G across this population in varying studies and the reported high penetrance of deafness following aminoglycoside administration, further investigation is warranted in this population.

1.4 Multifactorial pathways

The aetiology of hearing loss in preterm infants remains unclear. Whilst some variables might independently cause hearing impairment, there are more likely to be multifactorial relationships with indirect variables influencing the ototoxicity of more direct causative factors resulting in an increased risk of hearing loss (Figure 1-2).

Figure 1-2: Relationships between risk factors for hearing loss occurring independently or interactively in infants.



It is plausible that hearing loss is a consequence of a congruence of risk factors which previous studies have so far failed to adequately address. Several studies have suggested this. For example in the study of Vohr et al (2000) from the NICHD Neonatal Network database, 59% of infants had one or more risk factors for hearing loss, and two or more risk factors were found in 26% of NICU admissions [89]. However, hearing loss was not formally assessed in this study and the prevalence rates determined by this study provides only an indicator as to the number of children that could be at risk. Nevertheless, coexisting risk factors have been

demonstrated to increase the probability of hearing loss. Two risk factors or above have been found to increase the chances of SNHL in comparison to infants that have a single risk factor [128]. Furthermore, almost double the prevalence of children with hearing loss had five or more risks present in comparison to infants with 3 or 4 risks [129]. However, a greater prevalence of risk factors does not determine the relative importance of different factors. Salamy et al (1989) grouped infants based on the number of days of intensive care, ventilation, antibiotics and total number of blood transfusions and made comparisons within illness groups when investigating the aetiology of hearing loss. However, these variables are inextricably associated with other aspects of neonatal illness which could cause hearing loss, such as sepsis and BPD. Attempts to match groups based on risk factors ideally require these variables to be independent.

The increased risk of adverse outcome has also been expressed in terms of neonatal morbidities. Adverse neonatal outcome include delay in cognitive ability, neurodevelopmental functioning, and difficulties with sensory ability including vision and hearing impairment. In comparison to cognitive and motor impairments, difficulties with vision or hearing are less common across gestational age [1]. Although the rate of hearing loss is lower than other more prevalent impairment domains, it has a direct impact on language development and communication ability. Previous studies have investigated the risk of poor developmental outcome in respect of diagnoses received during neonatal care. Schmidt et al (2003) compared the rates of BPD, brain injury and ROP on poor neurodevelopmental outcome in extremely low birthweight infants (<1000g) following the Trial of Indometacin Prophylaxis in Preterms (TIPP) trial [130]. At 18 months, each morbidity was independently associated with an increased risk of neurosensory impairment, including severe hearing loss. These findings were replicated in a follow up of infants from the Caffeine for Apnoea of Prematurity (CAP) trial [131]. The risk of poor outcome at 5 years, increased incrementally with each additional morbidity (OR 2.4, 95% CI 2.0-2.9). The prevalence of

hearing loss was higher in children who had 2 or more morbidities in comparison to those without any morbidities (9% and 1% respectively). Further studies investigated BPD, brain injury and ROP with the addition of infection [82] or cholestasis following total parenteral nutrition [132]. Infection added to the prediction of poor neurodevelopmental outcome, but cholestasis was not significant. Although the total number of morbidities is suggestive of a more complex relationship, this is not specific to hearing loss but rather overall neurodevelopmental outcome.

Although the total number of risk factors or morbidities is suggestive of a more complex relationship, a timeline of coexisting risk factors rather than the prevalence of risk may be of greater benefit. Marlow et al (2000) compared a range of neonatal variables between 45 very preterm infants (<33 weeks), 15 of which had impaired hearing [4]. Infants with hearing loss required longer durations of respiratory support and treatment for poor circulation and oedema. However, they hypothesised that the coincidence of risk factors might provide a better understanding of the development of SNHL in preterm infants. The administration of furosemide when creatinine levels were raised, or in the presence of netilmicin was associated with hearing loss as was the administration of netilmicin when bilirubin was elevated. Furthermore, acidosis occurring when bilirubin levels were raised also increased the risk of hearing loss. These combinations highlight the potentiation of ototoxicity between medication and physiological instability, and there may be a further differential susceptibility depending on infant age or the duration of exposure. Netilmicin, bilirubin and creatinine levels were not significantly different between groups when comparing each risk factor individually. Coexisting risk factors for preterm hearing loss highlight the importance of timing in neonatal treatment.

The relative risk of different neonatal factors and their interactions are still not clearly understood. Studies are difficult to compare owing to a wide span of literature with differing

populations, definitions, criteria, outcome, and based on the time of the study and the subsequent type of care provided. This is further complicated by an often small sample size. There are a multitude of simultaneous factors which have separate effects and will influence other factors in complex ways; these relationships are still largely unknown. Time will play a role and there are problems with comparing babies when maturity will vary with gestational age, birthweight and additionally birthweight for gestation along with duration of treatment and exposure to risk factors.

1.5 Summary and aims

The aetiology of hearing loss in preterm infants is complex and likely to be influenced by a number of neonatal risks. It is well established that the babies born at the earliest gestational ages, with the lowest birth weights, have an increased prevalence of hearing loss. Hearing loss is derived from two aetiologies: SNHL as a result of damage to the outer hair cells of the cochlea, and ANSD as a consequence of inner hair cell or auditory nerve dysfunction. The aetiology of SNHL or ANSD is likely to be multifactorial; so far few studies have found discernible differences between the two. For this reason the current study will consider them as one population of preterm infants with hearing loss.

Despite many attempts to understand acquired hearing loss in a preterm population, eliciting the relationship between hearing impairment and clinical and therapeutic factors remains unclear. To some extent this is primarily due to the complexity of the interactions, and partly due to methodological heterogeneity in previous studies.

The pathological significance of m.1555A>G to deafness and the relationship between the mutation and aminoglycoside antibiotics has also yet to be established. Whilst this has not been thoroughly evaluated, m.1555A>G and an exposure to aminoglycosides could play a central role in the causative pathway of hearing loss in preterm infants.

The aim of this study is to evaluate the causative factors of hearing loss in children born prematurely over a geographic area. To achieve this the study will identify preterm infants with hearing loss and control infants with normal hearing, and compare risk factors and confounding factors between the groups. The specific objectives of this study are

1. To establish the role of the mitochondrial mutation, m.1555A>G in deafness in preterm babies.
2. To consider the frequency and penetrance of the genetic variant m.1555A>G between the two groups of infants.
3. Within the context of genetic status, to identify individual neonatal risk factors involved in hearing loss.
4. To identify combined risk factors in order to improve prediction by looking at occurrence rates and overlapping risks.

1.5.1 Hypotheses

The following hypotheses will be tested in this study.

1. The mutation, m.1555A>G, will make a significant contribution to deafness in preterm infants following neonatal intensive care even when levels of aminoglycosides have remained within the therapeutic range.
2. The frequency of the mutation will be higher in the group of infants with hearing loss.
3. Hearing loss in infants born prematurely is related to individual neonatal risk factors.
4. Exposure to multiple coexisting risk factors in the neonatal period will be associated with hearing loss.

2 Chapter 2: Method

2.1 Study design

This was a case control study, with multiple controls recruited per case.

2.2 Population

This study recruited children with confirmed hearing loss who were born at less than 31 weeks and 6 days gestational age. Children were born between 1st January 2009 and 31st December 2013 and were treated at a neonatal intensive care unit within the Greater London area. Initially all children with hearing loss of any severity were recruited to the study. Exclusions were subsequently made if there was a known genetic or "syndromic" cause of hearing loss or a neurological abnormality that could cause deafness. For purposes of clarity, children were excluded after genetic screening as a diagnosis of a syndrome or neurological abnormality did not predetermine a negative result for m.1555A>G. Methodology was informed by variations of inclusion and exclusion criteria in previous studies which led to limitations in interpreting data (as discussed in Chapter 1), therefore this study aimed to include as many children with hearing loss as possible.

For each child with hearing loss, up to five children with normal hearing were recruited. Control children were matched for sex, number of completed gestational weeks (plus or minus one week), calendar year in which they were born to minimise changes in neonatal treatment during the study period, and neonatal intensive care unit where they received the first two weeks of treatment after birth. Exclusion criterion for control children were missing pharmacological data, on the premise that other control participants with complete medical records could potentially be identified.

2.3 Identification of participants

Children with hearing loss were primarily identified by the national Newborn Hearing Screening Programme (NHSP) database. Initially the children born between 2009 and 2012 were identified, and a second cohort was identified for children born in 2013. The delay in identifying the 2013 babies meant that recruitment could start whilst enabling the babies born late in the year (who may have received many weeks of neonatal care) to have their hearing screen prior to discharge. The two birth cohorts aimed to reduce the number of children potentially missed from late 2013. Children were additionally identified through hearing assessment records, neonatal follow up services, and referral to the genetic deafness clinics. The study was advertised on the Action on Hearing Loss and Bliss websites, enabling parents to enquire about the study directly. As this study used multiple methods of ascertainment to identify eligible participants, new cases were screened for duplication following identification and prior to invitation letters being sent.

Children with normal hearing were partially identified through NICU admission books, where these were available, using matching criteria. This was supplemented by data from the National Neonatal Research Database (NNRD), through the Neonatal Data Assessment Unit (NDAU) who identified the remaining eligible control children. The NNRD is an 'opt out' database that contains demographic and clinical information about infants who have been admitted to any neonatal unit within the UK. No specific identifiers are held on this database, which is updated following cleaned quarterly downloads from the national 'real-time' database (BadgerNet Neonatal; managed by Clevermed Ltd). Having identified children using their unique NNRD badger identification numbers, NHS numbers were obtained subsequently through the original BadgerNet database. All children who met eligibility requirements were invited to participate to allow for potential recruitment failure.

2.4 Procedure

Children identified by NHSP with a failed newborn hearing screen had their hearing impairment confirmed by their Consultant Audiovestibular Physician/Paediatrician prior to being invited to participate in the study. Following confirmation of a permanent hearing loss, letters of invitation were sent to parents of all children by their Consultant (Appendix 4). In cases where it was not possible to identify a Consultant Audiovestibular Physician, the child's General Practitioner (GP) was approached and asked to send a letter of invitation instead. Parents of control children were invited by letter from the Neonatal Consultant acting as the local investigator for the study from the unit in which they received their first two weeks of neonatal intensive care. Invitations included a parent information leaflet providing details regarding the study, and parents were asked to return a reply sheet to the researcher if they would like to be contacted to participate (Appendix 5).

Parents of all children who responded, were contacted by phone or email, dependent on their stated preference, and a home visit was arranged. Parents who did not respond were contacted by telephone to ensure that they had received the study information. For children with an unconfirmed hearing loss, the diagnosis and severity of impairment was discussed with the parent/guardian, and the name of the Consultant Audiovestibular Physician leading their care was established for data collection purposes. Written consent was taken for all participants from a parent or legal guardian (Appendix 6). Consent was obtained to take a saliva sample for genetic screening for m.1555A>G where this had not already been done, to access medical notes to retrieve a clinical history from the neonatal period, and to obtain recent audiology data. Parents were given a £10 gift card to thank them for their time.

2.5 Hearing assessments

Hearing screening for all children was completed prior to discharge from neonatal units. The recommended initial screen for preterm babies is usually AOAE, followed by Automated ABR

in both ears, unless the baby has been previously diagnosed with meningitis or bilateral atresia. In these cases the baby will be referred for specialist ABR testing. Results of newborn hearing screening are recorded as either a pass (a clear response in both ears) or refer for outpatient follow up if there was no clear response in one or both ears. Follow up screening was carried out by an experienced audiological physician/paediatrician. A standardised audiological proforma for the study was completed by the audiology physician (Appendix 7). Hearing impairment was confirmed as being sensorineural hearing loss or auditory neuropathy spectrum disorder, and was either unilateral or bilateral. The definition for severity of hearing loss for this study was mild (21-40 dB HL), moderate (41-70 dB HL), severe (71-95 dB HL), or profound (>95 dB HL). The use of hearing aids or cochlear implant was recorded, along with any testing completed prior to the study exploring the aetiology of deafness. All data were entered onto the study database, which was built in the REDCap (Research Electronic Data Capture) environment (https://www.project-redcap.org).

2.6 Neonatal data collection

Data were collected from neonatal units following written parental consent. Data were abstracted from medical notes as a hard copy using a standardised proforma (Appendix 8), which was entered onto the electronic database (REDCap). To preserve anonymity, a unique study ID number was allocated to each child and used on both the proforma and computerised data entry, as well as the date of birth.

Variables for which data were collected were in accordance with previous research and were as follows; sex (male/female), date of birth, gestational age at birth (completed weeks and days), multiple birth, birth weight, intrauterine growth restriction (less than the 10th centile for gestational age), Apgar score (at 5 minutes), CRIB II scores [44, 133], all hospitals where treatment was received including transfer dates, acquired brain injury (IVH grades I-II, IVH with ventricular distension, intraparenchymal lesion, and periventricular leukomalacia) [53],

pneumothorax, pulmonary haemorrhage, PDA (no treatment needed, medical treatment, surgical intervention), NEC (medical treatment, surgical drain, laparotomy), BPD (oxygen at 28 days and in air at 36 weeks corrected GA, <30% oxygen at 36 weeks, >30% oxygen at 36 weeks) [44], septicaemia (positive blood culture), meningitis (positive cerebrospinal fluid), highest bilirubin level, highest creatinine level. The number of days were recorded as for each of the following; ventilation, CPAP, oxygen, long line in situ, level 1 care, level 2 care and level 3 care [134].

Data was collated in the form of a daily timeline for each variable/risk factor to calculate co-occurring risks, for the first 14 days and then weekly until the infant had either been discharged home or transferred to a paediatric ward. Variables were as follows; medication recorded if administered; amikacin, netilmicin, gentamicin, vancomycin, furosemide, indometacin, ibuprofen, inotropes (dopamine, dobutamine, adrenaline, norepinephrine), dexamethasone, hydrocortisone and methylprednisolone. Blood levels recorded; highest levels (for amikacin, netilmicin, gentamicin and vancomycin), highest serum bilirubin, highest creatinine, highest lactate, lowest pH level. Further variables documented; exchange blood transfusion, blood transfusion, highest mode of respiratory support (HFO, conventional ventilation, CPAP, oxygen), and the administration of total parenteral nutrition (TPN).

Many children received neonatal care at more than one hospital and therefore had multiple volumes of clinical notes. For those where the medical notes could not be accessed, information was derived from the daily and stay summaries on the BadgerNet system where available.

2.7 Genetic screening

Buccal swabs were taken from all children who had not been tested previously, to screen for the m.1555A>G mutation, using an Oragene OG-575 sample kit. Samples were labelled with the child's unique study ID number and date of birth. Children with hearing loss that had

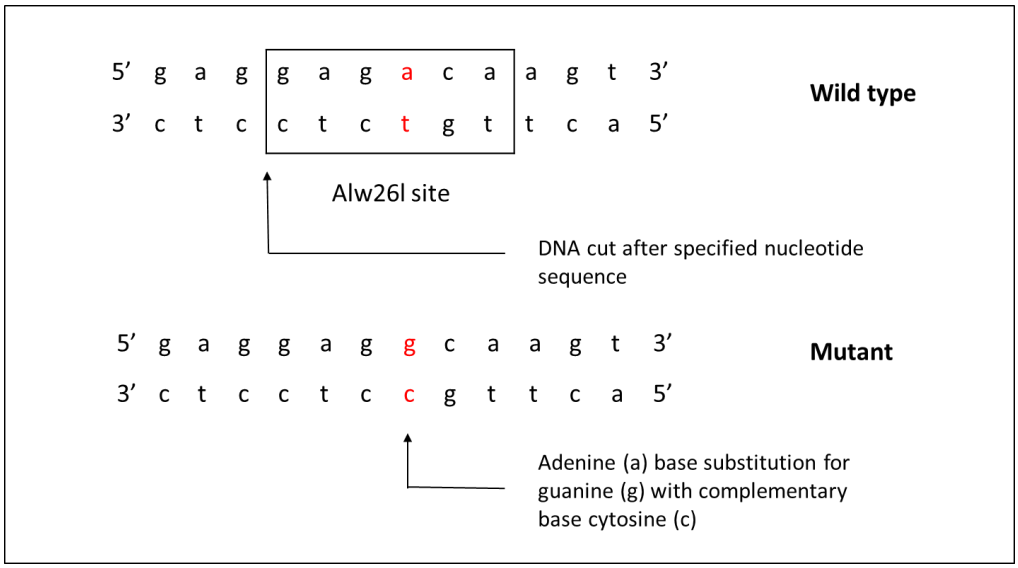
been tested clinically were not re-tested if their results were accessible from their medical notes.

Samples were analysed at the North Thames Regional Genetics Laboratory, Great Ormond Street Hospital. DNA was extracted from saliva using standard procedures and analysed by polymerase chain reaction (PCR) by laboratory personnel.

A primer mix and megamix (containing proprietary buffer, Mg and dNTPs and Taq polymerase) were added to the DNA sample for thermal cycling. The sample was heated to 95 ^{o}C for 5 minutes. Cycles of heating to 95 ^{o}C for 30 seconds and cooling to 56 ^{o}C for 1 minute for primer annealing and polymerase extension were repeated 33 times for amplification of the target sequence of DNA. After the 33 cycles the temperature was held at 72 ^{o}C for 5 minutes. The expected fragment size was 657 base pairs.

The DNA was cut at the target sequence ($GTCTC(N)_1\downarrow$) by the restriction enzyme Alw26I (figure 2-1) at 37^{o}C for a duration of 4 hours. The mutation causes a loss of the Alw26I site and therefore DNA sequences containing the mutation remained uncut and with a length of 657 base pairs.

Figure 2-1: Target site for enzyme restriction digestion in wild type and mutant DNA sequences



Wild type sequences were cut into two fragments of 405 base pairs and 252 base pairs. The digested DNA together with 10µl of dye were loaded on 3% agarose gel, separating the DNA by size and subject to electrophoresis. Any uncut fragments were then checked by bi-directional Sanger sequencing. A second sample was taken from children with the mutation for re-testing.

Families found to have the m.1555A>G mutation were offered a genetic clinic appointment at Great Ormond Street Hospital with the study Chief Investigator, Professor Maria Bitner-Glindzicz.

2.8 Statistical analysis

Data were double entered into a REDCap (Research Electronic Data Capture) database with a double coding error rate of less than 2%. REDCap is a secure website for developing an online database which enables audit trails for tracking data changes, and the export of data to statistical software packages. Only anonymised data was entered onto this database. The birth rate in London is approximately 120 000 babies per year (600 000 for 5 years of the

birth cohort for the study). Approximately 1% will have a birth weight under 1500g which gives a probable population of 3000 – 6000 infants born at less than 32 weeks gestation over the 5 year period. The prevalence of hearing loss is approximately 1-2% [4] which results in a possible 30-120 children with hearing impairment in this population. In a group of 3000 – 6000 preterm infants (less than 32 weeks of gestation) 4-10 children would be expected to carry m.1555A>G based upon a mutation frequency of 1 in 526 [121]. In the event, 93 eligible children were identified with hearing loss from all sources. It is possible that no child would be identified with m.1555A>G from such a small number, but if the thesis that neonatal acquired hearing loss resulted from aminoglycoside administration in the face of the mutation, we would expect that this group would be greatly enriched by children with m.1555A>G.

2.8.1 Baseline characteristics of case and control groups

Up to 5 matched normal hearing control children were recruited per case to increase power. To achieve a statistical power of 0.8, with a medium effect size and a p value of 0.05, the sample size required was 156 [135]. The expected sample size of cases was 30-60 children with hearing impairment, leaving an expected matched control group of normal hearing children as 3-5 children per case. A larger sample size was expected to narrow confidence intervals. All analyses were adjusted for matching criteria (sex and gestational age, as well as birthweight for gestational age to reduce additional confounding). Birthweight by gestational age was derived using a standard deviation score using UK standards (http://www.healthforallchildren.com/shop-base/shop/software/lmsgrowth/). Neonatal clinical characteristics were described using frequency and percentages for categorical data, and median and interquartile range for continuous variables. Differences between cases and controls were examined using chi square, independent t tests and non-parametric Mann Whitney U tests for binomial and continuous variables in Stata (version 13). The distribution

of the primary variables were plotted as histograms revealing a non-normal distribution in the majority of the variables, and therefore non-parametric testing was used where possible. All analyses were reported as the more conservative 2 tailed level of probability for each variable.

2.8.2 Risk factors as independent predictors of hearing loss

Neonatal data and clinical diagnoses were analysed individually using logistic regression for univariate analysis. Continuous variables were re-coded for the analysis of combined risk factors as follows, creatinine was considered a risk at greater than 90mmol/l, blood pH level less than 7.2, total bilirubin greater than 200micromol/l, and lactate greater than 2.0mmol/l [4]. Additional variables were added for aminoglycosides (amikacin, gentamicin and vancomycin) firstly as 'any aminoglycoside' denoting any drug given at any point during hospitalisation as a binary variable, and as a second variable to investigate the total number of individual drugs that had been given. Steroids (methylprednisolone, hydrocortisone and dexamethasone) were categorised in three ways: firstly the number given per day (and per week from week 3 onwards), secondly if any steroid had been given in the first 72 hours after birth, and thirdly, whether any steroid was received at any point during care.

2.8.3 Combinations of risk factors as independent predictors of hearing loss

Associations between simultaneously occurring combinations of clinical risk factors as predictors for hearing impairment were evaluated using multivariate regression. Models included baseline risk factors, diagnoses, treatments and physiological risk factors, and subsequently, with the exclusion of clinical diagnoses. Individual models for specific diagnoses (PDA, BPD, NEC/sepsis and IVH), and their associated treatments alongside potential effect modifiers in the form of physiological factors, were also run using multivariate regression analyses. Variables included in all models were predominantly

categorical. Sample size for multivariate regression models was based upon the largest regression model which included 22 predictors. To run this model the minimum sample size required was 50 + (8k) [135]; therefore 50 + (8x22) = 226. Data was available for 237 children meeting the minimum required sample size parameter.

Exposure to risk factors for hearing loss across the neonatal period, included 8 risks which had all reached significance in univariate analyses. A mean number of risk factors were derived for each day for the first 14 days and per week, using categorical variables.

The risk of ototoxic medication, including gentamicin, vancomycin and furosemide, was measured in two ways. Firstly as a binary variable, whereby infants had received any of the three medications in the first 14 days, or per week in the subsequent weeks. Logistic regression was used to compare each group per day, and then as an overall effect. Secondly, the total number of cumulative days of each medication (the total possible over the first 14 days was 42), as a continuous variable. Comparisons between the groups were made per day and as an overall effect using multivariate logistic regression. Comparisons were made between groups per week using logistic regression, and then as an overall comparison.

Multivariate regression analyses were again used to investigate the relationship between ototoxic medication, 'haemodynamic instability', clinical diagnoses and deafness in this population. The variables for ototoxic medication have been described above. Comorbid conditions diagnosed at any point during neonatal care included PDA, IVH/PVL, BPD and NEC and/or sepsis. Haemodynamic instability included variables indicating poor renal function or evidence of poor perfusion and need for circulatory support, namely: creatinine >90mmol/l, lactate >2.0mmol/l or the administration of inotropes. Similarly to ototoxic medication, a binary variable was created to ascertain whether any of the markers had been present per day. A cumulative total of the three variables was also created per day. Ototoxic medication and haemodynamic instability were analysed individually by day, and as an overall

comparison between cases and controls. The fit of all regression models was based upon the likelihood ratio chi-square significance value.

2.9 Ethical approval

The study received ethical approval by Central and East London Local Clinical Research Network (ref: 12/LO/0005). Approval was also granted by the Ethics and Confidentiality Committee of the National Information Governance Board to lift Section 251 of the NHS Act 2006 and the Health Service Regulations 2002. Approval enabled access to patient identifiable information without prior parental consent for the purpose of identifying eligible children.

Research and Development approval was sought by the student, and granted at 25 trusts covering 33 collaborating sites (Appendix 9), including both hospitals and community trusts. Substantial amendments were made to the parent information leaflet with updated contact information (dated 10.05.13), to the parent letter and protocol to enable GPs to be contacted (dated 20.03.14) and finally to the reply sheet and protocol to contact parents if no response was received from them (dated 09.12.14). Minor amendments were made to the consent form following approval for changes to the parent information leaflet (dated 20.05.13), to the protocol for the same reason (dated 28.05.13), and again to the protocol to update information regarding NNRD (dated 24.07.14). A research passport was obtained for the study which was required to gain a letter of access or honorary contract for each trust. It took approximately 9 months to receive the majority of the approvals and corresponding paperwork. The study was registered with the International Standard Randomised Controlled Trial Number (ISRCTN39982239) and was part of the National Institute for Health Research (NIHR) study portfolio. Action on Hearing Loss together with the Rosetrees Trust provided full funding for the study (ref: RNID G47).

3 Chapter 3: Influence of m.1555A>G on hearing loss in preterm infants

3.1 Introduction

Aminoglycoside antibiotics are widely used in the first line treatment of suspected sepsis in infants born prematurely. In line with NICE guidelines, 89% of the 160 neonatal units in England use gentamicin [136]. Susceptibility to the ototoxic side effects of aminoglycosides is increased in carriers of the maternally inherited m.1555A>G genetic variant, even when blood levels of aminoglycosides are within the normal clinical recommended range.

Owing to the frequency of gentamicin use in neonatal care, aminoglycoside induced hearing loss would be expected to be higher than the general population if penetrance is as high as previously reported (as discussed in Chapter 1). Methodology and population samples have varied between previous studies and the true risk of deafness caused by aminoglycosides in the presence of m.1555A>G remains unknown.

This study aimed to compare the difference in m.1555A>G prevalence between children with hearing impairment and those with normal hearing who were born prematurely. Children were expected to be more likely to have the mutation in the group with hearing loss, and to have received aminoglycoside antibiotics.

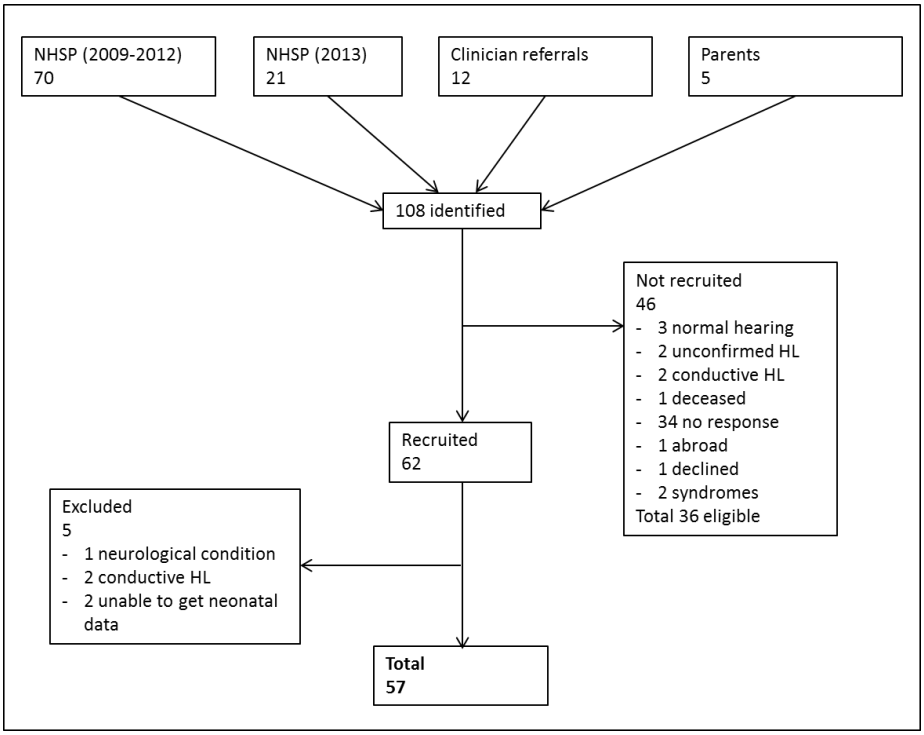
Before considering the frequency and penetrance of the mutation, it is firstly important to consider the number of children with hearing loss, recruitment, and demographic differences between the recruited and non-recruited groups of children identified with impaired hearing, before making comparisons between genetic influences on hearing loss.

3.2 Results

3.2.1 Participants

Between 2009 and 2013, there were 91 children identified with hearing loss by the Newborn Hearing Screening Programme, which was supplemented with parent and clinician referrals to give a total of 108 children. This is consistent with the predicted estimate of 30-120 preterm infants with hearing impairment over a 5 year period (see section 2.8). Sixty two children with hearing loss were recruited to the study, 5 of whom were subsequently excluded. The pattern of identification and recruitment is displayed in figure 3-1.

Figure 3-1: Flow diagram displaying the identification and recruitment of children with hearing loss born below 32 weeks of gestation



A total of 93 children were eligible from the 108. There were 5 children invited as matched controls that had not been identified as cases but parents reported impaired hearing. On confirmation of hearing loss (by Consultant Audiological Physician) they were recruited as cases. There were 3 children who had failed their hearing screen prior to discharge but at

follow up had normal hearing and were therefore not eligible for the study. This is consistent with an early study of newborn hearing screening which demonstrated a false positive rate of 1-2% [137]. Although the number of children in this study that went on to have normal hearing is slightly higher than the initial rate of false positives, 2 of the 3 children were seen in audiology clinics for multiple follow up appointments before they were discharged from the service. All 34 families from whom there was no response, were unable to be contacted in writing or by telephone.

Children excluded after recruitment were as follows. Two children were later found to have a conductive hearing loss and no confirmed permanent hearing impairment. One child received their neonatal care at a neonatal intensive care unit outside of Greater London. A further child received care at a hospital from which approval to access medical notes could not be obtained. As access to medical records to determine aminoglycoside exposure was a prerequisite to eligibility it was not possible to include them. Using data provided by NDAU, the distribution of sex, birth year and gestational age were compared between the recruited and non-recruited group of children with hearing loss (table 3-1).

Table 3-1: Comparison of core data in responders and non-responders for children with hearing loss

		Recruited (%) (n=57)	Not recruited (%) (n=36)	Total (n=93)
Sex	Male	38 (76)	12 (24)	50
	Female	19 (44)	24 (56)	43
Birth year	2009	16 (73)	6 (27)	22
	2010	13 (59)	9 (41)	22
	2011	9 (60)	6 (40)	15
	2012	8 (62)	5 (38)	13
	2013	11 (52)	10 (48)	21
Gestational age	23-25	16 (62)	10 (38)	26
(weeks)	26-28	19 (54)	16 (46)	35
	29-31	22 (81)	5 (19)	27
	Unknown (<32)	0	5 (100)	5

The proportion of males recruited to the study was higher than females, and the proportion of children identified with hearing loss was lower in 2011 and 2012 but relatively consistent across the other 3 years. More than half of the children identified at each gestational age were recruited, and there were 5 children from the non-recruited group with missing gestational age, but identified by the Newborn Hearing Screening Programme as having been born at less than 32 weeks of gestation.

3.2.2 Prevalence and penetrance of m.1555A>G

Genetic screening for m.1555A>G was carried out on 241 children (62 cases and 179 controls). One sample testing for a normal hearing control child failed (the laboratory was unable to process a result), and a repeat sample could not be obtained. The results for all children, including those that were later excluded, are displayed in table 3-2.

Table 3-2: Distribution of m.1555A>G for children with and without hearing loss

	Number of children tested	m.1555A>G positive
Cases*	62	0
Controls	179	1

*Total number of children tested before exclusion criteria applied

The prevalence of the mutation in this study is 1 in 241 which is 0.41% (95% CI 0.07-2.30). There were no children with hearing loss that tested positive for the mutation. Furthermore, the child with the genetic variant was recruited from the control group and had normal hearing.

The penetrance of permanent hearing loss following aminoglycoside exposure in this study cannot be established, as only one child was carrying the mutation. However, as hearing screening was normal despite aminoglycoside administration, penetrance is not as high as the previously reported 100%.

3.2.3 Case study

One child was found to be a carrier of m.1555A>G; for confidentiality purposes this child will be referred to as Child A. Child A was a male infant, born at 29^{+4} weeks gestational age with a birth weight of 956g (below the 10^{th} centile for gestational age), Child A received one course of amikacin (3 doses at 4mg/kg over 3 days) on admission to the neonatal unit and one course of gentamicin (dose and duration unknown as he was transferred to a local neonatal unit from which it was not possible to access medical records) in the third week after birth for suspected NEC. Blood cultures were negative throughout. No other ototoxic medication was received. During the first course of aminoglycosides creatinine level was >89mmol/l and

lactate was >3.5mmol/l for the duration of the course. Before being discharged from the neonatal unit, Child A had a newborn hearing screen which was passed.

Subsequently, genetic screening was completed for Child A's mother who also had m.1555A>G, and for an older female sibling (Child B). Child B was also preterm, born at 33^{+5} weeks gestation (not eligible for recruitment to the study) and also received gentamicin and vancomycin (dose and duration unknown for both antibiotics) during the neonatal admission for suspected sepsis. Child B also passed her newborn hearing screen prior to discharge from the neonatal unit. Known blood concentration levels for aminoglycosides in Child A and B were reported to be within the normal clinical range. Both children were offered a hearing assessment following confirmation of m.1555A>G, which indicated normal hearing for both children, at the age of 2 years for Child A (audiogram displayed in figure 3-1), and 5 years for Child B.

At a Principal Investigator follow up assessment 6 months later, a unilateral high frequency hearing loss was noted for Child A, at age 3 years (audiogram displayed in figure 3-2). OAE's were present bilaterally, indicative of functional outer hair cells. Child B continues to have normal hearing. This finding does not materially affect the conclusions of this study.

Figure 3-2: Audiogram for Child A at follow up audiology assessment, aged 2 years and 9 months

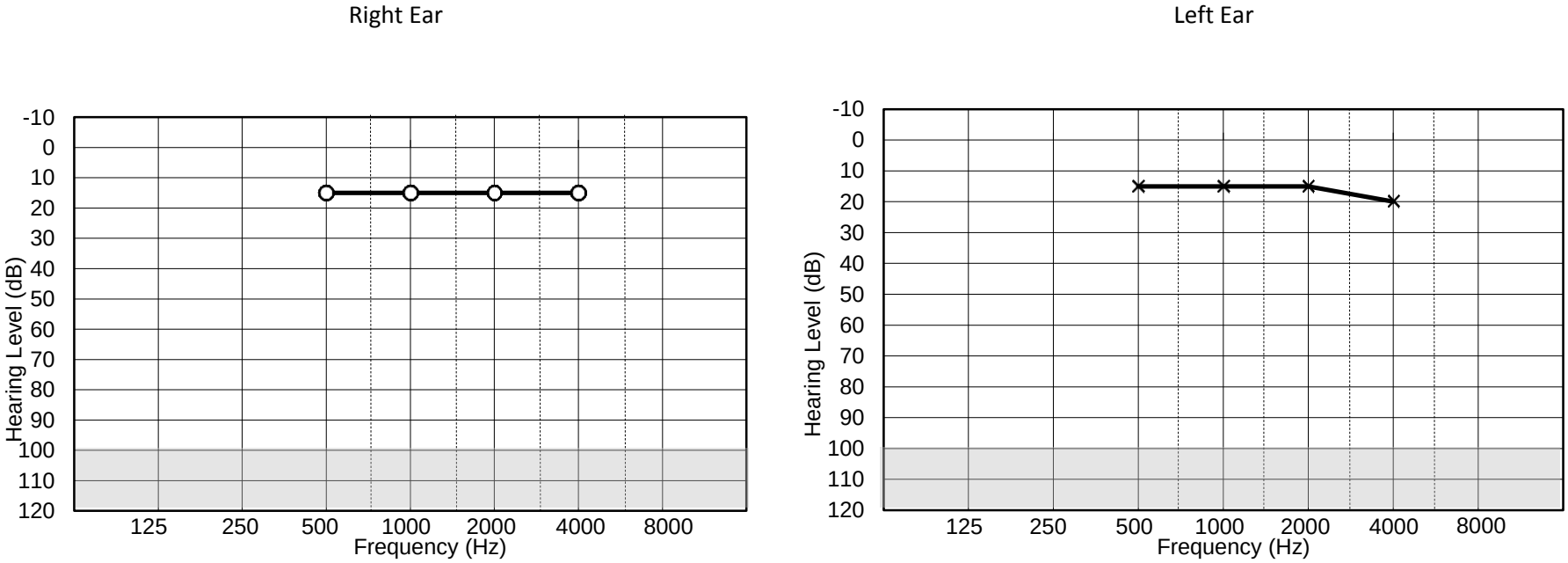
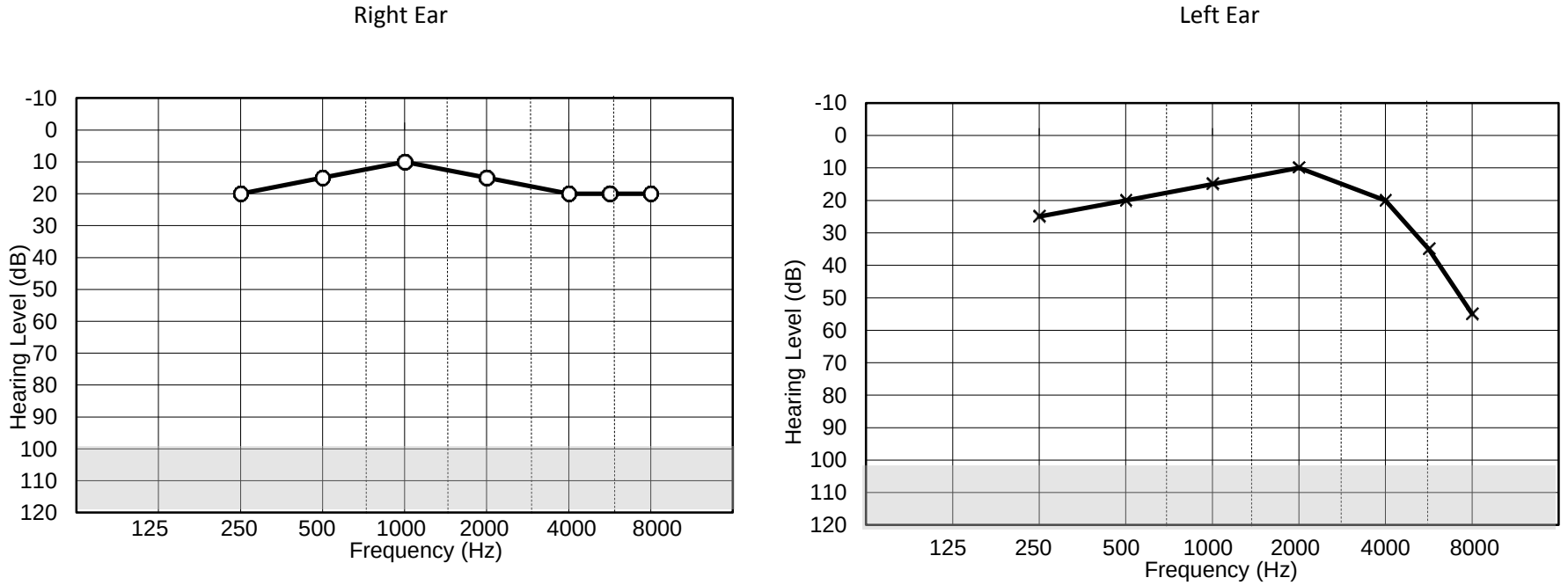


Figure 3-3: Audiogram for Child A at follow up audiology assessment, aged 3 years and 3 months



3.3 Discussion of results

The aim of the study was to determine the contribution of m.1555A>G to deafness in a preterm population. It was expected that this genetic variant would play a significant role in the loss of hearing in a premature population that is likely to have received aminoglycoside antibiotics. In the event, one child was found with the mutation, who had received amikacin and gentamicin during the neonatal admission but who had normal hearing throughout the study period. Hearing impairment was noted after the data collection period during continual follow up assessments. Although only detecting the mutation in one control child was an unexpected finding, his has been found by previous research (which will be discussed in relation to the penetrance of the mutation). Whilst there is a possibility that the mutation was present in children that were not recruited, there were no substantial clinical differences between the recruited and non-recruited children (table 3-1) therefore, it is unlikely that there will be a higher proportion of the mutation in the untested non-recruited children with hearing loss.

The overall prevalence of m.1555A>G in this study was 0.41% (95% CI 0.07-2.30), which fits within the trends that previous studies with neonatal populations have found (Appendix 3). Prevalence rates in studies in China, Germany and the US range between 0.12 and 0.69% [123-125, 138]. The US studies had the highest prevalence rates; Johnson et al (2010) from a population of 436 premature, low birth weight (<2500g) infants found 3 infants to be carriers of the mutation (0.69%) [125]. Ealy et al (2011) enrolled 703 infants who had previously been admitted to NICU, and found a prevalence rate of the mutation in this population to be 0.28% [123]. Gopel et al (2014) found 12 infants with m.1555A>G from 7056 (0.17%) recruited from neonatal intensive care units in Germany [124]. In a Chinese study of newborn infants, Wang et al (2011) recruited 14 913 infants and found the mutation in 18, giving a prevalence rate of 0.12% [138]. In contrast, a large Brazilian study of newborn infants found no mutations in

a sample size of 8974 infants that comprised almost 2000 more than the largest US or European neonatal studies [139]. There is therefore global variation in the prevalence of the mutation. In older children, a population study of European children (unselected for hearing status) demonstrated a prevalence of 0.19% (95% CI 0.10-0.28) [121]. The 95% confidence intervals in the current study overlap with those from the European study, indicating no significant difference between the populations which are therefore comparable. Although the range of prevalence varies according to geographical region, the current study follows the trend found in US and other European studies.

The penetrance of deafness in carriers of m.1555A>G following a single dose of aminoglycosides has previously been reported to be 100% [117]. The current study found one child (and their sibling, both with normal hearing throughout the study period as discussed in section 3.2.3) with m.1555A>G making penetrance impossible to establish with this dataset, although it would certainly appear to be lower than 100%. If hearing loss had been evident during the study period, this child would have been recruited as a case. Subsequently, this would have resulted in a 1 in 57 frequency which is not significantly different to the expected mutation frequency of 4 ($p>0.05$), and in retrospect does not alter the overall study conclusions. Even when taking into account the late onset hearing loss in this child, the sibling had normal hearing and therefore penetrance remains below 100%. The London Operational Delivery Network discharged home 1475 infants <32 weeks in 2014 (unpublished source: NHS England). Admission rates at these gestational ages have been stable leading to an estimated discharge population of 7375 infants over the five year study period (full yearly data are not readily available for the whole period). We would anticipate, therefore, 14-15 children discharged home at <32 weeks with m.1555A>G (based on a prevalence of the mutation of 1 in 525 in the UK). Of the babies recruited to this study 93% received gentamicin, amikacin, or vancomycin (table 4-3). Given 100% penetrance, we would

have anticipated 14-15 infants with the mutation among those with hearing loss or 8-9 within our study cohort, or 6 patients if based on gentamicin administration alone (73%: table 4-3).

Since this study began, other studies have also found children to have normal hearing despite having the mutation and receiving gentamicin, again indicating 100% penetrance is an overestimate. Ealy et al (2011) identified 2 children born prematurely who both received gentamicin for 9-13 days, carried the m.1555A>G mutation and had normal newborn hearing screens [123]. Gopel et al (2014) identified 12 preterm infants with m.1555A>G, 10 of whom had received gentamicin, 9 of 12 children passed their initial newborn hearing screen [124]. All 3 babies who failed their hearing test had been exposed to gentamicin. Follow up hearing assessments to confirm hearing loss have not yet been reported for either study, and therefore their hearing status is unconfirmed. Given that the child in the current study had a late onset hearing loss, confirming hearing loss in previous neonatal studies is pivotal. Furthermore, 2 of the 3 infants from Gopel et al's study that failed their hearing screen had low birth weights, 2 of the 3 infants were also the smaller infant of a twin pair (the siblings having a normal hearing screen despite gentamicin exposure), which raises the possibility of additional risk factors along the pathway to hearing loss in those with m.1555A>G. Johnson et al (2010) also found carriers of m.1555A>G had normal hearing after having been exposed to 2 days of gentamicin (2 out of 3 infants) [125]. The infant with impaired hearing had received a longer course of aminoglycosides (4 days) but was also noted to have an extremely low birthweight. Furthermore, the child in the current study was also born below the 10th centile which coincides with previous findings.

A low penetrance suggests other unidentified genetic or environmental factors are involved in the progression of hearing impairment [140]. Low birth weight as mentioned, appears to be a common occurrence when looking at the children with hearing loss across these studies. However, there are other neonatal factors that could increase susceptibility to

aminoglycosides in these individuals. The mutation enables aminoglycosides to bind more readily to mitochondrial ribosomes; protein synthesis is decreased and therefore cellular energy also declines to below the required rate. Moreover, the infiltration of aminoglycosides to the inner ear hairs cells can be potentiated by additional medications such as furosemide which would increase the availability of aminoglycosides binding to mitochondrial ribosomes (discussed in section 1.2.8). Whilst there were no further ototoxic medications given to the child in this study, there were raised physiological markers that reduce the clearance of aminoglycosides. This has also previously been discussed in relation to preterm hearing loss, but not specifically in the presence of m.1555A>G. Cumulative doses and blood levels of aminoglycosides have previously not been associated with hearing loss in the presence of the mutation [120], therefore the influence of additional factors must be explored. Studies often do not report the cause of aminoglycoside exposure in children. Proven sepsis from blood cultures would indicate increased physiological stress that again can impede elimination of aminoglycosides. In the current study both child A and B were treated for suspected sepsis only, none was proven, however child A was unwell following birth as indicated by the administration of respiratory support, antibiotics and elevated creatinine and lactate levels. The presence or absence of sepsis (and associated physiological stress) in the presence of m.1555A>G following aminoglycosides might explain why, in some families, individuals may or may not be affected.

A further point for consideration is the age of onset of hearing loss in previous research. In the absence of aminoglycosides, a prospective study in Finland looked at the audiological screening of 19 children from one family aged 2-13 with m.1555A>G [141]. Thirteen out of these 19 children passed their newborn hearing screen, the remaining 6 did not receive a newborn hearing test. Of these 19 children, 10 (8 of which were male) later developed a hearing impairment ranging from high frequencies to severe progressive losses, with an average age of diagnosis 3.7 years. Non-exposure to ototoxic medication was self-reported

and it is therefore possible that this could be incorrect. Nevertheless, this study demonstrates the risk to hearing from the mutation even without aminoglycosides. In one of the early studies looking at aminoglycoside induced hearing loss, 763 deaf mutes were retrospectively investigated for exposure to aminoglycosides and familial inheritance [142]. Inheritance was exclusively through the maternal line. Over half of participants with aminoglycoside exposure in the presence of a family history of deafness, had an age of onset of hearing loss before 3 years. The group with the positive family history of deafness also received shorter durations of treatment, indicating a higher susceptibility to aminoglycosides existed in this group in comparison to the group without a family history. Not only have shorter durations been observed, but the use of aminoglycosides may hasten the appearance of hearing loss. In a study of 70 families with severe SNHL, 19 were found to carry the m.1555A>G mutation [117]. The age of onset of deafness for patients with m.1555A>G in the presence of aminoglycoside exposure was much lower (median age of 5 years) in comparison to those not treated with aminoglycoside antibiotics (median age 20 years). Again, exposure to aminoglycosides were self-reported as was the onset and progression of hearing impairment. With a probable median time to deafness of 3-5 years in the aforementioned studies in the presence of aminoglycosides, caution should be taken interpreting normal hearing in studies with a neonatal population in those with m.1555A>G; as they could still be at a higher risk of developing hearing loss than those who have the genetic change but have not been exposed.

Since data collection was completed for this study, the control child with m.1555A>G developed a mild high frequency loss of hearing. This was noted around the age of 3 years and is therefore consistent with the findings of previous research.

3.3.1 Summary

The contribution of m.1555A>G was predicted to play a significant role in the development of deafness in a preterm population who are likely to have received aminoglycoside

antibiotics. However, no children from 57 with hearing loss were observed to carry the mutation, and only one child amongst 179 controls carried the mutation. Therefore, the overall prevalence of the m.1555A>G mutation was 1 in 241, but this is unlikely to be significantly different from population estimates from previous studies. It would appear that the m.1555A>G mutation contributes little, if at all, to the excess of hearing loss observed in survivors of very preterm birth.

The penetrance of aminoglycoside induced hearing loss in the presence of m.1555A>G is unlikely to be 100%, given the widespread use of aminoglycosides in a neonatal population (see chapter 3.3). Owing to the unexpectedly low number of children observed to carry the mutation, in contrast to the anticipated finding of there being at least 6 children (see section 2.8), this interaction is unlikely to be responsible for the increased prevalence of deafness among very preterm children. Hence, the following chapter will evaluate the potential role of other risk factors in the causation of hearing loss in this population.

4 Chapter 4: Neonatal influences on hearing loss

4.1 Introduction

Previous studies have evaluated risk factors either from neonatal illnesses or treatments, as independent or combinations of factors, with few consistent conclusions. The clinical course and treatment exposures of very preterm infants are frequently complex and often spread across a number of weeks of hospitalisation.

The aim of the study was to investigate independent and coincident risk factors for hearing loss. Analysis was therefore developed to assess baseline characteristics, diagnoses, intensive care measures and potential ototoxic medications, before evaluating the coincidence of risk factors assessed on a timeline that specified daily occurrences for 14 days and weekly thereafter until discharge.

This section will address the two hypotheses:

1. Hearing loss in infants born prematurely are related to individual neonatal risk factors.
2. Exposure to multiple coexisting risk factors in the neonatal period will be associated with hearing loss.

4.2 Results

4.2.1 Characteristics of children with hearing loss

Fifty-seven children with a confirmed hearing loss were included in the study. This gives an estimated prevalence of 13 children with hearing loss per 1000, in infants born at less than 32 weeks gestation. Data were collected regarding their hearing diagnosis, severity,

detection of impairment and treatment. The audiological characteristics in terms of hearing loss and severity of the sample is summarised in table 4-1.

Table 4-1: Audiological characteristics of hearing impairment in 57 children born at less than 32 weeks of gestation

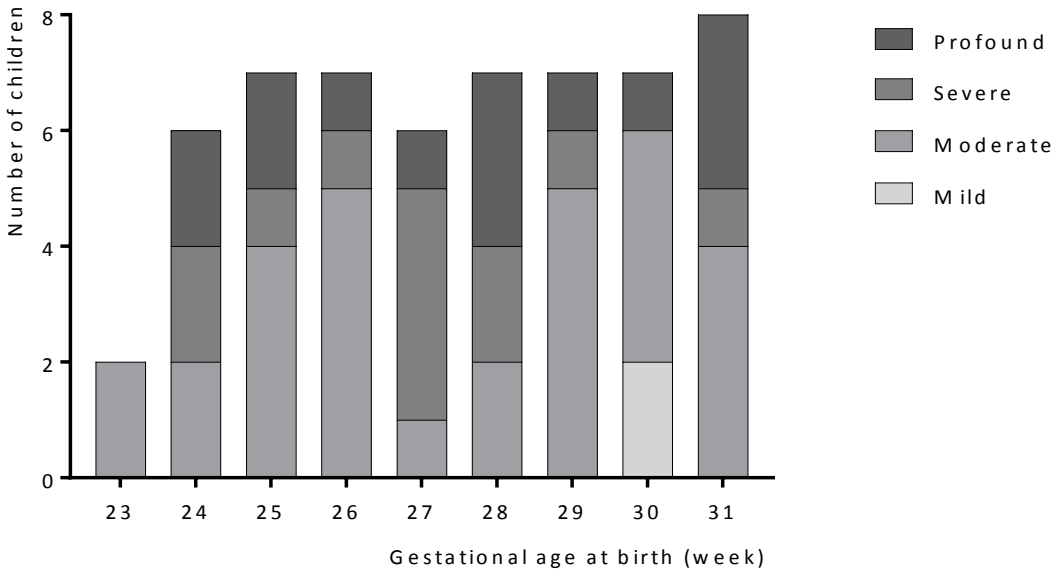
	Data available (n)	n	(%)
SNHL	57	57	(100)
Unilateral	57	8	(14)
Bilateral	57	49	(86)
ANSD			
Confirmed	54	15	(27.8)
Suspected	54	6	(11.1)
Not recorded	54	7	(13)
Severity of hearing loss			
Mild (21-40dB)	57	2	(3.5)
Moderate (41-70dB)	57	29	(50.9)
Severe (71-95dB)	57	12	(21.1)
Profound (>95dB)	57	14	(24.6)
Newborn hearing screen			
Passed	55	6	(10.9)
Referred	55	49	(89.1)
Hearing aids	56	47	(83.9)
Cochlear implant/referral	57	15	(26.3)
Intellectual disability	53	29	(54.7)
Additional disability	51	33	(64.7)

Abbreviations; SNHL, Sensorineural hearing loss; ANSD, Auditory neuropathy spectrum disorder

All children were reported by their consultant audiological physician as having SNHL, but for 15 children there was evidence that this might be ANSD. A further 11% were suspected as having ANSD but this was unconfirmed, and a further 13% had reportedly not been tested with ABR; ANSD could not therefore be confirmed. Of the 57 cases, 49 had a bilateral impairment. The majority of children had a moderate to profound loss and had received treatment in the form of hearing aids. The distribution of severity by gestational week is displayed in figure 4-2. At the point of data collection, 26% of children had been referred for, or had undergone a cochlear implant. Almost 90% of children were identified at the Newborn Hearing screen prior to neonatal discharge and were referred for follow up audiology assessments. Aside from hearing impairment, 33 children had an additional disability. Investigations into the aetiology of hearing impairment had been undertaken prior to the study as follows: CMV testing (78.7%), GJB2 testing (54.8%), m.1555A>G prior to this study (47.4%), MRI of inner ear (76.6%) and ophthalmic review (71.4%).

As all children were reported to have SNHL, for the purposes of these analyses, in order to differentiate between the SNHL and ANSD, children that did not show signs of ANSD will be referred to as having Cochlear Hearing Loss (CHL).

Figure 4-1: The distribution of the severity of hearing impairment by week of gestation



The severity of hearing impairment was not significantly different across gestational weeks within the study range ($p>0.05$). Of the 57 children, 3% had a mild loss (21-40dB), 51% had a moderate loss (41-70dB), 21% had a severe loss (71-95dB) and 25% had a profound impairment (>95dB).

4.2.2 Neonatal clinical differences between CHL and ANSD

Based on my review of the literature, discussed in chapter 1, differences in clinical condition and diagnosis between infants with CHL or specifically ANSD have been reported. CHL and ANSD have been associated with different patterns of clinical risk. As already identified in section 4.2.1, ANSD was difficult to determine in some children. Therefore, the distribution of clinical factors in those at a high risk of ANSD (confirmed or suspected) were compared to those of children with CHL in a univariate analysis (table 4-2). There were few differences in the distribution of clinical risk factors between the two. Children with cochlear losses were more likely to have a low birthweight for gestation but were less frequently found to be from

a multiple pregnancy or to have any evidence of serious neonatal brain injury, specifically PVL. The frequency of other risk factors did not vary significantly between the two conditions. Given the small numbers of individuals in each group and the use of multiple comparisons, it seems unlikely that there are any important clinical risk differences between the two groups, which for the purposes of further investigation, have subsequently been treated as one.

Table 4-2: Distribution of neonatal risk factors in 57 children with CHL and ANSD born at less than 32 weeks gestational age

Risk factor	CHL (n=36)		ANSD (n=21)			
	n/median	(%/range)	n/median	(%/range)	OR (95% CI)	Significance
Male sex	25	(69.4%)	13	(61.9%)	1.40 (0.45-4.33)	
Gestation (week^{+days})	28^{+1}	(25^{+6}-29^{+6})	27^{+5}	(25^{+6}-30)		
Birthweight (g)	894	(751-1135)	1000	(800-1400)		
Birthweight (sd)	-0.51	(-1.71-0.21)	0.12	(-0.6-0.82)		*
IUGR	12	(36.4%)	2	(9.5%)	0.21 (0.04-1.05)	
Multiple pregnancy	3	(8.3%)	9	(42.9%)	8.25 (1.90-35.7)	**
Apgar (5 mins)	7	(6-9)	8	(7-9)		
CRIB-II	9	(7-11)	8.5	(4.5-11)		
Diagnoses						
IVH	17	(47.2%)	8	(61.9%)	0.97 (0.57-1.64)	
IVH I-II	8	(22.2%)	4	(19.1%)		
IVH with ventricular distension	5	(13.8%)	1	(4.8%)		
Intraparenchymal lesion	3	(8.3%)	3	(14.3%)		
Periventricular leukomalacia	1	(2.8%)	5	(23.8%)	10.9 (1.18-101.4)	*
Pneumothorax	5	(13.9%)	4	(19.1%)	1.45 (0.35-6.17)	
Pulmonary haemorrhage	4	(11.1%)	2	(9.5%)	0.84 (0.14-5.04)	
PDA	25	(69.4%)	14	(66.7%)	0.98 (0.57-1.67)	
No treatment	12	(33.3%)	7	(33.3%)		
Medical treatment	9	(25%)	4	(19.1%)		
Surgical treatment	4	(11.1%)	3	(14.3%)		
NEC	17	(47.2%)	10	(48.6%)	0.92 (0.56-1.52)	
Medical treatment	10	(27.8%)	7	(33.3%)		
Surgical drain	0		0			
Surgical treatment	7	(19.4%)	3	(14.3%)		
BPD	29	(80.6%)	16	(80%)	0.88 (0.56-1.38)	
Mild	2	(5.6%)	3	(15%)		
Moderate	6	(16.7%)	3	(15%)		
Severe	21	(58.3%)	10	(50%)		

Septicaemia	21	(58.3%)	11	(52.4%)	0.79 (0.27-2.32)
Meningitis	2	(5.6%)	0		N/A
Treatment					
Ventilation (/d)	12	(2-33)	17	(5-52)	
CPAP (/d)	36	(15-61)	37.5	(10-68.5)	
Oxygen (/d)	22	(5-63)	22	(8.5-38)	
Long line (/d)	17	(11-44)	20	(12-40)	
ITU (/d)	22.5	(7-43)	29	(12.5-60)	
HDU (/d)	37.5	(20-55)	31	(18-52)	
SCBU (/d)	35	(23-44)	26.5	(18.5-41.5)	
Aminoglycoside (any) $^{\Phi}$	36	(100%)	21	(100%)	N/A
Amikacin	17	(47.2%)	7	(33.3%)	0.56 (0.18-1.71)
Gentamicin	30	(83.3%)	19	(90.5%)	1.90 (0.35-10.40)
Vancomycin	23	(63.9%)	18	(85.7%)	3.39 (0.84-13.73)
Steroid$^{\Phi ®}$	15	(41.7%)	7	(33.3%)	0.70 (0.23-2.15)
Furosemide$^{\Phi}$	29	(80.6%)	14	(66.7%)	0.48 (0.14-1.65)
Inotropes$^{\Phi ¤}$	22	(61.1%)	11	(52.4%)	0.70 (0.24-2.08)
Indometacin$^{\Phi}$	3	(8.3%)	2	(9.5%)	1.16 (0.18-7.56)
Ibuprofen$^{\Phi}$	8	(22.2%)	3	(14.3%)	0.58 (0.14-2.49)
Maximum creatinine	111	(86-128)	104.5	(93.5-125)	
Maximum serum bilirubin	203	(172-247)	185	(157-215)	
pH$^{\Psi}$	29	(80.6%)	14	(66.7%)	0.48 (0.14-1.65)
Creatinine$^{\Psi\Psi}$	22	(61.1%)	17	(81.0%)	2.70 (0.75-9.72)
Total bilirubin$^{\Psi\Psi\Psi}$	18	(50.0%)	10	(47.6%)	0.91 (0.31-2.67)

Categorical variables; Mann Whitney U test, continuous variables; Odds ratio (95% confidence interval)

$^{\Phi}$medications given at any point during treatment

$^{¤}$ includes use of dopamine, dobutamine, adrenaline and nor-adrenaline infusions

$^{®}$ includes use of methylprednisolone, hydrocortisone and dexamethasone

$^{\Psi}$pH <7.2, $^{\Psi\Psi}$creatinine >90mmol/l, $^{\Psi\Psi\Psi}$total serum bilirubin > 200micromol/l – at any point during treatment

* P<0.05; **p<0.01

4.2.3 Distribution of risk factors

Neonatal data was obtained for 237 children (57 cases with impaired hearing and 180 controls with normal hearing). As the majority of variables were not normally distributed; non parametric testing was used (table 4-3). Logistic regression was used to identify univariate risk factors. Risk factors for hearing loss include baseline data (sex, gestational age, multiple birth, birthweight, birthweight for gestation, SGA, Apgar and CRIB-II scores), diagnoses occurring during neonatal care (septicaemia, meningitis, IVH, PDA, NEC, pneumothorax, pulmonary haemorrhage, BPD), treatment variables (ventilation, CPAP, oxygen, long line, ITU, HDU and SCBU) and drug treatments received at any point during the neonatal stay (antibiotics, steroid, diuretics, inotrope, indometacin, ibuprofen). Markers of haemodynamic impairment were included as indicators of circulatory risk (elevated creatinine, acidosis and bilirubin levels). Raised bilirubin was included as it may compete for binding sites on albumin, as does low pH, thereby increasing the availability of other risk factors.

Table 4-3: Distribution and univariate analysis of neonatal risk factors in hearing impaired and normal hearing children born at less than 32 weeks of gestation

	Hearing loss		Normal hearing			
	n/median	(%/IQR)	n/median	(%/IQR)	OR (95% CI)	Significance
Male sex	38	(67.7%)	130	(72.2%)	1.30 (0.69-2.47)	
Gestation (week^{+days})	28	(25^{+6}-30)	28^{+2}	(26^{+2}-30)		
Multiple pregnancy	12	(21.1%)	54	(30%)	0.62 (0.31-1.27)	
Birthweight (g)	900	(795-1150)	1090	(855-1333)		*
Birthweight (sd)	-0.26	(-1.18-0.49)	-0.1	(-0.63-0.43)		
SGA	14	(24.6%)	23	(12.8%)	2.22 (1.05-4.68)	*
Apgar (5 mins)	8	(7-9)	9	(7-9)		*
CRIB-II	9	(7-11)	8	(5-10)		
Diagnoses						
Septicaemia	32	(56.1%)	61	(34.5%)	2.43 (1.32-4.47)	**
Meningitis	2	(3.5%)	4	(2.3%)	1.56 (0.28-8.77)	
IVH	30	(42.1%)	51	(28.3%)	1.45 (1.05-2.00)	*
IVH I-II	12	(21.1%)	34	(19.3%)		
IVH with ventricular distension	6	(10.5%)	9	(5.1%)		
Intraparenchymal lesion	6	(10.5%)	8	(4.5%)		
Periventricular leukomalacia	6	(10.5%)	5	(2.8%)	4.02 (1.18-13.73)	*
PDA	39	(68.4%)	60	(34.1%)	1.55 (1.18-2.05)	**
No treatment	19	(33.3%)	23	(13.1%)		
Medical treatment	13	(22.8%)	20	(11.4%)		
Surgical treatment	7	(12.3%)	17	(9.7%)		
NEC	27	(47.4%)	52	(29.7%)	1.51 (1.12-2.04)	**
Medical treatment	17	(29.8%)	39	(22.3%)		
Surgical drain	0		1	(0.6%)		
Laparotomy	10	(17.6%)	12	(6.9%)		
Pneumothorax	9	(15.8%)	8	(4.6%)	3.94 (1.44-10.76)	**
Pulmonary haemorrhage	6	(10.5%)	7	(4.0%)	2.84 (0.91-8.83)	
BPD	45	(80.4%)	126	(71.2%)	1.66 (1.26-2.19)	**
Mild	5	(8.9%)	48	(27.9%)		

Moderate	9	(16.1%)	35	(19.8%)		
Severe	31	(55.4%)	43	(24.3%)		
Treatment						
Ventilation (/d)	12	(4-41)	2	(1-11)		**
CPAP (/d)	36	(11-63)	16	(2-32)		**
Oxygen (/d)	22	(6-49)	16	(2-32)		**
Long line (/d)	18	(12-40)	11	(2-23)		**
ITU (/d)	27.5	(8-52)	11.5	(4-33)		**
HDU (/d)	36	(18-55)	22	(9-39)		**
SCBU (/d)	31	(20-44)	30	(23-39)		
Aminoglycoside (any) $^{\Phi}$	57	(100%)	164	(91.1%)	N/A	
Amikacin	24	(42.1%)	67	(37.2%)	1.23 (0.67-2.25)	
Gentamicin	49	(86%)	127	(70.6%)	2.56 (1.13-5.76)	*
Vancomycin	41	(71.9%)	76	(42.2%)	3.51 (1.83-6.71)	**
No. of aminoglycosides$^{\Phi}$					N/A	
1	13	(22.8%)	80	(44.4%)		
2	31	(54.4%)	62	(34.4%)		
3	13	(22.8%)	22	(12.2%)		
Steroid$^{\Phi\circledR}$	22	(38.6%)	16	(8.9%)	6.44 (3.07-13.5)	**
Furosemide$^{\Phi}$	43	(75.4%)	83	(47.1%)	3.59 (1.84-7.02)	**
Inotropes$^{\Phi\text{¤}}$	33	(57.9%)	45	(25%)	4.13 (2.21-7.70)	**
Indometacin$^{\Phi}$	5	(8.8%)	7	(3.9%)	2.37 (0.72-7.80)	
Ibuprofen$^{\Phi}$	11	(19.3%)	30	(16.7%)	1.20 (0.56-2.57)	
Maximum serum bilirubin	203	(171.5-246.5)	185	(158-214)		**
Maximum creatinine	105	(87.5-125)	90	(77-102)		**
pH <7.2$^{\Psi}$	43	(75.4%)	101	(56.1%)	2.40 (1.23-4.70)	**
Lactate >2.0	57	(100%)	172	(95.6%)	N/A	
Creatinine >90$^{\Psi\Psi}$	39	(68.4%)	91	(50.6%)	2.12 (1.13-3.98)	*
Bilirubin >200$^{\Psi\Psi\Psi}$	28	(49.1%)	70	(38.9%)	1.52 (0.83-2.76)	

Categorical variables; Mann Whitney U test, continuous variables; Odds ratio (95% confidence interval)

$^{\Phi}$medications given at any point during treatment

¤ includes use of dopamine, dobutamine, adrenaline and nor-adrenaline infusions

® includes use of methylprednisolone, hydrocortisone and dexamethasone

$^{\Psi}$pH <7.2, $^{\Psi\Psi}$creatinine >90mmol/l, $^{\Psi\Psi\Psi}$total serum bilirubin > 200micromol/l – at any point during treatment

*p<0.05; **p<0.01

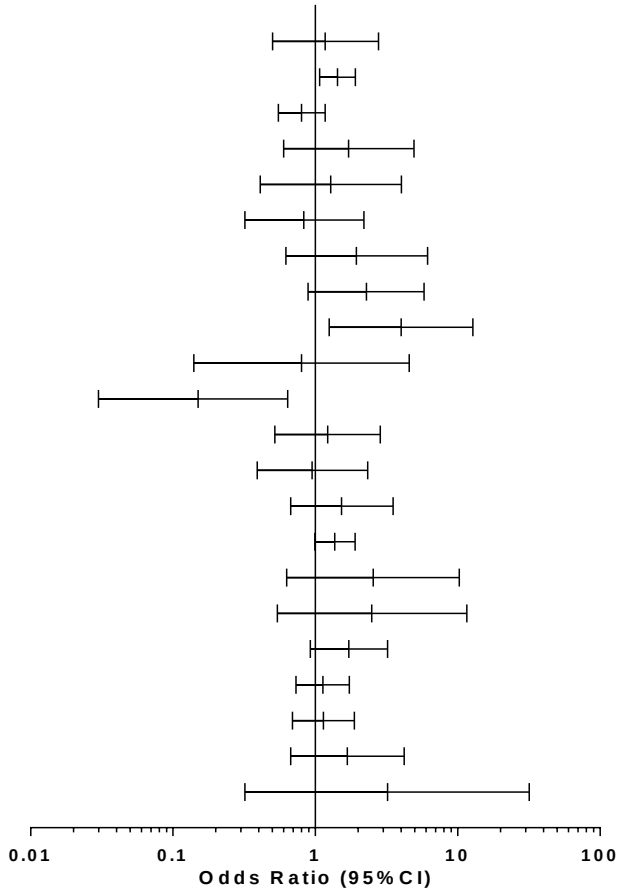
From baseline data, sex and gestational age were similarly distributed between groups. Children who developed hearing loss were more likely to have a lower birthweight, a smaller weight for gestation and have a lower Apgar score at birth. Children with hearing impairment were more likely to have been diagnosed with sepsis, IVH of all grades, PVL, PDA, NEC, pneumothorax and BPD. Only meningitis and pulmonary haemorrhage were not significantly different between groups. Index children received increased periods of respiratory support (ventilation, CPAP and days of oxygen therapy), and longer durations of both intensive and high dependency care. Gentamicin, vancomycin, steroid, diuretics, and inotrope were all received more frequently in the case group. Haemodynamic impairment was also more frequent in the hearing loss group. Renal function as indicated by levels of creatinine were higher, peak bilirubin was also elevated and acidosis was more likely.

4.2.4 Diagnoses and treatment risk factors for hearing loss

The combination of neonatal risk factors for hearing loss were analysed using multivariate logistic regression (figure 4-2). Factors include baseline risks (sex, gestational age, birthweight for gestation), medications received at any point during the neonatal stay inotrope, diuretics, antibiotics, steroid, indometacin, and ibuprofen), presence of physiological risk factors (elevated creatinine, acidosis and bilirubin levels), and diagnoses (IVH, pneumothorax, pulmonary haemorrhage, PDA, NEC, BPD, septicaemia, and meningitis).

Figure 4-2: Multivariate logistic regression analysis: odds ratio (95% CI) for independent risk factors for hearing impairment in children born at less than 32 weeks of gestation

	Hearing loss (n=57)		Normal hearing (n=180)				
Risk factor	n/median (%/IQR)		n/median (%/IQR)		OR	95% CI	
Sex (male)	38	(67.7)	130	(72.2)	1.17	0.50-2.78	
Gestation (/w)	28	(25^{+6}-30)	28^{+2}	(26^{+2}-30)	1.43	1.07-1.92	*
BW (/sd)	-0.26	(-1.18-0.49)	-0.1	(-0.63-0.43)	1.98	0.55-1.17	
Inotropes$^{\Phi}$	33	(57.9)	45	(25)	1.72	0.60-4.93	
Furosemide	43	(75.4)	83	(47.1)	1.28	0.41-4.02	
Amikacin	24	(42.1)	67	(37.2)	0.84	0.32-2.19	
Gentamicin	49	(86)	127	(70.6)	1.95	0.62-6.15	
Vancomicin	41	(71.9)	76	(42.2)	2.28	0.89-5.81	
Steroid®	22	(38.6)	16	(8.9)	4.00	1.25-12.79	*
Indometacin	5	(8.8)	7	(3.9)	0.80	0.14-4.57	
Ibuprofen	11	(19.3)	30	(16.7)	0.15	0.03-0.64	*
Creatinine$^{\Psi}$	39	(68.4)	91	(50.6)	1.22	0.52-2.86	
pH$^{\Psi\Psi}$	43	(75.4)	101	(56.1)	0.95	0.39-2.33	
Bilirubin$^{\Psi\Psi\Psi}$	28	(49.1)	70	(38.9)	1.53	0.67-3.51	
IVH/PVL	30	(53.0)	56	(32.0)	1.37	0.99-1.90	
Pneumothorax	9	(15.8)	8	(4.6)	2.55	0.63-10.27	
Pulm. Haem	6	(10.5)	7	(4.0)	2.49	0.54-11.59	
PDA	39	(68.4)	60	(34.1)	1.72	0.92-3.22	
NEC	27	(47.4)	52	(29.7)	1.13	0.73-1.73	
BPD	45	(80.4)	126	(71.2)	1.14	0.69-1.88	
Septicaemia	32	(56.1)	61	(34.5)	1.68	0.67-4.21	
Meningitis	2	(3.5)	4	(2.3)	3.21	0.32-31.75	



$^{\Phi}$ includes use of dopamine, dobutamine, adrenaline and norepinephrine infusions

® includes use of methylprednisolone, hydrocortisone and dexamethasone

Ψ creatinine >90mmol/l, ΨΨpH <7.2, ΨΨΨtotal bilirubin >200micromol/l – at any point during treatment

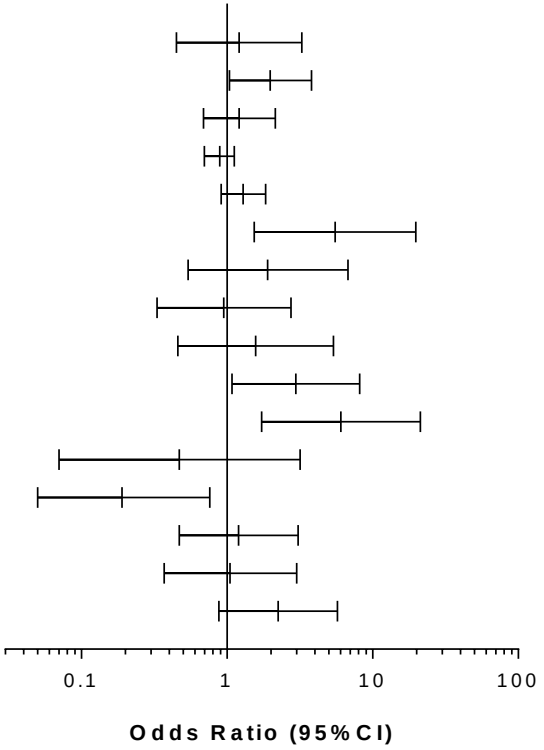
* P<0.05; **p<0.01

Gestational age was significantly associated with hearing loss, increasing risk at lower gestational ages. Of the postnatal clinical factors only two associations remained: the use of steroids was associated with an increased risk of hearing loss, whilst ibuprofen remained protective of hearing impairment. None of the diagnostic groups were independently associated with hearing loss but the relationship was not consistent among the contributing items. For example, within the brain injury subset, PVL was independently associated with hearing loss (p=0.05), whereas IVH was not. The relationship with hearing loss was more complex within the items making up the PDA subset, and will be investigated further below. Variables that were not significantly distributed in the univariate analysis were removed and the multivariate analysis repeated with no real change in the overall picture.

Due to potential interactions between conditions and treatments, treatment variables were analysed following the exclusion of diagnoses during neonatal care (figure 4-3). Factors in this analysis were: baseline risks (sex, gestational age, birthweight for gestation, CRIB-II and Apgar scores), medications received at any point during the neonatal stay (inotrope (including dopamine, dobutamine, adrenaline and norepinephrine), diuretics, antibiotics, steroid (including hydrocortisone, methylprednisolone and dexamethasone), indometacin, ibuprofen), and the presence of physiological risk factors (elevated creatinine, acidosis and bilirubin levels).

Figure 4-3: Multivariate logistic regression analysis: odds ratio (95% confidence interval) for independent risk factors for hearing impairment in children born at less than 32 weeks of gestation

Risk factor	Hearing loss (n=57) n/median	(%/IQR)	Normal hearing (n=180) n/median	(%/IQR)	OR	95% CI	
Sex (male)	38	(67.7)	130	(72.2)	1.21	0.45-3.26	
Gestation (/w)	28	(25^{+6}-30)	28^{+2}	(26^{+2}-30)	1.98	1.04-3.80	*
BW (/sd)	-0.26	(-1.18-0.49)	-0.1	(-0.63-0.43)	1.21	0.69-2.15	
Apgar (5 min)	8	(7-9)	9	(7-9)	0.89	0.70-1.12	
CRIB-II	9	(7-11)	8	(5-10)	1.29	0.91-1.84	
Inotropes$^{\Phi}$	33	(57.9)	45	(25)	5.23	1.54-19.83	**
Furosemide	43	(75.4)	83	(47.1)	1.90	0.54-6.76	
Amikacin	24	(42.1)	67	(37.2)	0.95	0.33-2.75	
Gentamicin	49	(86)	127	(70.6)	1.57	0.46-5.39	
Vancomycin	41	(71.9)	76	(42.2)	2.96	1.08-8.13	*
Steroid®	22	(38.6)	16	(8.9)	6.06	1.73-21.22	**
Indometacin	5	(8.8)	7	(3.9)	0.47	0.07-3.17	
Ibuprofen	11	(19.3)	30	(16.7)	0.19	0.05-0.76	*
Creatinine$^{\Psi}$	39	(68.4)	91	(50.6)	1.20	0.47-3.01	
pH$^{\Psi\Psi}$	43	(75.4)	101	(56.1)	1.05	0.37-3.01	
Bilirubin$^{\Psi\Psi\Psi}$	28	(49.1)	70	(38.9)	2.24	0.88-5.72	



Abbreviations; BW, birthweight for gestation

$^{\Phi}$ includes use of dopamine, dobutamine, adrenaline and norepinephrine infusions

$^{\Psi}$ creatinine >90mmol/l, $^{\Psi\Psi}$pH <7.2, $^{\Psi\Psi\Psi}$total bilirubin >200micromol/l – at any point during treatment

® includes use of methylprednisolone, hydrocortisone and dexamethasone

* P<0.05; **p<0.01;

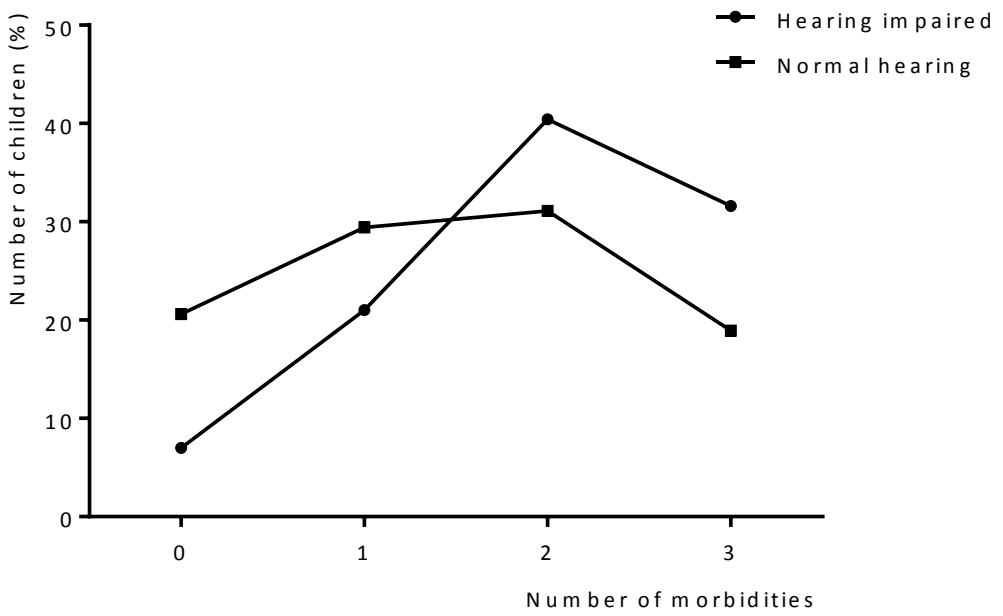
In contrast to the global regression analysis, new independent associations were found. Of the baseline risk factors, only week of gestation was still associated with hearing loss. None of the physiological risk factors independently increased the risk of hearing loss. However, the use of three medications (inotrope, vancomycin and steroid) were associated with hearing loss and the use of ibuprofen appeared to reduce the risk. Specifically, previously identified risk factors such as furosemide, gentamicin and indometacin within this dataset appeared to have little independent effect. The strongest associations were with the use of inotrope and steroid medications. The association with inotropic medication may relate to the drugs used or to the presence of low blood pressure for which they are prescribed. The use of steroid is more complex, as it can be prescribed to treat hypotension resistant to inotropic medication, alongside its use to support extubation in children with airway problems or with severe BPD. The protective effect of ibuprofen was unanticipated, and interestingly in this population indometacin use also decreased the risk of hearing loss, although this did not reach significance.

Several of the courses of treatment in table 4-3 are associated with specific diagnoses, therefore exploratory analyses were conducted grouping these related risk factors. Further targeted analyses to investigate associations with hearing loss in these situations were undertaken and are presented below. Firstly, diagnoses will be considered independently.

4.2.4.1 Morbidities as factors for hearing loss

Previous research has used the measurement of comorbidities as a predictor of neurodevelopmental impairment, but none were specific to hearing loss in isolation. Table 4-3 shows diagnoses including brain injury, NEC/sepsis and BPD are more frequent in children with hearing loss in comparison to children with normal hearing, the association between hearing loss and the presence of these morbidities was therefore analysed (figure 4-4).

Figure 4-4: Comorbid diagnoses as risk factors for hearing loss in children born at less than 32 weeks of gestation



Children with impaired hearing were less likely to have had no diagnoses (7%) compared to children with normal hearing (21%). The diagnosis of any morbidity increased the risk of hearing loss (OR 1.64, 95% CI 1.20-2.26), but the likelihood of hearing loss was more strongly associated with two (OR 3.80, 95% CI 1.21-11.88) or three morbidities (OR 4.90, 95% CI 1.51-15.92). In a comparison of the 3 diagnoses in the presence of the others, only acquired brain injury was significant (OR 1.46, 95% CI 1.14-1.89).

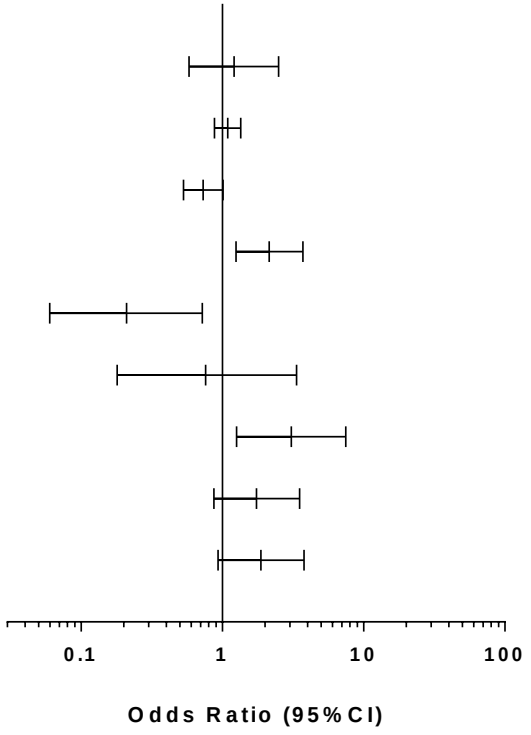
Each of these diagnoses are associated with very different courses of treatment, therefore analyses were conducted grouping these related risk factors to investigate associations with hearing loss. This included risk factors relating to acquired brain injury (IVH/PVL), BPD, NEC/sepsis, and PDA.

4.2.4.2 PDA and associated treatments as risk factors for hearing loss

A PDA was more frequent in the case group and reached statistical significance in independent analyses. Treatment methods for PDA (ibuprofen, indometacin and furosemide) were analysed separately along with baseline risks (sex, gestation and birthweight for gestation), and physiological factors (raised creatinine and bilirubin) as potential effect modifiers to ascertain whether the likelihood of hearing loss is related to the PDA or the treatment received (figure 4-5).

Figure 4-5: Multivariate logistic regression analysis: odds ratio (95% confidence interval) for independent risk factors for hearing impairment associated with PDA in children born at less than 32 weeks of gestation

Risk factor	Hearing loss (n=57) n/median (%/IQR)		Normal hearing (n=180) n/median (%/IQR)		OR	95% CI	
Sex (male)	38	(67.7)	130	(72.2)	1.21	0.58-2.51	
Gestation (/w)	28	(25^{+6}-30)	28^{+2}	(26^{+2}-30)	1.09	0.88-1.35	
BW (/sd)	-0.26	(-1.18-0.49)	-0.1	(-0.63-0.43)	0.73	0.53-1.01	*
PDA	39	(68.4)	60	(34.1)	2.15	1.25-3.71	**
Ibuprofen	11	(19.3)	30	(16.7)	0.21	0.06-0.72	**
Indometacin	5	(8.8)	7	(3.9)	0.77	0.18-3.35	
Furosemide	43	(75.4)	83	(47.1)	3.07	1.26-7.46	*
Creatinine$^{\Psi}$	39	(68.4)	91	(50.6)	1.74	0.86-3.52	
Bilirubin$^{\Psi\Psi}$	28	(49.1)	70	(38.9)	1.87	0.93-3.79	



Abbreviations; BW, birthweight for gestation; PDA; patent ductus arteriosus

$^{\Psi}$ creatinine >90mmol/l, $^{\Psi\Psi}$total bilirubin >200micromol/l – at any point during treatment

* P<0.05; **p<0.01

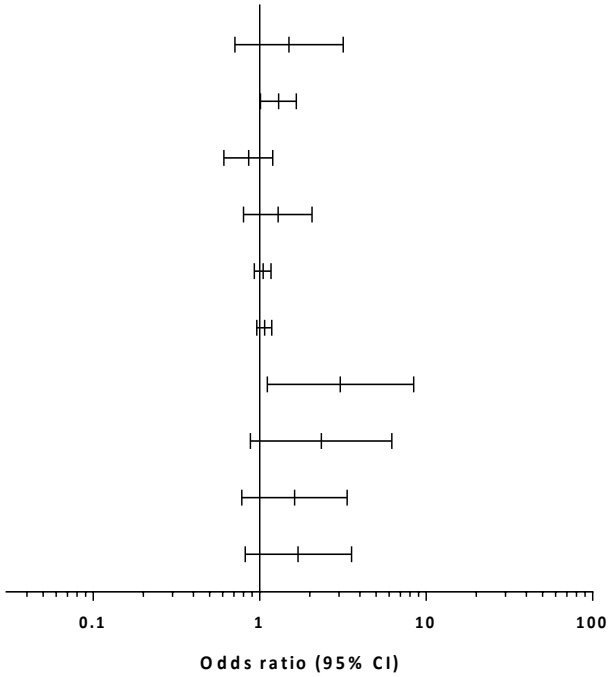
The diagnosis of PDA independently confers a greater risk for hearing loss than for infants without a diagnosis of PDA. Low birthweight for gestational age and receiving furosemide were also independently associated with hearing loss. Furosemide may also be prescribed during blood transfusions; 72% of infants (86% of cases and 68% of controls) received at least one transfusion. It is not possible to determine whether hearing loss associated with furosemide occurs with PDA or with blood transfusion. However, infants with a diagnosis of PDA (including untreated, medically and surgically treated PDA) were analysed separately, whereby only furosemide was independently associated with hearing loss (p=0.04). Treatment of PDA with ibuprofen remained protective of hearing loss.

4.2.4.3 BPD and associated treatments as risk factors for hearing loss

Bronchopulmonary dysplasia (BPD) defined as the receipt of supplemental oxygen at 36 weeks postmenstrual age, is a somewhat artificial concept as the lung disease suffered by very preterm infants is a continuum. However, BPD has been used as a diagnosis for some considerable time and co-occurs with many other risk factors. These were also analysed in a separate regression model (figure 4-6). Factors included baseline variables (sex, gestational week and birthweight for gestation), treatment risk factors (days of ventilation and oxygen, receiving steroid (including hydrocortisone, methylprednisolone and dexamethasone, from week 3 onwards) or furosemide), and physiological risk factors (elevated creatinine and bilirubin). Steroid is administered to aid the weaning of respiratory support but also as an alternative to inotropes in treating hypotension in the first few days after birth. For this reason, only steroid given from week 3 onwards were included in this model.

Figure 4-6: Multivariate logistic regression analysis: odds ratio (95% confidence interval) for independent risk factors for hearing impairment associated with BPD in children born at less than 32 weeks of gestation

Risk factor	Hearing loss (n=57) n/median (%/IQR)	Normal hearing (n=180) n/median (%/IQR)	OR	95% CI	
Sex (male)	38 (67.7)	130 (72.2)	1.50	0.71-3.18	
Gestation (/w)	28 (25^{+6}-30)	28^{+2} (26^{+2}-30)	1.30	1.01-1.66	*
BW (/sd)	-0.26 (-1.18-0.49)	-0.1 (-0.63-0.43)	0.86	0.61-1.21	
BPD	45 (80.4)	126 (71.2)	1.29	0.80-2.06	
Ventilation (/w)	1.71 (0.57-5.86)	0.29 (0.14-1.57)	1.05	0.93-1.17	
Oxygen (/w)	3.14 (0.86-7.00)	2.29 (0.29-4.57)	1.07	0.96-1.18	
Steroid®	18 (31.6)	13 (7.2)	3.05	1.11-8.41	*
Furosemide	43 (75.4)	83 (47.1)	2.35	0.88-6.23	
Creatinine >90$^{\Psi}$	39 (68.4)	91 (50.6)	1.62	0.78-3.35	
Bilirubin >200$^{\Psi\Psi}$	28 (49.1)	70 (38.9)	1.70	0.82-3.56	



Abbreviations; BW, birthweight for gestation; BPD; bronchopulmonary dysplasia

® includes use of methylprednisolone, hydrocortisone and dexamethasone

$^{\Psi}$ creatinine >90mmol/l, $^{\Psi\Psi}$total bilirubin >200micromol/l – at any point during treatment

* P<0.05; **p<0.01

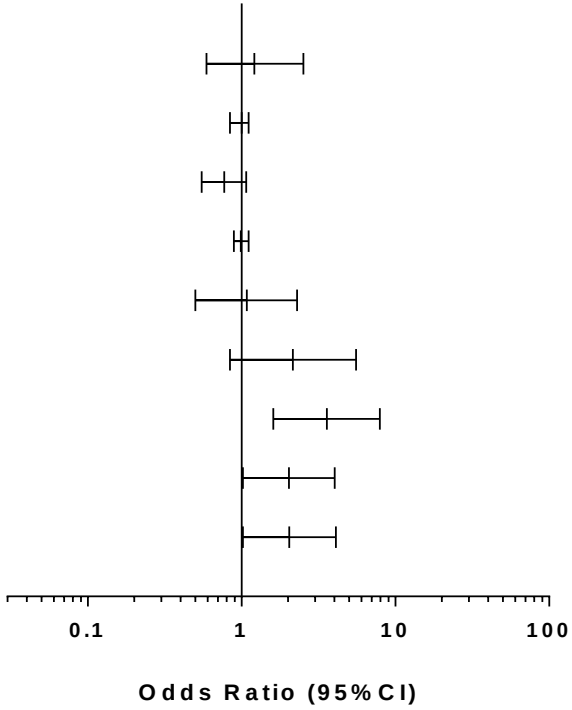
Although BPD was associated with hearing loss in univariate analysis, it was not an independent predictor in the presence of other related risk factors. The administration of steroid remained independently associated with an increased risk of hearing impairment. All three types of steroid were more frequently given to infants with hearing loss, with dexamethasone and hydrocortisone being used more frequently than methylprednisolone. Hydrocortisone was the most commonly prescribed in 28% of cases and 6% of controls; 21% of cases received a course of dexamethasone compared to 4% of controls, only 1 child with hearing loss received methylprednisolone. Of note in this model, in contrast to the PDA model, furosemide was not associated with hearing loss. Apgar scores, the total number of days of CPAP and a low pH level were included in an additional analysis and were not significant.

4.2.4.4 Sepsis and/or NEC and associated treatments as risk factors for hearing loss

Neonatal sepsis was associated with hearing impairment in univariate analyses. The types and frequency of sepsis between the two groups are listed in table (Appendix 10). NEC was also associated with hearing loss in univariate analyses but has an overlapping risk with sepsis. These were therefore combined as an either/or variable and analysed along with other known risk factors for hearing loss including baseline variables (sex, gestational week and birthweight), treatment factors (amikacin, gentamicin, vancomycin), and physiological risk factors (elevated creatinine and bilirubin) associated with neonatal sepsis (figure 4-7).

Figure 4-7: Multivariate logistic regression analysis: odds ratio (95% confidence interval) for independent risk factors for hearing impairment associated with NEC and/or sepsis in children born at less than 32 weeks of gestation

Risk factor	Hearing loss (n=57) n/median (%/IQR)		Normal hearing (n=180) n/median (%/IQR)		OR	95% CI	
Sex (male)	38	(67.7)	130	(72.2)	1.21	0.59-2.52	
Gestation (/w)	28	(25^{+6}-30)	28^{+2}	(26^{+2}-30)	1.00	0.84-1.19	
BW (/sd)	-0.26	(-1.18-0.49)	-0.1	(-0.63-0.43)	0.77	0.55-1.07	
NEC/Sepsis	37	(64.9)	85	(47.2)	0.99	0.89-1.11	
Amikacin	24	(42.1)	67	(37.2)	1.08	0.50-2.30	
Gentamicin	49	(86)	127	(70.6)	2.16	0.84-5.54	
Vancomycin	41	(71.9)	76	(42.2)	3.58	1.62-7.94	**
Creatinine$^{\Psi}$	39	(68.4)	91	(50.6)	2.03	1.02-4.03	*
Bilirubin$^{\Psi\Psi}$	28	(49.1)	70	(38.9)	2.04	1.02-4.10	*



Abbreviations; BW, birthweight for gestation; NEC, necrotising enterocolitis

$^{\Psi}$ creatinine >90mmol/l, $^{\Psi\Psi}$total bilirubin >200micromol/l – at any point during treatment

* P<0.05; **p<0.01

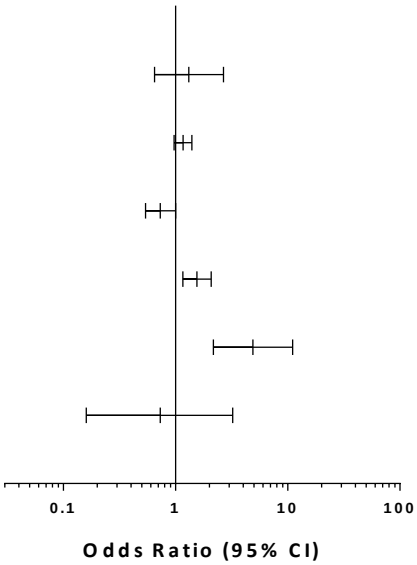
In this group of babies, none of the baseline risks were associated with hearing loss and the frequency of septicaemia and/or NEC was not significantly different between the hearing loss and normal hearing groups when controlling for other variables. The use of vancomycin was significantly associated with hearing loss. However, vancomycin is used in the treatment of infection, suspected sepsis and prophylactically by way of continuous infusion to prevent long line sepsis. An elevated creatinine level and high bilirubin were also independently associated with the outcome of hearing loss, both of which had been associated with hearing loss in previous research, and neither of which had been predictive in the initial regression model including baseline variables, diagnoses and treatments (figure 4-2). Meningitis has previously been implicated as causative of hearing loss and so was added to the above model. Meningitis was not associated with hearing loss in the univariate analysis and did not change the overall model ($p>0.05$), with vancomycin, raised creatinine and bilirubin remaining associated with the outcome. In summary, the use of vancomycin and two physiological markers increased the risk of hearing loss amongst babies with sepsis; which will be explored further.

4.2.4.5 IVH/PVL and associated treatments as risk factors for hearing loss

IVH and PVL has been associated with hearing loss as described above, and was significantly more common (inclusive of all grades of IVH) in infants with hearing loss than normal hearing controls. Risk was therefore explored within this group of babies (figure 4-8), with relevant associated variables, including the early use of steroid (<72 hours from birth, including hydrocortisone, methylprednisolone and dexamethasone) and inotrope (including dopamine, dobutamine, adrenaline and norepinephrine).

Figure 4-8: Multivariate logistic regression analysis: odds ratio (95% confidence interval) for independent risk factors for hearing impairment associated with IVH/PVL in children born at less than 32 weeks of gestation

Risk factor	Hearing loss (n=57) n/median (%/IQR)		Normal hearing (n=180) n/median (%/IQR)		OR	95% CI	
Sex (male)	38	(67.7)	130	(72.2)	1.32	0.65-2.68	
Gestation (/w)	28	(25^{+6}-30)	28^{+2}	(26^{+2}-30)	1.17	0.97-1.40	
BW (/sd)	-0.26	(-1.18-0.49)	-0.1	(-0.63-0.43)	0.73	0.54-1.00	*
IVH/PVL	30	(53.0)	56	(32.0)	1.55	1.15-2.08	**
Inotrope$^{\Phi}$	33	(57.9)	45	(25)	4.90	2.17-11.06	**
Steroid®	22	(38.6)	16	(8.9)	0.73	0.16-3.24	



Abbreviations; BW, birthweight for gestation; IVH, intraventricular haemorrhage; PVL, periventricular leukomalacia

$^{\Phi}$ includes use of dopamine, dobutamine, adrenaline and norepinephrine infusions

® includes use of methylprednisolone, hydrocortisone and dexamethasone in the first 72 hours of life

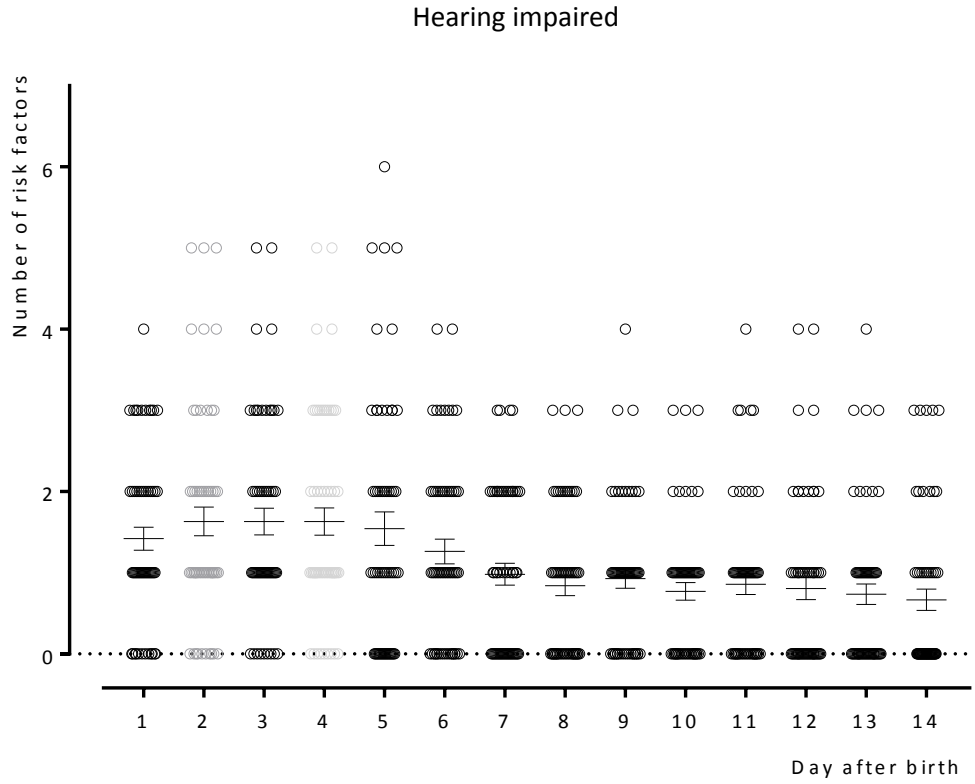
* P<0.05; **p<0.01

Within the subset of babies who had identified brain injury, birthweight was inversely related to the risk of hearing loss. In contrast, the presence of brain injury and use of inotropic medication were independently positively associated with hearing loss. Among the constituent injuries PVL conferred the major risk. The strongest association was with the use of inotropic drugs, but it is unclear whether it is the underlying poor perfusion, the medication, or the elevation in perfusion pressure by the drug that leads to the increase risk of impaired hearing.

4.2.5 Coincidence and timing of neonatal risk factors for hearing loss

The relationship between hearing loss and neonatal illness is complex. Previous research has suggested that an accumulated, or coincident risk may be more important than single identifiable risk factors [4]. Infants may experience an increased sensitivity to ototoxic agents in the initial period after birth when multiple risk factors may coincide, or as the newborn adapts to life outside the womb. To address this, a timeline of clinical risk factors across the neonatal period was compared between children with and without impaired hearing. Coinciding risk factors within a 24 hour period were explored across the first 14 days after birth for children with hearing loss and normal hearing children (figure 4-9). Mean number of risk factors for children with and without hearing loss with confidence intervals were also compared (figure 4-10). In this analysis we identified key independent factors and three physiological risk factors. As previous studies have identified aminoglycosides as part of the co-incident risk, gentamicin was included in the analysis. Thus, risk factors included treatments; gentamicin, vancomycin, steroid, inotrope, furosemide, and physiological risk factors; creatinine >90mmol/l, total serum bilirubin >200microm/l and pH<7.2. Diagnoses were removed from this analysis as they are not independent from treatment variables.

Figure 4-9: Daily number of risks in non-hearing and hearing children born at less than 32 weeks of gestation, over the first 14 days after birth



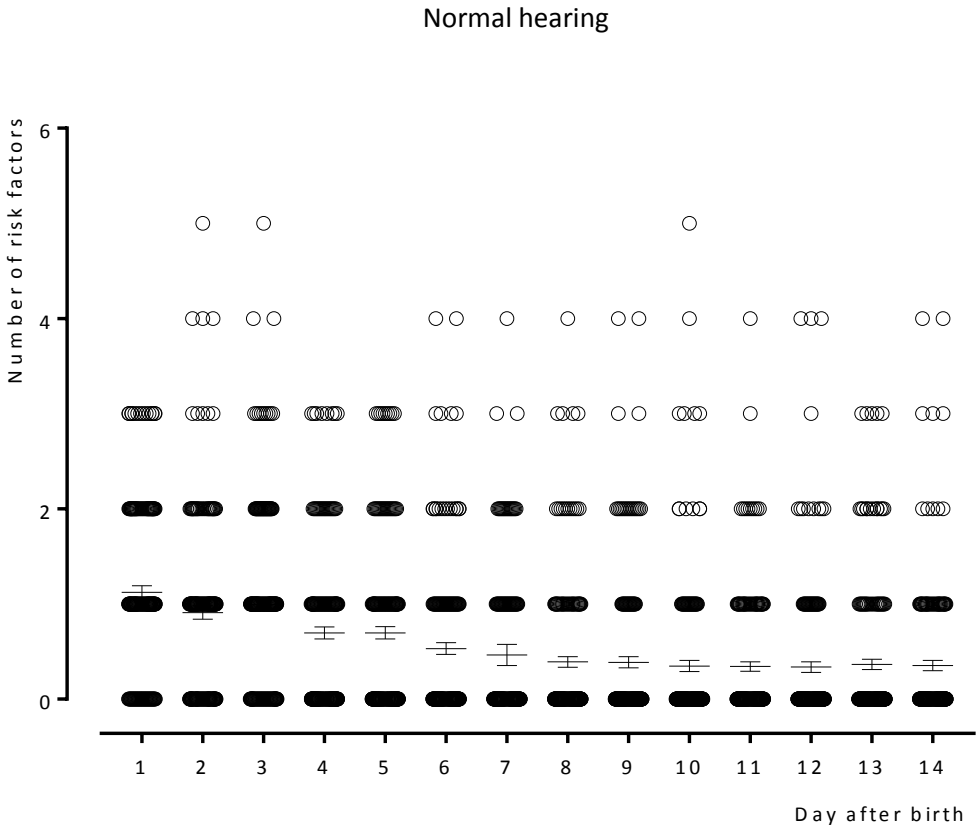
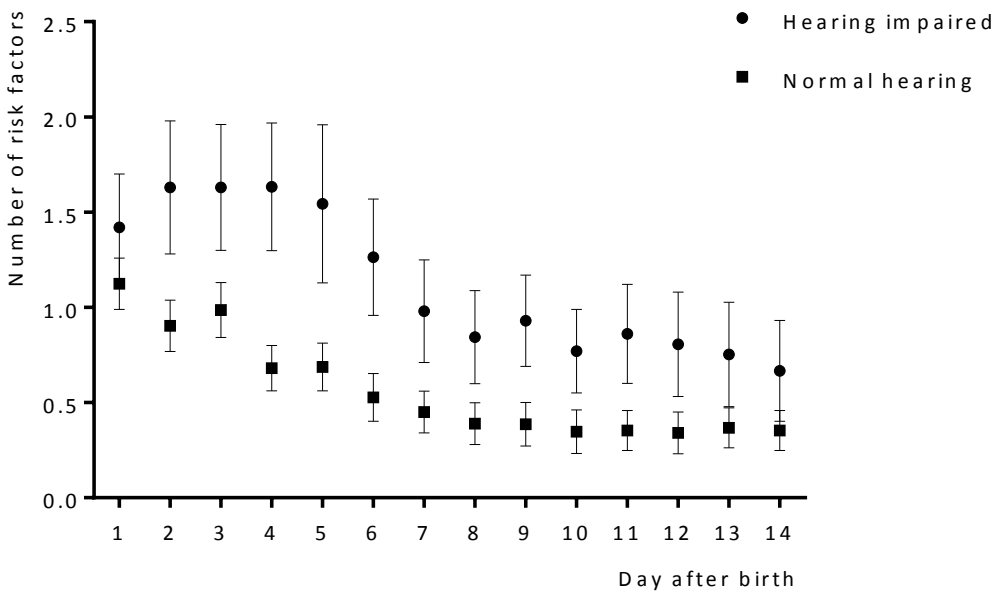


Figure 4-10: Mean number of risk factors (with 95% CI) for children with and without hearing loss born at less than 32 weeks gestation over the first 14 days after birth



Children with hearing loss were exposed to a greater number of risk factors for the first 14 days after birth, reaching significance every day. Cumulative number of risks were higher for children with impaired hearing (mean 15.7, range 1-41) in comparison to children with normal hearing (mean 7.8, range 0-43; OR 1.10, 95% CI 1.06-1.15). Exposure to 9-18 risks was incurred by 42.1% of hearing impaired children compared to 23.3% of normal hearing children, and greater than 19 risks by 28.1% of children with hearing loss compared to 8.3% of control children. Greater exposure to risk factors continued until week 23 where there was a higher incidence of mean risk factors every week until discharge for infants who were later found to have an audiological impairment.

4.2.6 Ototoxic medication as a risk factor for hearing loss

The impact of ototoxic medication including gentamicin, vancomycin and furosemide, on neonatal acquired hearing loss has been long debated in the literature. Gentamicin use is widespread in neonatal care but was given significantly more frequently to children with

hearing loss than control children. In the first 14 days, 73.7% of index cases and 62.2% of control children received at least one dose of gentamicin. Children with hearing loss received a higher number of doses (mean 3.2 days of exposure/child (range 0-12)) compared to normal hearing children (mean 2.1 days of exposure (range 0-10; OR 1.19 (95% CI 1.06-1.33))). Subsequent weeks of exposure to gentamicin was also more frequently received by children with hearing loss (mean 1.2; range 0-7) in comparison to children with normal hearing (mean 0.7; range 0-7). The highest gentamicin levels recorded were similar in cases (median 1.8, IQR 1, 2.9) in comparison to control children (median 1.5, IQR 1, 2.6; $p>0.05$).

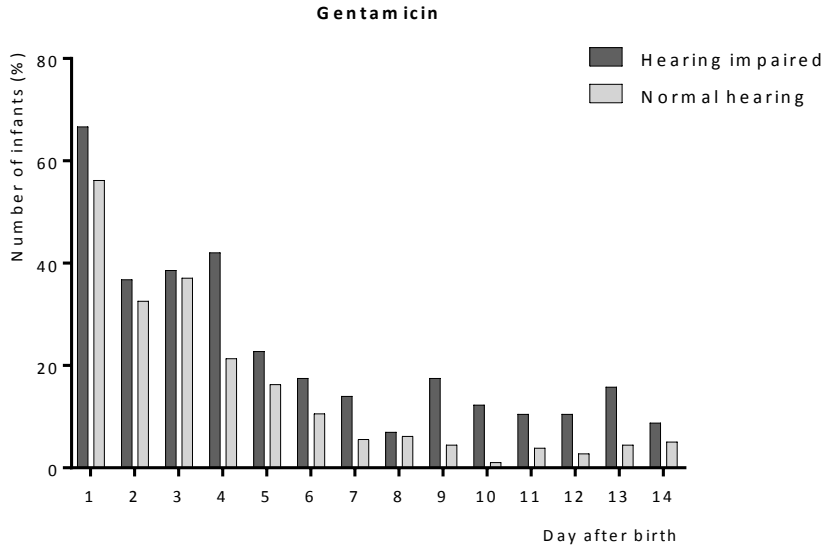
Vancomycin exposure was significantly more frequent and for longer durations in children with hearing loss. A course of vancomycin (>4 days) was given in 24.6% of the hearing loss group in the first 14 days compared to 11.7% of control infants (OR 1.17, 95% CI 1.05-1.30), but courses were of similar duration (6.6 days and 7.0 days, respectively). Subsequently, courses of vancomycin of greater than 14 days were more frequent in the hearing loss group (45.6%) compared to controls (21.7%; $p<0.01$). Additionally, children that later developed hearing loss were more likely to have received a course of vancomycin in the first two weeks and subsequently (19.3%), compared to controls (7.8%; $p=0.01$). Peak concentration levels of vancomycin did not significantly differ between groups ($p>0.05$).

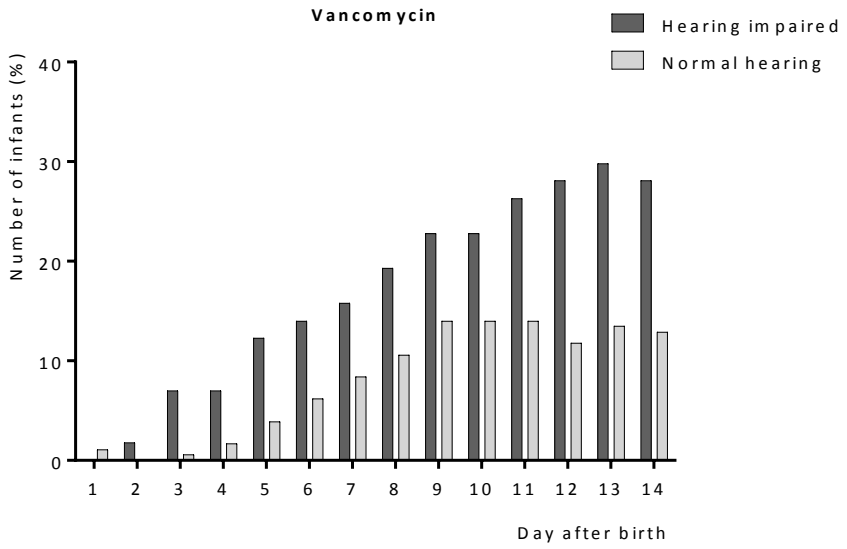
Finally, furosemide use was also significantly more frequent during the first 14 days in children with hearing loss. Of the children with impaired hearing, 38.6% (mean 1.2; range 0-11) received furosemide in comparison to 20% of hearing controls (mean 0.4; range 0-5 (OR 1.41, 95% CI 1.14-1.75)). Similarly, weeks of exposure to furosemide were greater in the hearing impaired children (mean 2.9; range 0-10) in comparison to normal hearing children (mean 1.3; range 0-10 (OR 1.33, 95% CI 1.17-1.50)).

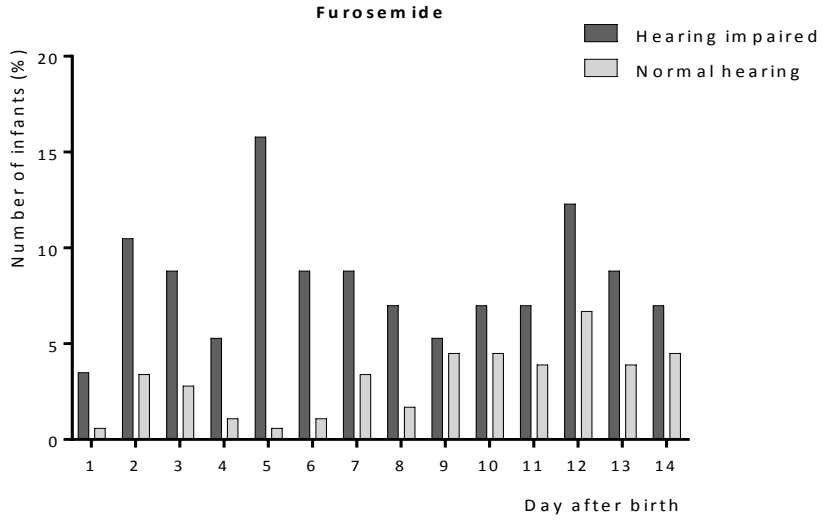
During the first 14 days of treatment, the number of days of exposure to gentamicin (OR 1.23, 95% CI 1.09-1.39), vancomycin (OR 1.15, 95% CI 1.03-1.29) and furosemide (OR 1.39, 95% CI 1.11-1.74) were all independently associated with hearing loss in the presence of the others.

Exposure to ototoxic medication (including gentamicin, vancomycin and furosemide) were compared between index cases and controls, for the first 14 days after birth are reported in figure 4-11.

Figure 4-11: Comparison of profiles of the use of individual ototoxic medications in children with and without hearing loss born at less than 32 weeks of gestation







The profile of ototoxic medication use differed between the two groups, mainly in terms of the use of vancomycin and furosemide (figure 4-12). Only 5.3% of children with hearing loss did not receive any ototoxic medications compared to 27.8% of control children. Over the first 14 days, the mean number of days of treatment using individual ototoxic medications was 6.7 for the hearing impaired (maximum 19), compared to 3.6 for the hearing controls (maximum 15). The median number of doses received by cases was 6 (IQR 3-10), in comparison to control children (3; IQR 0-6). Gentamicin and furosemide were given to a higher percentage of infants with hearing loss every day for all 14 days, and vancomycin from day 2 onwards.

The association between days of ototoxic medication administered in the first 14 days and hearing loss increased with each additional day that either gentamicin, vancomycin or furosemide were given (OR 1.21, 95% CI 1.12-1.31). However, children who received ototoxic medication between 1-7 days out of the first 14 had an increased probability of hearing loss (OR 5.61, 95% CI 1.65-19.12), than children who had not received ototoxic medication. Furthermore, the odds were highest if 8 or more dose days had been given in the first 14 days in comparison to children that had not received any ototoxic medication (OR 12.18, 95% CI 3.30-44.99).

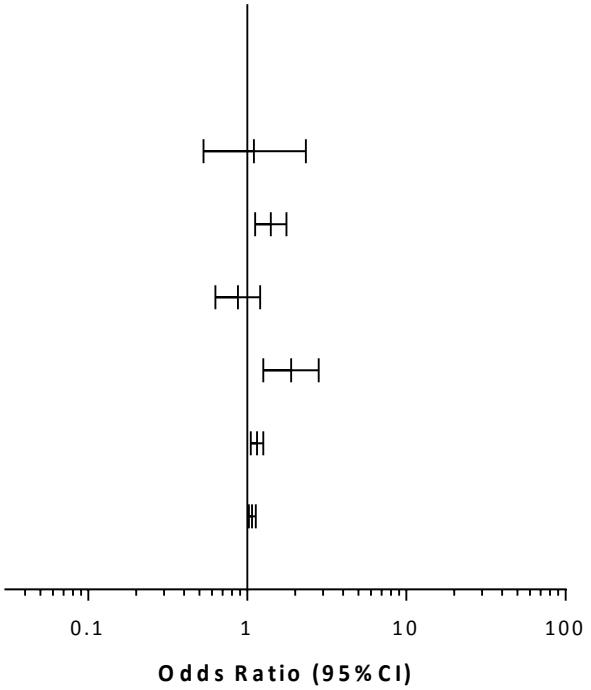
Cumulative episodes of ototoxic medication (including gentamicin, vancomycin and furosemide) from week 3-12 were also higher for children with hearing loss (maximum 20; IQR 2-10) compared to normal hearing children (maximum 16 aside from one child who received 21 episodes; IQR 0-5.5). Ototoxic medication was measured as having received each individual medication at any point that week. Children with hearing loss received more vancomycin and furosemide every week until week 12, and more gentamicin in all but 2 weeks. Hearing loss was significantly more likely with each increasing week of gentamicin, vancomycin and furosemide in this time frame (OR 1.18, 95% CI 1.10-1.26).

4.2.7 Time related risk of ototoxic medication and coincidental risk factors

The relationship between ototoxic medication and hearing loss has been investigated as a cumulative risk (section 4.2.6). However, the condition of the baby at the time of exposure to medications, specifically in terms of clinical diagnoses and the physiological clearance of medication might influence this further. Ototoxic medication in this section therefore included gentamicin, vancomycin and furosemide received in the first 14 days of life. Comorbid clinical diagnoses included PDA, IVH/PVL, NEC/sepsis and BPD. A priori measures of physiological instability, included lactate >2.0mmol/l, use of inotrope, and creatinine >90mmol/l which were used as measures of potential haemodynamic instability in the first 14 days of life (figure 4-12).

Figure 4-12: Multivariate logistic regression analysis: odds ratio (95% confidence interval) for independent risk factors for hearing impairment associated with ototoxic medication, comorbid diagnoses, and haemodynamic instability in preterm children

Risk factor	Hearing loss (n=57) n/median (%/IQR)		Normal hearing (n=180) n/median (%/IQR)		OR	95% CI	
Sex (male)	38	(67.7)	130	(72.2)	1.11	0.53-2.33	
Gestation (/w)	28	(25^{+6}-30)	28^{+2}	(26^{+2}-30)	1.40	1.12-1.76	**
BW (/sd)	-0.26	(-1.18-0.49)	-0.1	(-0.63-0.43)	0.87	0.63-1.20	
Morbidities$^{\Psi}$	3	(2-4)	2	(1-3)	1.89	1.26-2.81	**
Ototoxic medication$^{\Phi}$	6	(3-10)	3	(0-6)	1.15	1.05-1.26	**
Haemodynamic instability®	8	(4-14)	4	(1-7)	1.07	1.02-1.13	**



Abbreviations; BW, birthweight for gestation

$^{\Psi}$includes PDA, IVH/PVL, NEC/sepsis and BPD

$^{\Phi}$ includes use of gentamicin, vancomycin and furosemide in the first 14 days of life

® includes creatinine >90mmol/l, lactate >2.0mmol/l and the administration of inotropes in the first 14 days of life

* P<0.05; **p<0.01

Infants born at the lowest gestational age were at the greatest risk of hearing loss. Cumulative days of ototoxic medication remained associated with hearing loss independent of comorbid diagnoses and haemodynamic instability. Children at the greatest risk of hearing impairment were more likely to have been diagnosed with comorbid conditions during the neonatal period. Children with hearing loss were more frequently diagnosed with all four conditions (PDA, IVH, BPD and NEC and/or sepsis) than normal hearing children (28.1% and 13.9% respectively (OR 7.89, 95% CI 2.08-29.94)). Only 5.3% of index cases had none of these diagnoses during their neonatal care in comparison to 20.6% of normal hearing children. Cumulative days of haemodynamic instability was also independently associated with the outcome. The total number of times each individual marker of haemodynamic instability was observed in the first 14 days, was again significantly higher in children who were later found to have impaired hearing (creatinine OR 1.26, 95% CI 1.13-1.42, lactate OR 1.24, 95% CI 1.11-1.39, and inotrope OR 1.21, 95% CI 1.09-1.34). The total number of days of ototoxic medication in the presence of haemodynamic instability on the same day was added to the analysis in place of the individual variables. Low gestational age, comorbid diagnoses and the combination of ototoxic medication and haemodynamic instability were independently associated with hearing impairment.

4.3 Discussion of results

The aim of the study was to investigate the individual and combined influence of risk factors for hearing loss in the neonatal period, in infants born at less than 32 weeks of gestation. Acquired hearing loss is a common complication of prematurity in long term developmental studies. Among this population, a consistent challenge in previous research has been the overlap of risk factors; this study aimed to establish some of the relationships between them.

Based on previous findings this study expected to find firstly, individual clinical risks for hearing impairment, and secondly multiple coinciding risk factors.

In the current study, there were 57 children with hearing loss born below 32 weeks of gestation; the estimated preterm birth rate over the 5 year study period was 7375, giving a prevalence of 0.77%. In comparison to previous studies with a gestational age below 32 weeks, this is slightly lower [22, 28], however studies have shown wide variance in prevalence rates. This could be due to the method of identifying children with a hearing impairment which was based upon the newborn hearing screening programme, so some children with later onset hearing loss may not have been identified.

4.3.1 Neonatal differences between types of hearing loss

The prevalence of cochlear hearing loss was 73% of all children with a hearing impairment, giving an incidence of 5.7 per 1000. The prevalence of ANSD in this study was 27% but a further 10% of hearing loss was potentially attributable to ANSD. The estimated incidence of ANSD from the population of preterm births is between 2-2.8/1000 which is slightly higher than Dowley et al (2009), who reported an incidence of 0.67 per 1000 infants for SNHL and 0.27/1000 for ANSD in the UK [15]. This may be explained by population differences, the current study looked exclusively at preterm hearing impairment and included all severities of hearing loss, in comparison to a newborn study of severe to profound impairment. However, the higher incidence of cochlear hearing impairment compared to ANSD follows the trends shown in previous studies [14, 15].

Literature has suggested CHL and ANSD in preterm infants may have differences in aetiology, however comparisons between clinical factors in this study found few differences. Birthweight was lower for CHL indicating the smallest babies are at greatest risk for CHL, contradicting the findings from previous research whereby infants with ANSD were found to

have lower birthweights [14]. Infants from a multiple pregnancy were also more likely to have a diagnosis of ANSD. IVH occurred more frequently in the ANSD group, but was not significantly different which is consistent with previous research [14], although PVL was significantly higher in the ANSD group in this study. PVL is characterised by damage to the cerebral white matter which could be associated with the central audiological impairment of ANSD.

In this study, there were no substantially distinguishing factors between CHL and ANSD in terms of any diagnoses or exposure to treatment factors. Literature might have expected raised peak bilirubin levels, which is a known neural toxin, to be associated with ANSD [13, 15], which was not supported. Aminoglycosides and furosemide have also been associated with increasing the risk of ANSD in preterm infants [14], however findings in this study also did not support this. Some of the differences in results could be accounted for by differences in sample population, or the uncertainty surrounding the diagnosis of hearing impairment in this study.

4.3.2 Independent clinical risks for hearing loss

Neonatal risk factors for hearing loss revealed almost all diagnoses and treatments to be more frequent for infants with hearing loss than normal hearing controls. The most frequent risks for hearing loss in the literature were supported by the findings in this study. Children with hearing loss were more likely to have been diagnosed with NEC, PDA, IVH, sepsis and BPD. Duration of intensive care treatment and days of respiratory support were longer for the index group. Each medication was more likely to have been received by the hearing loss group, most notably the use of aminoglycosides, steroid, furosemide and inotrope, and they had a greater presence of physiological risk factors, reflecting a higher level of illness for infants who were later found to have impaired hearing.

Level of illness was further demonstrated by investigating co-morbidity frequencies. Previous research has used morbidity counts to express the increased risk of adverse neurodevelopmental outcome inclusive of hearing impairment, but none to date, had considered hearing loss as an independent outcome. The overall risk of hearing loss increased incrementally with each additional morbidity (OR 1.64, 95% CI 1.20-1.26) although the greatest risk of hearing loss was found in infants with two or three morbidities which again coincides with the results from previous studies [82, 131]. Both studies included ROP which the current study did not include but the findings were similar regardless. Bassler et al (2009) found the inclusion of blood culture proven infection or NEC to improve the prediction of adverse outcome [82], which this study also included however when comparisons were made between each morbidity in combination, only brain injury was significantly associated with hearing impairment, which will be discussed further below. Whilst the use of morbidity counts provide an indicator of infants at the greatest risk for impairment, little further is gained in terms of understanding the underlying processes that are associated with each morbidity which this study aimed to unravel.

Reported individual risks for hearing loss in previous studies have been difficult to elucidate as preterm infants are likely to have encountered a number of risk factors, as demonstrated by the morbidity frequencies. Specifically, distinguishing between a diagnosis and subsequent related treatments has proved challenging. However, in this study there were interesting findings, related to the management of several clinical conditions.

Diagnoses and medications as risk factors for preterm hearing loss

The youngest and smallest babies are known to be at an increased risk of poorer developmental outcomes. The babies with the smallest gestational age were at the greatest risk for developing hearing loss when adjusting for all diagnoses and treatment (figure 4-2). The use of steroid was independently associated with hearing impairment in the presence of

diagnoses for which it can be prescribed. The relationship between ibuprofen and hearing loss was also significant but in the opposite direction.

The administration of individual medications used regularly in neonatal care was investigated independently of diagnoses (figure 4-3), the smallest gestational age, again, was a risk for hearing impairment. Steroid, as mentioned above, and also the use of vancomycin and inotrope were independently associated with hearing loss. Each of which were included in further sub-analyses alongside clinical conditions for which they might be associated, which will be discussed below.

PDA and associated risk factors for preterm hearing loss

Low birthweight for gestational age was associated with hearing loss in babies when investigating PDA and treatments associated with the management of this diagnosis. Babies with the lowest birthweights are known to be at a higher risk of poor outcome. Infants that are born small for dates are more physiologically immature than their appropriate weight for age comparisons. Low birthweight also confers a greater risk of other preterm conditions. PDA is also more common in the youngest and smallest babies, and is one of the known morbidities likely to influence later outcome. A PDA increased the risk of hearing loss in this study. PDA can cause oxygenation perfusion to fall which may interrupt normal cochlear function; the cochlea is dependent on an adequate oxygen supply. Studies of infants born very preterm (less than 32 weeks [96], and less than 28 weeks gestation [12]) found similar results, in which there was an association between PDA ligation and hearing impairment. One difficulty in excluding babies for which medical management of a PDA has been successful, is that they are likely to have been exposed to similar oxygenation issues and medications as a baby that then requires surgery, in these studies it could be the surgery and associated complications that are being measured, rather than the PDA. The current study

included untreated and medically treated infants and found the same association, indicating the PDA rather than the additive risk of surgery are linked to hearing loss.

Furosemide is a long loop diuretic often used in the management of a PDA. In the current study, furosemide increased the risk of hearing loss. Loop diuretics are thought to change the composition of the endolymph within the inner ear resulting in decreases in endocochlear potential [95]. Previous studies have been inconclusive in determining the independent risk of furosemide; Eras et al (2014) found furosemide to increase the risk of hearing loss in multivariate analyses [96] but Rais-Bahrami et al (2004) had contrasting findings [97]. Furosemide has been found to potentiate the ototoxic side effects of other medications, namely aminoglycosides which will be discussed in terms of cumulative exposure to ototoxic medication (section 4.3.3). Importantly, furosemide administration when creatinine levels are raised has also been demonstrated to increase the risk of hearing loss [4]; results in this study found that furosemide was independently associated with the development of hearing impairment in the face of elevated creatinine, but creatinine was not independently increasing the risk of hearing loss.

Most surprisingly, was the protective nature of ibuprofen, and although indometacin did not reach significance the correlation was in the same protective direction. Indometacin has been associated with increased cerebral vasoconstriction whilst ibuprofen is not thought to affect cerebral oxygenation, and therefore is used preferentially in comparison to indometacin. However, both medications have been associated with decreases in renal function which could be associated with hearing loss when occurring alongside the administration of ototoxic medication. Previous studies have not found a protective relationship between ibuprofen or indometacin with hearing impairment but neither have they been associated as being a cause of hearing loss [99]. The unusual findings in this study could be representative of the inclusion of children with untreated and medically treated PDA in the analyses.

BPD and associated risk factors for preterm hearing loss

BPD is a further comorbid diagnosis that increases the chances of poor outcome. BPD is most common in the youngest babies, and as expected, gestational age correlated with an increased risk of hearing loss. Also in this study, whilst BPD occurred more frequently in children with hearing loss than in normal hearing children, it did not independently increase the risk. BPD involves the long term dependence on respiratory support and prolonged oxygen therapy, and whilst the duration of each was longer for the children with hearing loss these risk factors were also not associated with developing impaired hearing. This contrasts with previous research that have correlated hearing loss with BPD [12], mechanical ventilation [28, 49, 50], and the number of days of respiratory support [32]. The need for prolonged respiratory support is often a result of severe birth asyphyxia; Hille et al (2007) and Eras et al (2014) found birth asphyxia to be independently associated with hearing impairment, as well as prolonged respiratory support [28, 96]. Disrupted oxygen supply to the cochlear is likely to be the cause, however condition following birth (as indicated by a low Apgar score) in this study was also not an indicator of later hearing impairment.

Exposure to increased and prolonged noise levels has also been implicated in the development of hearing loss [85], particularly in infants that require long periods of ventilation [86]. Noise was not monitored in this study, but as measures of time spent ventilated, and respiratory support required at 36 weeks of age were not associated with hearing impairment, it could be inferred that exposure to noise associated with respiratory support in this study is unlikely to be having a direct impact, but this would not rule out a potentiation of drug induced ototoxicity as previously purported [87].

The administration of steroid in neonatal care is used to wean respiratory support, or in early care, to improve circulation. Previous research has been inconclusive in the association between steroid and hearing loss, however in the current study steroid was not only more

frequently given to the children with hearing impairment but was consistently associated with hearing loss when accounting for both BPD and respiratory support, contrasting with previous studies [14]. Dexamethasone in particular is used to reduce dependency on mechanical ventilation. However, since being associated with the development of cerebral palsy, it is used less frequently in clinical practice. Nevertheless, dexamethasone was given to 5 times as many cases as controls who also received a greater number of days of respiratory support. Steroid was also independently associated with impaired hearing when controlling for all types of medication in this study. It is unclear as to how steroid affects the ear, but with a known risk of cerebral palsy, it is likely to be a result of neurological damage.

Furosemide is frequently used in the management of BPD, but was not associated with hearing loss in the presence of BPD. This contrasts with the significant association with hearing loss in the presence of PDA. The use of furosemide has already been discussed, and the ototoxicity of this was considered further alongside antibiotics which will be discussed in section 4.3.3.

Infection and associated risk factors for preterm hearing loss

Hearing loss is a known complication of many infections such as CMV and meningitis, and up to 20% of preterm infants are thought to be affected by neonatal sepsis [80]; the prevalence of septicaemia was much higher in this study with over 50% of children with hearing loss having had at least one episode of neonatal infection. The association between infection and hearing loss has been demonstrated previously [55]. Meningitis in particular, was an independent predictor of preterm hearing loss in previous studies [49], however these findings were not supported by this study. Meningitis is a rare condition but is a known cause of hearing loss, the small numbers in both groups of participants may account for these results. Sepsis and NEC have a poor effect on outcome and were both associated with hearing impairment in univariate analyses which corresponds with previous research [30]. Infants

following a diagnosis of NEC have shown a delay in neural conduction likely to result from impaired myelination or synapse dysfunction [79]. However, it has not been discernible as to whether the cause of hearing loss is due to the sepsis itself, the medication used to treat infection, or the two in combination.

In this study, in the presence of a range of antibiotics previously associated with hearing loss, and markers of physiological instability commonly associated with ill health in premature infants, sepsis was not independently associated with hearing loss. However, vancomycin increased the risk of impaired hearing. Exposure to vancomycin occurred frequently in this study with 54% of all children receiving at least one dose during their neonatal care. The ototoxicity of vancomycin has previously been attributed to prolonged exposure; children in this study were more likely to have received vancomycin earlier than normal hearing children (in the first 14 days after birth), and had received longer courses of treatment. The effect of vancomycin is complicated further by the varied uses for which it is administered, which will be discussed further as part of the cumulative effect of ototoxic medication (section 4.3.3).

The impact of gentamicin on hearing loss in preterm infants has provided mixed results in previous studies. However, gentamicin in therapeutically controlled doses did not increase the risk of hearing loss in recent research [92], which is consistent with this study. Gentamicin and amikacin were the most frequently administered antibiotics in this sample and neither were independently associated with hearing loss in multivariate analysis in the presence of infection. Clinical measures have been taken to reduce the risk of harmful blood levels of these medications, and whilst the babies with hearing loss had longer durations and a greater overall exposure than normal hearing children, neither of these medications showed any increased relationship to preterm hearing loss.

Elevated creatinine and bilirubin were however associated with hearing loss. Both of which have been related with preterm hearing impairment previously [4]. Interestingly, both of

these markers of physiological instability were not associated with hearing loss when investigating risks associated with a diagnosis of a PDA or BPD, only infection in the presence of aminoglycosides showed a relationship. Bilirubin ototoxicity increases the risk of neuronal damage [62], in particular unconjugated bilirubin that has been displaced from albumin and can cross the blood brain barrier. In low birthweight infants, longer durations of raised bilirubin have been previously associated with hearing loss [31, 69], which increases the time period of potential exposure to concomitant antecedents, decreasing bilirubin binding capacity and increasing circulating unconjugated bilirubin. Relationships between bilirubin and acidosis [69], and ototoxic medication [4] have been proposed, resulting in competition for albumin binding sites. Despite using peak bilirubin (>200micromol/l) as a measure of hyperbilirubinaemia rather than duration in this study, the relationship with hearing loss persisted. Peak bilirubin was higher in children with hearing loss than normal hearing children, it is likely that this is related to the number of concomitant risk factors they were exposed to, such as acidosis and ototoxic medication as found in previous studies. A lack of association between bilirubin and hearing loss in previous studies could be due to the inclusion of term infants that are less likely to be exposed to the same number of risk factors as a very preterm baby [65].

Elevated creatinine levels were also associated with an increased risk of hearing loss in this study. Previous research has related this to a synergistic interaction with exposure to additional risk factors such as furosemide [4]. Creatinine levels are indicative of decreased renal function for which diuretics may be prescribed. In this study, there is evidence of a relationship between bilirubin and creatinine with preterm hearing impairment, however it is likely that they are effect modifiers rather than predictors of hearing loss. This will be discussed further amongst the cumulative risk of ototoxic medication and markers of haemodynamic instability (section 4.3.3).

Acquired brain injury and associated risk factors for preterm hearing loss

The relationship between brain injury and hearing loss is complex. In the presence of other diagnoses (pneumothorax, pulmonary haemorrhage, PDA, NEC, BPD, septicaemia, and meningitis) and their associated treatments (figure 4-2), brain injury including IVH and PVL did not predict a later hearing impairment. However, when investigating individual morbidities, alongside neonatal infection and BPD, brain injury was the only independent predictor. The same association was found when investigating the effect of brain injury in the presence of associated treatments (figure 4-8). Acquired brain injury is most common in the youngest and smallest infants. In this study, babies that were born with a low birthweight for gestational age conferred the greatest risk of impaired hearing. All grades of IVH were included in analyses; grade I-II IVH is the most commonly diagnosed neurological complication of prematurity and was the most frequently incurred by both groups of infants in this study. A correlation between low grades of neurological injury and hearing loss is consistent with previous research which specifically considered children with mild haemorrhages [52]. Martinez-Cruz et al (2008) also found cerebral haemorrhage to be a risk factor for hearing loss, along with serum bilirubin levels and exchange transfusion [49]. It was postulated that the relationship between brain injury and hearing impairment could be influenced by the location of the bleed or the physiological process of reabsorption of the bleed resulting in an increase in bilirubin. Bilirubin has been associated with neurotoxicity causing damage to the auditory pathway. Bilirubin was not associated with hearing loss in the presence of IVH, but as already discussed maximum bilirubin was higher in children with impaired hearing. Studies that had not reached significance were underpowered in terms of both the number of children that had a hearing impairment, and with the number that had also been diagnosed with an IVH [31, 55].

The complex aetiology of IVH and the effects on audiological outcome are further compromised by the use of treatment for hypotension. Faranoff et al (2006) in a study of extremely low birthweight infants found hearing loss was almost 10% greater in the treated group for low blood pressure, as was severe IVH (grade III-IV) [57]. Early low blood pressure and treatment in the first 3 days for hypotension is more likely to result in an IVH, and poorer neurodevelopmental outcome inclusive of hearing loss. The use of inotropes are administered to increase circulating volume and low blood pressure. Inotropes were also independently associated with preterm hearing loss in the presence of IVH in this study which coincides with previous research [57]. However, the use of steroid in the first 3 days, which is typically when an IVH is most likely to occur was not associated with hearing loss. Similarly to inotrope, steroid is also likely to be administered early to correct poor circulation; both medications can cause bleeds as a result of rapid increases of cerebral blood volume, but steroid did not present the same risk to hearing as the use of inotropes. Whilst brain injury, small for dates babies, and inotropes increase the risk of hearing loss, it remains unclear as to whether the bleed, location of the bleed, medication, changes in cerebral blood volume or interactions with the subsequent acidosis underlie the relationship.

This study has built upon previous research and used an alternative method of analysis which aimed to establish whether a diagnosis or the clinical treatment of such, could be differentiated. This methodology is somewhat novel and therefore challenging to make direct comparisons to previous findings. Nevertheless, this showed some interesting findings which were then investigated as cumulative risks.

4.3.3 Combined clinical risk factors for hearing loss

A timeline of risk factors (including medications; gentamicin, vancomycin, steroid, inotrope, furosemide, and physiological risk factors; creatinine >90mmol/l, total serum bilirubin >200microm/l and pH<7.2) showed infants with hearing impairment experienced a greater

number of risk factors throughout their care (figure 4-9). It was hypothesised that exposure to multiple coexisting risk factors in the neonatal period would be associated with hearing loss, and this was supported. The infants at the greatest risk for hearing loss encountered a greater incidence of risk factors from birth throughout their neonatal care until discharge. Whilst other studies have looked at the number of risk factors [128, 129], morbidity counts as predictors of neurodevelopmental outcome including hearing loss [82, 130-132], or used multivariate analyses to identify independent risk in the presence of other factors [14, 71], few have looked at the coincidence of variables across a timeline of care. This was investigated further in terms of ototoxic medication, concomitant diagnoses and the risk of physiological risk factors as discussed below.

4.3.3.1 Ototoxic medication

Ototoxic medication such as gentamicin, amikacin and vancomycin are known to be harmful to the inner ear, hence strict adherence to blood level monitoring in neonatal practice. In this study, children with impaired hearing were more likely to receive 2 or 3 types of antibiotic (amikacin, gentamicin and vancomycin), in comparison to normal hearing children (77% and 47% respectively). Furthermore, furosemide has been associated as potentiating the effect of ototoxic antibiotics. Yet, the impact of ototoxic medication on hearing loss in preterm infants has been controversial in previous studies.

Exposure to gentamicin, vancomycin and/or furosemide in cumulative doses was associated with hearing loss in the current study. Within the first 14 days of life children were almost 6 times more likely to be in the group of children with impaired hearing if they had received up to 7 doses of any of the ototoxic medications within that time frame.

The risk of increased exposure to these medications coincides with the results of previous studies [31, 93]. Total number of days of treatment and cumulative doses were higher in the case group than the normal hearing group, in a sample of preterm infants (<34 weeks

gestation) [98]. Similarly, but in term or near term infants, the use of vancomycin was higher, and cumulative doses and duration of diuretics was greater for children with hearing loss [71]. Concomitant administration of aminoglycosides and furosemide was also found to increase the risk of hearing loss.

Methodological variation is likely to explain the differences between the findings in the current study and studies that have failed to find an association between hearing impairment and drug induced ototoxicity. Combinations of tobramycin, vancomycin and furosemide were not related to hearing loss in a sample of 45 infants who failed their hearing screen [94]. There are several reasons for which these results might underestimate the ototoxicity of these medications. Firstly, conclusions were based upon hearing screening results whereas, the current study recruited children with hearing loss up to the age of 7 with a confirmed hearing loss. Secondly, reliance on the newborn hearing screen is likely to include children that do not have hearing loss at follow up. In the absence of confirmed hearing impairment, the safe use of gentamicin is a conservative finding. Furthermore, high frequency losses were not tested. Impaired hearing as a result of ototoxic medication commonly start with a high frequency loss and can cause delayed onset impairment due to slow clearance from the inner ear which progresses to the lower frequencies. Finally, tobramycin is used less frequently in the UK, and therefore gentamicin which is a commonly used antibiotic in neonatal care which also has ototoxic properties, was included in the analysis instead; the ototoxicity levels of tobramycin are understood to be lower than that of gentamicin which could play a role in the differing results [143].

A further study, with a similar population of preterm infants (<32 weeks gestation or <1500g) to the current study, also found contrasting results [92]. Cumulative doses and total number of days of aminoglycosides posed no additional risk to hearing function. The frequency of exposure to gentamicin (case group 86% in the current study, 76% in Fuchs et al's study,

control groups 71% and 70% respectively) and vancomycin (case group 72% in the current study, 64% in Fuchs et al's study, control groups 42% and 50% respectively) were comparable between the studies, but furosemide administration was substantially lower than the current study (double the number of cases and controls received furosemide), as was the frequency of BPD for which furosemide will often be prescribed as part of the treatment course. Published literature has indicated a cumulative effect of ototoxic medication leads to an accrual within the inner ear that potentiates hearing loss. Furthermore, in laboratory studies, an interaction between an accumulation of aminoglycosides within the inner ear and loop diuretics increase the damage to cochlear hair cells [90]. As discussed in chapter 1, aminoglycosides not only present an ototoxic risk but also a nephrotoxic risk, which can increase the circulating volume of aminoglycosides and also the need for diuretics. Therefore, infants with the greatest exposure are the most at risk of hearing loss.

The critical period for preterm infants is typically the first two weeks of intensive care following birth. Therefore, it was thought that this time frame would pose a greater risk, however the risk of ototoxic medication did not appear to be time critical. Infants received a cumulatively higher amount of ototoxic medication and were at as much risk of hearing loss in subsequent weeks of care as they were in the first two weeks of life. This extended on previous research that has found an association between ototoxic medication and hearing loss but has not looked at the influence of time.

Gentamicin is frequently used as a first line antibiotic in neonatal care, and while it has been deemed innocuous to the functioning of the inner ear, possibly as a direct consequence of stricter monitoring of both prescribed doses and serum blood levels during treatment, the results in this study suggest otherwise. The coincidence of gentamicin with other ototoxic medications, namely vancomycin and furosemide have been cumulatively associated with hearing loss in infants born at less than 32 weeks gestation. The clinical uses of vancomycin

vary between babies; it is prescribed as a second line antibiotic for proven sepsis, suspected sepsis or prophylactically to prevent long line sepsis. Long line duration was longer for children with hearing loss (mean 31 days, range 0-155) in comparison to children with normal hearing (mean 17 days, range 0-133). This could be indicative of the increased prevalence of gastrointestinal disturbances such as NEC in children with impaired hearing, for which long line nutrition may be required.

4.3.3.2 Ototoxic medication and coincidental risk factors for hearing loss

Multifactorial relationships are likely to increase the relative risk of hearing loss in preterm infants. Published literature suggested exposure to a coincidence of risk factors exacerbates the risk of hearing loss [4]. Combinations of furosemide in the presence of elevated creatinine levels, furosemide and netilmicin, netilmicin when bilirubin levels were raised, and acidosis in the presence of hyperbilirubinaemia posed a risk to hearing. The potentiation of ototoxic medication by physiological instability partially informed the analysis for the current study.

Cumulative ototoxic medication, clinical diagnoses and haemodynamic instability were all independently predictive of hearing loss (figure 4-12). Furthermore, the combination of ototoxic medication and haemodynamic instability occurring on the same day was also associated with an increased chance of hearing impairment which coincides with previous findings [4]. Ototoxicity may be more severe in those with haemodynamic instability compared to those with exposure to ototoxic medication alone or haemodynamic instability alone, but the study was not powered to detect this. Small numbers of infants with no exposure to ototoxic medication or haemodynamic instability in the first 14 days following birth meant that analyses could not determine whether exposure to both in combination increased the risk of hearing loss more than to exposure to either of these alone.

This study provides an indication of the exposure to multiple risk factors which were greatest during the first two weeks of life (section 4.2.5). The complexity of the interactions between

these risk factors are commonly recognised in previous studies mainly in terms of ototoxic medication [71, 92, 93, 98]. Ototoxic medication, namely antibiotics used to treat sepsis, administered at safe doses is dependent on adequate renal function which is often impaired in very preterm infants. Creatinine levels are a marker of inadequate elimination, and for which loop diuretics are often prescribed. Diuretics are often also used to treat BPD and occasionally PDA. Inotrope is administered for poor perfusion (often associated with a diagnosis of PDA and IVH) which is more common in preterm infants, but is also associated with acidosis and raised lactate levels. Inotrope has been independently associated with hearing loss in the current study and acidosis is known to compete for albumin binding sites along with ototoxic medication. Few studies have considered a time based aspect to analyses and the overlap of a multitude of risk factors. The current study has provided an insight into the risks for hearing loss encountered by preterm infants in the critical period after birth.

Overall, the hypothesis was supported in terms of the most unwell children being at the greatest risk for hearing loss.

4.3.4 Summary

In this study of preterm infants, a number of risk factors for hearing loss were elicited. Infants with the lowest gestational ages, the smallest birthweights and born in poorer condition are at the greatest risk of hearing impairment. Infection, acquired brain injury, PDA, NEC, BPD and pneumothorax were all more frequent in the hearing loss group. Duration of respiratory support, oxygen therapy and intensive care treatment were also more common in children with impaired hearing, as was exposure to almost all medications. Children with hearing loss were therefore expected to have been exposed to a higher number of individual risk factors than children with normal hearing, across a timeline of neonatal care. The findings in this study support that expectation.

Individual risks for preterm hearing impairment include low gestational age, low birth weight, acquired brain injury, a diagnosis of PDA, the use of steroid, inotrope, vancomycin, and furosemide, and raised creatinine and bilirubin independently increased the risk of hearing loss. The rate of comorbid diagnoses tended to be higher in children that developed hearing loss, whom were subsequently also exposed to an increased number of individual risk factors on a daily basis throughout the first 14 days of their neonatal care.

The proportion of infants receiving ototoxic medication was higher, however, the ototoxicity of gentamicin was only evident in accumulation with vancomycin and furosemide. Cumulative doses of ototoxic medication increased the risk of audiological harm. Coinciding risk factors were also expected to play a role in increasing the risk of hearing loss. The use of ototoxic medications in the presence of haemodynamic instability was found to increase the likelihood of audiological impairment, supporting this hypothesis.

It is evident that there is a continuum of exposure to risk factors which lasts beyond the first two weeks of life. It could be that there is an initial insult to the ear and then consequent exposure exacerbates the damage that has already occurred, particularly in the use of aminoglycosides. Suppressed renal function and perfusion necessitate the use of diuretics which further exacerbates the ototoxicity of aminoglycosides.

In conclusion, we found multiple risk factors over a timeline of neonatal care, both independent and combined risks which interact to increase the chances of hearing loss in this vulnerable population.

The final chapter will consider implications for research, implications for clinical practice and the strengths and limitations of the study.

5 Chapter 5: General discussion

The risk of hearing loss for children born prematurely is widely known and reported by developmental outcome studies [1, 4]. The neonatal course of infants born prematurely is complex and elucidating individual risk factors for hearing loss is therefore challenging. Ten risk factors for impaired hearing were identified by JCIH including: family history of hearing loss, congenital infections, craniofacial abnormalities, low birth weight (less than 1500g), hyperbilirubinaemia, ototoxic medications, bacterial meningitis, low APGAR scores at 1 or 5 minutes, assisted ventilation for 5 days or more, and syndromes associated with hearing loss. Whilst many of the children with hearing loss were more frequently exposed to the neonatal variables amongst these risks in comparison to children that have normal hearing, additional risk factors were also evident in this sample of preterm infants. Low gestational age, acquired brain injury, PDA and an individual and cumulative risk of ototoxic medication increased the outcome of impaired hearing.

The risk of ototoxic medication to hearing in preterm infants is exacerbated in individuals with m.1555A>G which provides a predetermined susceptibility to hearing loss following exposure to aminoglycosides. The contribution of aminoglycosides has been unclear in previous research, and remains somewhat unclear in this study. A late onset mild hearing impairment became evident in one control child, following aminoglycosides in the presence of m.1555A>G, although this was not apparent in an older sibling who had also been exposed to aminoglycosides.

The rest of this chapter will consider the implications of the findings in this study, as well as limitations and directions for further research.

5.1 Implications for research

5.1.1 Genetic research

The implications for research surrounding the effect of m.1555A>G to deafness are primarily methodological. Results from this study can be used to reflect on previous studies and steer future research in the following ways.

Research has previously found hearing loss in individuals with m.1555A>G following exposure to aminoglycosides, however from the current study it is evident that the methodology chosen to investigate deafness as an outcome impacts the overall findings. This study has revealed flaws in some previous studies which have focussed only on individuals with severe to profound deafness [126], used self-reporting of the progression of hearing loss [117], self-reported aminoglycoside exposure [141], and used the failure of early newborn hearing screens as an outcome measure [124]. The results from this study emphasise firstly, the need to include normal hearing children and lower severities of impairment including high frequency losses, and not just a subset of children with impaired hearing. As a progressive form of hearing loss has been associated with m.1555A>G many affected children with a later onset of impairment could have been missed, subsequently underestimating not only the age of onset of hearing loss but also the impact of the mutation. Secondly, the current study has shown that the timing of monitoring outcome measures is influential on the research findings. A newborn hearing screen is completed too early to detect a late onset hearing loss as used in previous research. Longitudinal or retrospective studies with a complete audiological and pharmacological history are paramount to reviewing the causes of hearing loss in a susceptible population.

In summary, implications for the research of m.1555A>G in children is predominantly methodological. Reliance on an early hearing screen is liable to underestimate the influence

of m.1555A>G and the impact of aminoglycosides which affects clinical care, this will be discussed in section 5.2.1.

5.1.2 Neonatal research

From the research explored to date, this appears to be the most comprehensive analysis of overlapping risk factors for hearing loss, particularly along a timeline of neonatal care. The number of risk factors for hearing loss encountered by children with impaired hearing in the first 2 weeks of neonatal care are crucial, but these continue until discharge.

A strength of this study is the longitudinal nature of recruiting children born over a 5 year period, and recruited over a three year period which meant that hearing loss was confirmed, whilst changes to clinical care were likely to be minimal. The rates of preterm hearing loss are much higher than term born populations but overall prevalence rates are still relatively low (0.77% in this study), and to reach adequate sample size recruitment will often need to be multicentre and/or over a prolonged period of time to make reliable inferences as to the manifestation of hearing loss. The number of children with hearing loss participating in this study was at the higher end of the target group, this again is an achievement.

Some of the discrepancies between the results in this study and previous research could surround the reliance on cross sectional data from hearing screening rather than confirmed hearing loss from follow up appointments [94]. The inclusion of children with suspected and confirmed ANSD is advantageous. Given the uncertainty surrounding diagnosis, excluding these children would have reduced the power of the study, and given the lack of discernible neonatal differences in characteristics between the ANSD and CHL groups (section 4.2.2), exclusion would not have been viable.

A further strength is the cut off of gestational age (<32 weeks) for study inclusion. The risk factors that a very preterm infant is exposed to are very different to those of a term baby

and are therefore not comparable in the same ways. Furthermore, maturational differences lead to inconsistencies in the relationships between risk factors when comparing babies born at the lowest gestations to those born near or at term. Studies including the two as an individual group, or making comparisons between a term and preterm group are likely to miss influential interactions [65].

Statistical analyses have incorporated a multitude of variables associated with neonatal care and attempted to tease apart complex interactions between these risk factors for hearing loss. This again, is a strength of the current study. Previously, a reliance of descriptive and univariate analysis has been adopted by studies [30] which does not account for the influence of confounding factors and co-dependence between risk factors, therefore limiting the ability to apply these results to neonatal practice.

In summary, future research can be informed by the methodology adopted in this study which features many strengths, namely using confirmed hearing impairment, restricting inclusion of very preterm infants and considering a breadth of overlapping risk factors which has previously been overlooked.

5.2 Implications for practice

5.2.1 Genetic practice

The current study has found one child with m.1555A>G who developed a late onset hearing impairment following exposure to aminoglycosides, which may be a progressive loss. Current practice does not screen for m.1555A>G prior to the administration of aminoglycosides in neonatal care, but is screened for prior to the commencement of paediatric oncology treatment. Reliance on the newborn hearing screen could delay diagnosis and therefore treatment for children that are not identified as at risk. Furthermore, children with a late onset hearing impairment with a history of preterm birth and no family history of deafness,

may be assumed to have an acquired loss as a result of neonatal complications rather than undergo genetic investigations.

Based on findings from this study, and previous research into the increased risk of hearing loss in those with the mutation following exposure to aminoglycoside antibiotics [117], there are several considerations to be discussed. Firstly, taking a family history prior to the administration of aminoglycosides has been previously suggested, however due to the constraints of providing rapid neonatal care this is not always possible. Furthermore, a mild hearing loss is not always obvious, and so a self-reported family history may not provide much more detailed information.

Secondly, a change in first line antibiotics for suspected sepsis in preterm infants could be implemented. An alternative option to aminoglycosides is to use cephalosporin agents instead. However, an advantage to the use of aminoglycosides antibiotics in preterm infants is due to the low drug resistance and cost effectiveness. In comparison, cephalosporin agents are less economically viable and there is a potential to lower the effect of the antibiotic and increase drug resistance. In a study of paediatric hospital admissions with antibiotic administration, children who had received 3rd generation cephalosporin's were significantly more likely to develop extended-spectrum β-lactamase (ESBL) [144]. Since the current study did not find a confirmed association between m.1555A>G and aminoglycoside use, a change in aminoglycoside protocol would not be recommended. However, a reduction in the use of antibiotics may be more appropriate considering 95% of infants that are treated prophylactically for sepsis do not in fact have positive blood cultures and are therefore in receipt of at least 48 hours of antibiotics unnecessarily [145]. A decrease in exposure would reduce the risk of aminoglycoside induced hearing loss in general, as well as benefitting carriers of m.1555A>G.

Thirdly, the use of rapid screening could be undertaken prior to the administration of aminoglycosides in preterm infants that are likely to receive antibiotics within the first few hours of life. As observed from the results in this study, aminoglycoside exposure in the presence of m.1555A>G can cause a late onset decline in hearing which is preventable following screening and the avoidance of aminoglycosides. If there is a threshold effect a dose or short course might not have implications for hearing, however testing would still be required. Cot side testing of saliva samples is non-invasive and fast; currently taking around an hour to process. However, an infant born prematurely is likely to be born unwell, and a saliva sample will not be given priority over stabilisation; given that the first dose of antibiotics will be administered within an hour of life to treat suspected early onset sepsis, a result would be unlikely to be available.

Lastly, specific antenatal rapid response screening for m.1555A>G in preterm labouring women and women showing signs of infection during labour, whose babies are likely to be prescribed antibiotics following delivery. The result of which could be available prior to birth for avoidance of aminoglycosides in infants of women testing positive for m.1555A>G. This is as cost effective as testing babies, but provides a wider time frame for obtaining a result in comparison to screening the babies once born. Early screening and detection of individuals with the mutation, would also enable follow up audiology assessments which are critical in detecting impairment as early as possible to reduce the effect on development. Rapid screening would enable alternative antibiotics to be given where possible and for identification of at risk patients to be followed up even upon passing their newborn hearing screen. This study found a late onset presentation of hearing loss, which, based on previous studies, is likely to deteriorate with time.

Preterm infants are just one group of patients exposed to repeated courses of aminoglycosides. As discussed in section 1.3.2, cystic fibrosis patients are also frequently

treated with aminoglycosides, and hearing loss following exposure to ototoxic antibiotics in the presence of m.1555A>G has been found [120]. The use of pre-treatment screening for m.1555A>G in paediatric oncology patients is already in current practice, it would be useful to see if this has been cost effective before applying to other areas of patient care, this will be discussed further in section 5.4.

5.2.2 Neonatal care

Preterm hearing loss is up to 10 times higher than in the term born population. The prevention of neonatal hearing loss can only be achieved by understanding the underlying relationships between risk factors. This study aimed to investigate those and derived individual and coinciding risk factors for hearing loss in preterm infants as expected. However, the greatest risk of hearing loss appears to surround the use of medication which is consistent along a continuum of neonatal care. There are important implications for future neonatal treatment.

The findings from this study have identified the babies at greatest risk of hearing impairment; infants with the highest exposure to risk factors. Therefore, the management of these vulnerable babies is dependent on the reduction of exposure to risk factors, one of the most commonly presented being ototoxic medication.

Almost all babies in this study were exposed to at least one dose of antibiotics (100% of children with impaired hearing and 91% of children with normal hearing). Interestingly, given the high rates of exposure to aminoglycosides, the relative risk of sepsis was much lower; confirmed by positive blood culture or NEC in 65% of children with hearing loss in comparison to 47% of normal hearing children, yet sepsis was not independently predictive of hearing impairment in the presence of aminoglycosides. Therefore, treatment for suspected sepsis or prevention of central line sepsis accounted for 35% of children with hearing loss and 44%

of normal hearing children in this study. In context, it is also important to consider the rate of sepsis progression in preterm infants with an immature immune system for which antibiotics are given until sepsis is disproven. Antibiotics are a lifesaving treatment in this vulnerable population, which overrides the risk of hearing loss, but it nevertheless is important to consider the long term risks of prolonged exposure as well as the palpable benefits.

Gentamicin alone appeared to not increase the risk of hearing loss, yet a cumulative effect was found. Vancomycin in particular, was associated with hearing loss in multiple analyses and questions arise surrounding the high use of this antibiotic. In a Cochrane review of the prophylactic use of vancomycin in infants with a birthweight <1500g, no evidence was found to support low dose continuous therapy and therefore recommendations concluded that prophylactic use should not be undertaken [81]. Attempts to lessen the impact of ototoxic medication on hearing have already been implemented in current practice by the strict monitoring of blood levels during courses of treatment of amikacin, gentamicin and vancomycin. However, negative effects can be further diminished by minimising exposure to all ototoxic medication with short durations, and monitoring for coinciding risk factors, in particular furosemide and indicators of haemodynamic instability.

One marker of haemodynamic instability is raised bilirubin. A previous nationwide study found the monitoring and treatment of such to be inconsistent across neonatal units [66], of particular interest as a consequence of this study is the sickness line and the commencement of early therapeutic intervention. Whilst findings in this study did not indicate a consistent association between bilirubin and hearing loss, there were indicators of an underlying role (section 4.2.4.4) which corresponds with previous studies. This supports the notion that the therapeutic level for the commencement of phototherapy should be lower when infants are

exposed to a number of risk factors that compete for binding affinity, therefore lowering the risk of hearing impairment when there are likely to be other risk factors present.

The monitoring of cumulative risk factors could also be addressed in clinical practice. BadgerNet provides an electronically accessed summary of daily treatment, however diagnoses could be added to the timeline. At present, diagnoses are listed and are not cohesive with the daily timeline which would increase the awareness of coinciding risks, particularly in relation to comorbidities and associated treatments. Long term medication use could be monitored more easily to assess previous exposure such as a running total of days or doses, specifically for ototoxic medication. This is also beneficial for future research investigating later outcome measures, whereby retrospective methodologies are employed for data collection.

In summary, an integrated approach to future care that utilises daily summaries already in clinical practice will reduce the risk of hearing impairment in preterm infants. Prescribing of medication especially for suspected sepsis needs to take into account other coinciding risk factors. Furthermore, given the predisposition to the ototoxicity of aminoglycosides which was discussed in section 5.2.1; a review of current practice surrounding antibiotic prescribing in the absence of confirmed sepsis may be required.

5.3 Limitations

During the three year study period a number of limitations were experienced during the set-up of the study, recruitment and data collection which could have impacted on the results. This will be discussed in relation to sample size, data collection and analysis.

5.3.1 Sample size

The first limitation related to sample size, is the time taken to establish the study and begin recruitment. A faster set up time would have enabled a greater length of time to identify,

recruit and collect data which would have strengthened the findings of the study. Subsequent difficulties with the identification and recruitment of children with hearing loss was a further constraint on the study. The target sample size of cases was 30-60 children with hearing loss, which was achieved at the upper end. However, one third of children identified with impaired hearing were not recruited and this could have affected overall results.

Children with hearing loss were identified by NHSP, which was thought likely to remove the risk of selection bias typically associated with case control studies. However, this could have impacted on the findings of the study, specifically, the number of children that were found to carry m.1555A>G. Although small numbers were expected, the target mutation was thought to be more prevalent in a population of children with impaired hearing who were very likely to have received aminoglycosides. However, it appears evident that this study is less likely to have captured information on preterm children with a later onset of hearing loss. Only 10% of cases in this study passed their initial hearing screen and presented with a late onset of impaired hearing, all of whom were identified by clinicians. Whilst the prevalence of the mutation in this study fits within the trend of previous studies, it is possible that the low number of children with the mutation is due to this under-represented group, especially, as the only child identified with m.1555A>G in this study was recruited as a control but was later found to have a late onset impairment. Therefore, it is possible that these results underestimate the impact of m.1555A>G following exposure to aminoglycosides.

A further potential source of bias is from the families who did not respond to letters of invitation to participate. Follow up strategies to increase responses were employed in this study, however, not all of the cases or controls could be contacted, which could have introduced an unforeseen bias.

The identification and recruitment of matched control children was also challenging, and reduced the total number of controls enrolled to the study. The children with hearing loss

born at the very lowest gestational ages had a fewer number of potential controls to match with, given that sex and neonatal unit for which care was received, were also controlled for. Due to the low numbers of children to invite to participate coupled with a low response rate, it was not possible to recruit five matches for every case. Despite difficulties with recruitment, the study had three times the number of control children to cases. Measures were taken to control for the lower number of matched children with normal hearing born at the lowest gestational ages by adjusting analyses for sex, gestational week, and birthweight in statistical analyses.

This study recruited a higher proportion of male than female infants, whilst this was not a significant difference, males have a tendency to be more unwell [23, 24], and this could have been reflected in the results. The number of females recruited to the study was too low to make comparisons between male and female infants in terms of neonatal treatment.

In summary, the study experienced predictable difficulties with recruitment that may have inadvertently impacted on the overall sample size and results.

5.3.2 Data collection and analysis

The design of the study intended to include as many children with confirmed hearing loss as possible within the study time frame. However, in order to achieve this, data (particularly neonatal information) was collected retrospectively. This was a limitation in itself and will be discussed further in terms of audiological, and neonatal data collection.

Audiology data was collected as soon after the time of consent as possible, however this differed between children across sites. As a consequence, there was an age difference between the children regarding when the data was collected and ultimately how recent their last hearing assessment had been; hearing could have improved or worsened during the course of the study. The level of data obtained was also somewhat problematic. Information

concerning which hearing tests were used and when, was simplified to encourage response rates from clinicians. In this respect, the data might be slightly crude and would have benefitted from more in depth information regarding the nature, and onset of hearing loss. Furthermore, it was difficult to make conclusions without a firm diagnosis of ANSD for some of the children, which is partly accounted for by the challenges surrounding data collection.

With regard to neonatal data collection, changes in data storage and tracking mechanisms in recent years led to difficulty in tracing some of the children's medical notes. Many children were treated in a number of hospitals during the neonatal period, resulting in multiple sets of clinical records whereby data needed to be abstracted from several volumes of notes across different sites. Inaccessibility to medical notes, especially from neonatal units outside of London, was supplemented with data collection from BadgerNet summaries (neonatal daily summaries where available), however this is somewhat less accurate and less detailed than clinical notes. Therefore, study findings may be underestimated. Medical notes were also accessed for data abstraction by the student only. Whilst care was taken in designing the data collection proforma to enable data to be accurately transcribed from the clinical notes, it is possible that errors could have occurred during this process. This would have been reduced by having a second person to check the data transcription, however ethical approval had stated only the research nurse should be able to access medical records from outside the direct medical team. Owing to the time taken to collect data from clinical notes, it was not feasible to have a staff member from each neonatal unit second check data collection.

Furthermore, as a multi-site study, there are differences in protocol between units. Although this was controlled for by recruiting matched controls based on which neonatal unit they received the first two weeks of treatment, this may still have had a minor impact, and may explain some of the discrepancies in findings between this study and previous research. For example antibiotic prescribing across the Trusts in this study ranged in terms of the first line

choice of medication, dose per kg and in the number of doses given per day. To reduce the differences between sites, data was collected on the number of days of doses in the first 14 days rather than the amount prescribed, it is therefore slightly more difficult to compare to single site research protocols, or studies.

In addition, it might have been beneficial to collect more information regarding the number of drug doses, and dosages for each baby. This would have enabled a calculation regarding the total amount of ototoxic medication received, as opposed to the yes/no data collected per day and then per week. Certainly for the child with m.1555A>G, greater pharmacological data would have provided more accuracy as to the total exposure to aminoglycosides. Whilst most medications investigated in this study are given once daily, some may have a 6, 8 or 12 hourly dosing interval which would potentially underestimate the results reported. Although this would have provided a more accurate picture, there would have been a consequent relative rise in missing data. As already mentioned it was not possible to access all medical notes and therefore the depth of data would have cancelled out the breadth.

Similarly, peak and trough levels of antibiotics were measured differently across sites, and were not available for all children. Therefore, it was not possible to report detailed aminoglycoside levels, due to obtainability issues. However, only one infant with hearing loss had a reported high level during antibiotic treatment, and it is unlikely that this would have influenced the result given the number of other risk factors present.

Bilirubin measurements were collected as a total serum bilirubin level which was based on previous research [4], however toxicity is strongly correlated with unbound bilirubin and collecting this may have led to more accurate analysis [146]. Again, this information was not available from all sites, and so a decision was made to collect the information that was most likely to be available.

Associations in this study have found the aetiology of preterm hearing loss to occur across a continuum. Inferences from this data are limited by the lack of antenatal data which will also influence the outcome of hearing loss. The early use of early ototoxic medication particularly vancomycin as a second line antibiotic could have been a consequence of maternal infection which in itself is a risk factor for hearing loss.

A further drawback during data collection was the inability to make any reliable inferences as to the role of CMV in this cohort. Symptomatic infants were screened for CMV during their neonatal care, and some children with impaired hearing were tested later as part of their audiology treatment to investigate aetiology. However, not all children and been tested and it was therefore not possible to make comparisons to the normal hearing group. Given the currently uncertain impact of CMV on hearing loss which is known to be an independent cause of childhood hearing loss, this would have added to current understanding regarding hearing impairment in this population.

A limitation of data analysis was the use of preselected risk factors for multivariate regression, which was partly based on results, and partly from current knowledge from previous research. There is a chance that this could have introduced bias. It was not possible to run an analysis comparing groups of children with ototoxic medication only, haemodynamic instability only, ototoxic medication and haemodynamic instability and none (section 4.2.7) without introducing a Type 1 error. Only 1.8% of children with hearing loss and 4.4% of children with normal hearing did not receive any aminoglycosides or have any haemodynamic instability during the first 14 days of their neonatal care; this was too low to form a baseline comparison group with which to compare the risk of ototoxic medication and haemodynamic instability to the other groups. Whilst this demonstrates the widespread use of aminoglycosides, a larger number of children who had not been exposed would have increased the power of the analyses.

It is also very unlikely that the risk factors are completely independent; relationships are complex and difficult to differentiate, and analysis underestimates the relationships despite attempts to tease them apart. It was initially thought that critical period was during the first 14 days but when considering drug induced ototoxicity it appeared this was not the case. Collecting data on a daily basis during each diagnosis for example PDA if it occurred during weekly data collection (after the first two weeks), would have enabled the observation of independent risks to emerge. Daily measurements during critical periods might have been as informative as the collection of data for the first 14 days, although as already mentioned this would increase the risk of missing data. Most relationships had declined by week 12 although at this point a large number of infants had already been discharged.

In summary, despite incurring a number of limitations associated with sample size and data collection, most of which could not be anticipated, these were controlled for as much as possible. Given that the results follow similar trends to those of previous studies, it is unlikely that the findings have been adversely affected.

5.4 Future research

Drug induced ototoxicity is one of the main findings from this study. Given the large number of preterm infants treated with ototoxic medication, future research should investigate this further. Individuals with m.1555A>G are predisposed to the audiological side effects of aminoglycosides, and cumulative aminoglycosides with furosemide have also been prominent in the risk of hearing impairment. As a result of the findings in this study, and methodological strengths and limitations, the following section will discuss potential avenues for further research.

The ongoing monitoring of the child in this study found to carry m.1555A>G and their sibling will be a priority following this study and will inform future research. Long term audiological follow up will be required for both children. Whilst m.1555A>G initially appeared benign in

this study, a later onset high frequency hearing impairment questions the long term audiological outcome. Progressive hearing loss has been associated with m.1555A>G following aminoglycosides; therefore it is important to observe the course that this hearing loss takes. Furthermore, as the sibling also received aminoglycosides based on previous research this would also be expected to result in a hearing impairment, time will tell.

Follow up of preterm studies that have used the newborn hearing screen as a measure of hearing loss in infants with m.1555A>G and a history of aminoglycosides, would also be informative, and are understood to be in progress [124]. This would enable a direct comparison to the results in this study. Late onset impairments would coincide with our findings and would highlight the importance of investigating childhood hearing loss as opposed to a failed hearing screen.

Owing to the small number of children affected by m.1555A>G, a meta-analysis of recent preterm literature [123-125], preferably following confirmation of hearing loss, would increase power and improve the estimates of the effect. In section 5.2.1, the screening of infants or mothers in labour was discussed, however this would require a cost benefit analysis requiring greater numbers than we found in this study alone. A systematic review including the findings of major studies investigating aminoglycoside induced deafness in carriers of m.1555A>G might provide greater clarity. Already in practice is genetic screening for m.1555A>G in paediatric oncology. A review of the data surrounding the detection of the mutation would also be crucial before implementing in neonatal care. Screening was introduced prior to the commencement of aminoglycosides to prevent drug induced ototoxicity, an audit would aid cost benefit analysis.

The addition of other contributing risk factors has also been discussed in section 5.2.1. An extension of this research might consider the reason behind aminoglycoside administration and the potential for proven sepsis as opposed to suspected sepsis to determine deafness in

the presence of m.1555A>G. Hu et al (1991) listed potential sources of infection as well as exposure to aminoglycosides [142]. Studies consistently fail to report the type of infection, which could also be pivotal in the presentation of hearing loss in these individuals. Septicaemia increases bodily demand for energy and could cause mitochondrial inhibition and subsequent functional errors. This could not be explored in this study as neither the case, nor their sibling, had confirmed sepsis when they were treated with aminoglycosides. As hearing loss has become evident following suspected sepsis it is unlikely that the cause is septicaemia, however interactions between the aminoglycosides, the mutation and increased bodily stress are likely to coexist.

A wide variety of antibiotics have been reported as ototoxic throughout the literature although some are used less frequently now, for example kanamycin. As both children with the mutation were treated with two different types of aminoglycoside, it is therefore not possible to differentiate between the two as to which was the most ototoxic. Tobramycin has previously been deemed less harmful than gentamicin, but given the variation used in neonatal care future research could investigate which is most toxic in the presence of m.1555A>G. Based on the results discussed in section 4.2.7, cumulative doses of aminoglycosides could be pivotal in the manifestation of hearing loss in individuals with m.1555A>G. Again, it was not possible to report in this study due to inaccessibility to medical notes. Moreover, the potentiation of furosemide to aminoglycosides also found in this study should also be measured in future research investigating drug induced ototoxicity and m.1555A>G.

From a neonatal perspective, ototoxic medication, namely vancomycin, was consistently associated with hearing loss in this study. Exposure to vancomycin in this study was measured in terms of whether it had been received per day or per week during neonatal care. Given the direct association with hearing loss, future research should consider the total dosages of

vancomycin administered per kg. However, this would also need to be considered relative to the exposure and dosages of other ototoxic medications, it was evident in this study that cumulative exposure to ototoxic risk factors also increase the likelihood of hearing loss. The specific use of vancomycin should also be explored further. It was noted that some of the infants in this study were given vancomycin for the prevention of long line sepsis, which a Cochrane review of prophylactic vancomycin did not recommend. Furthermore, few of the studies included in the review had considered hearing loss as an outcome and no conclusions could be drawn regarding later antibiotic resistance [81]. The uncertainty surrounding this highlight the need for alternative options to be explored, which would involve cost analysis reviews and long term follow up on outcome, specifically in terms of antibiotic resistance. Similarly, the long term use of furosemide, which is a fast working diuretic was noted for some infants. The cumulative effect of furosemide with aminoglycosides was one of the main findings from this study. Changes to clinical practice resulting in the decreased exposure to ototoxic medication would warrant a similar review to investigate the consequent reduction in hearing loss in preterm infants.

The relationship between the unanticipated protective effect of ibuprofen and hearing impairment should also be explored further. Similarly, indometacin showed a protective tendency although this did not reach significance which may be due to the small numbers in both groups. This study considered the presence of a PDA and the number of days or weeks of exposure to ibuprofen and indometacin only; it may be beneficial to consider the size of the duct, velocity of blood flow, time taken for the duct to close and the physiological markers of changes in perfusion such as bradycardias, acidosis, apnoea's and desaturations. As this study included an untreated and a medically treated PDA in analyses it is possible that these had a lesser clinical impact than a surgically treated PDA which is typically measured in previous studies; subsequently influencing the overall findings.

One of the limitations of the results from this study was the small number of children that had not been exposed to ototoxic medication or haemodynamic instability. Based on published literature ototoxicity is likely to be increased as a consequence of haemodynamic instability, but with a very small unexposed group it was not possible to make this comparison. An extension of this study could investigate the predictive nature of ototoxicity in the presence of haemodynamic instability as a primary outcome so that recruitment could be made to each group (no exposure, haemodynamic instability only, exposure to ototoxic medication only, or exposed to ototoxic medication in the presence of haemodynamic instability). Although this would be challenging given the difficulties faced with recruitment, this would enable a comprehensive evaluation of these risks in association with hearing loss.

5.5 Conclusion

Hearing loss in preterm infants is influenced by a multitude of interacting factors. Ototoxic medication presents an increased risk of hearing loss to infants born at less than 32 weeks gestation. The impact of ototoxic medication on hearing can be reduced by giving short durations, and monitoring for coinciding risk factors, in particular indicators of haemodynamic instability. Aminoglycosides in particular, appear to present an increased risk when given in the presence of m.1555A>G. Further research could build upon the results from this study to develop a cost benefit analysis for changes to clinical practice with regard to genetic screening prior to exposure to aminoglycosides.

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Appendices

Appendix 1: Prevalence of hearing loss following neonatal care (Publications from 2004-2014)

Study	Definition of hearing loss	Population size	Gestational age/weight	Prevalence	
Coenraad et al [30], 2010	ABR >40dB	3316 NICU graduates (>24hr stay)		58/3316	1.75%
Vella-Brincat et al [93], 2011	OAE failure	2347 NICU admissions (>48hr stay)		153/2347	6.51%
	OAE and ABR failure			30/2347	1.28%
Rastogi et al [86], 2013	Receiving therapy or hearing assistance	344	<1500g	19/344	5.52%
Declau et al [19], 2008	AABR >35dB	87000 infants		116/87000	0.13%
Xoinis et al [14], 2007	ABR >20dB	4250 NICU infants		95/4250	2.24%
Synnes et al [147], 2011	>21dB	586 extremely low birth weight infants (ELBW)	<800g	50/586	8.53%
Martinez-Cruz et al, [51] 2012	SNHL (BAEP >45dB) AN (TEOAE/DPOAE pass, BAEP fail)	93 extremely low birth weight infants (ELBW)	<750g	6/93	6.45%
Johnson et al [125], 2010	ABR >25dB	256	<2500g	10/256	3.9%
Robertson et al [12], 2009	ABR >25dB	1279	<28 weeks <1250g	40/1279	3.12%
Hille et al [28], 2007	AABR	2186 infants	<30 weeks and/or <1000g	71/2186	3.2%
Gopel et al [124], 2014	OAE/BAER	7056 NICU infants	<37 weeks <1500g	788/7056	11%

Bielecki et al [129], 2011	TEOAE >30dB	5282 infants		280/5282	5.3%
Dowley et al [15], 2009	>60dB	45050 infants		30/45050	0.07%
Van Dommelen et al [22], 2015	ABR >35dB	18564 NICU infants	<32 weeks	403/18546	2.17%

Appendix 2: Prevalence of hearing loss following neonatal care in selected groups of infants (publications 2004-2016)

Study	Definition of hearing loss	Population size	Gestational age/weight	Prevalence	
Amin et al [68], 2016	Absent or abnormal ABR, normal OAE	44 with total serum bilirubin >20mg/dL	>34 weeks	5/44	11.36%
Martines et al [148], 2012	ABR >40dB	412 at risk infants		47/412	11.41%
Martinez-Cruz et al [49], 2008	BAEP >40dB	418		146/272	53.68%
Morini et al [48], 2008	SNHL >20dB	82 NICU infants with congenital diaphragmatic hernia		40/82	48.78%
Patra et al [52], 2006	Unknown	362 NICU infants 104 with grade I-II IVH	<1000g	15/362 9/104	4.14% 8.65%
Robertson et al [71], 2006	ABR >25 dB	81 NICU infants following severe respiratory failure	>34 weeks	43/81	53.09%
Yoshikawa et al [149], 2004	Referred following AABR and ABR	226, 102 NICU graduates, 124 healthy newborn controls		9/226	3.98%

Appendix 3: Prevalence of m.1555A>G in neonatal populations (publications from 2010-2014)

Study	Population size	Prevalence of m.1555A>G		Hearing loss and m.1555A>G	Aminoglycoside exposure and m.1555A>G
Ealy et al [123], 2011	703 NICU infants	2/703	0.28%	0/2	2/2
Gopel et al [124], 2014	7056 preterm infants <37 weeks gestation <1500g	12/7056	0.17%	3/12	10/12 3/3 with HL
Johnson et al [125], 2010	436 infants with birthweight <2500g	3/436	0.69%	1/3	3/3
Nivoloni et al [139], 2010	8974 newborn infants	0/8974	0	0	0
Wang et al [138], 2011	14913 newborn infants	18/14913	0.12%	0/18	Not measured

Appendix 4: Consultant letter (audiology/neonatal)

Consultant Letter Version 1; 2 November 2011

Address

Research Study into Hearing Loss and Prematurity

Patient name
Date of birth

Dear Parent

I am writing to introduce a research study to you and to ask if you would consider assisting the research team in working out the relationship between deafness and the use of antibiotics after birth on the neonatal unit in babies who were born prematurely. This is an important study because it will help us to decide which antibiotics to use for newborn babies.

I enclose a letter and leaflet introducing the study from Professor Neil Marlow who is a newborn specialist working at University College Hospital and is leading the research team. Please read his letter and the information leaflet they designed, to help you decide whether you want to join this study.

Yours sincerely

Consultant Audiological Physician / Consultant Neonatologist

Appendix 5: Parent reply sheet

Reply Version 2; 9 December 2014

Professor Neil Marlow,
Professor of Neonatal Medicine,
Institute for Women's Health,
74 Huntley Street,
London WC1E 6AU

Are you happy for us to contact you? Yes ☐ No ☐

If we do not receive a reply, we will contact you to check you have received the study information.

Your name …….....

Your child's name …….....

Your child's date of birth …….....

NICU where your child was treated ..…….....

Your GP's name and address …….....

Your contact details

Phone number …….....

Mobile number …….....

Email address …….....

How may we contact you (please circle phone number/mobile number/email as appropriate)?

Alternatively, if you wish to get in touch directly with the study Research Nurse, the contact details are:

Kathy Chant
Institute for Women's Health,
74 Huntley Street, London WC1E 6AU

Tel: 020 7679 6031 or 07580 219408
Email: k.chant@ucl.ac.uk

Appendix 6: Parent consent form

Consent Form Version 4; 30 August 2013

MITOGENT PARENTAL CONSENT FORM

Project ID:
REC Number: 12/LO/0005

Name of Principal Investigator: Dr M Bitner-Glindzicz

Please tick

Yes or No

1.	I confirm that I have read and understood the information sheet dated 20 May 2013 for the above study and have had the opportunity to ask questions and these questions have been answered satisfactorily.	**Yes** ☐	**No** ☐
2.	I understand that my child's participation is voluntary and that we are free to withdraw at any time, without giving any reason, without affecting my child's medical care or legal rights.	**Yes** ☐	**No** ☐
3.	I understand that sections of any of my child's medical notes may be looked at by responsible individuals from UCLH, UCL or from regulatory authorities where it is relevant to our taking part in research. I give permission for these individuals to have access to my child's records.	**Yes** ☐	**No** ☐
4.	I agree that the research team can contact my child's General Practitioner to inform him/her if my child is found to have the MitoGent variant.	**Yes** ☐	**No** ☐
5.	I understand that all provided tissue samples (eg. saliva and cheek swabs) will be considered a 'gift' to UCL.	**Yes** ☐	**No** ☐
6.	If my child is found to have m.1555A>G during the research I would like to be told this information.	**Yes** ☐	**No** ☐
7.	I agree for my child to take part in the above study.	**Yes** ☐	**No** ☐

_____
Name of Child (print)

_____ _____ _____

Name of Parent (print) Date Signature

_____ _____ _____

Name of Person taking consent Date Signature

1 form for Parent; 1 to be kept as part of the study documentation, 1 to be kept with hospital notes

Appendix 7: Audiology data collection proforma

Patient Demographics	
Child's NHS number	
Sex	
Date of birth	
Referring Hospital	
Date of completion	

Audiometric data ***(Please circle)***		
Sensorineural Hearing loss (SNHL)[1] detected?	YES	NO
Unilateral/Bilateral hearing loss	UNILATERAL	BILATERAL

Newborn Hearing Screen		
Passed	YES	NO

Severity of Hearing Loss*			
Mild	Moderate	Severe	Profound

Hearing Aids	YES	NO
Cochlear Implant	YES	NO
Intellectual disability	YES	NO
Additional disability	YES	NO

Did the test results fit with Auditory Neuropathy Spectrum Disorder (ANSD)[5]?			
RIGHT	Yes	No	Not recorded
LEFT	Yes	No	Not recorded

Additional information: ***(please fill in)***		
Any known causes of SNHL?		
Aetiological tests completed	CMV status	Y/N
	GJB2 (Connexin26)	Y/N
	m.1555A>G before this study	Y/N
	MRI/CT of inner ear	Y/N
	Ophthalmology review	Y/N

Notes

*Mild hearing loss	21 - 40 dB
Moderate hearing loss	41 - 70 dB
Severe hearing loss	71 - 95 dB
Profound hearing loss	> 95dB

Appendix 8: Neonatal data collection proforma

Mitogent study ID –

Date of birth –

Variable	Value	Code
Sex		0=Male 1=Female
Gestational age		
Birth weight		
Apgar @ 5 mins		
CRIB – II		Admission temp Base excess

Diagnoses

Variable	Value	Code
IVH/PVL		0=None 1=IVH I-II 2=IVH with vent distension 3=Intraparenchymal lesion 4=Perivent leukomalacia
Pneumathorax		0=No 1=Yes
Pulmonary Haemorrhage		0=No 1=Yes
PDA		0=No 1=Yes-no treatment 2=Yes-medical treatment 3=Yes-surgical
NEC		0=No 1=Yes-medical treatment 2=Yes-surgical drain 3=Yes-laparotomy
CLD/BPD		0=No 1=02 28d off 36w 2=02 36w <30% 02 LF 3=02 36w >30% HF/CPAP/vent
Septicaemia (+ve BC) (bug)		
Meningitus (+ve CSF) (bug)		

Variable	Value	Code
Bilirubin (highest)		
Creatinine (highest)		
Days of ETT		
Days of CPAP		
Days of 02		
Days of Long Line (PIC)		
Days Level 1		
Days Level 2		
Days Level 3		

Mitogent study ID –

Date of birth –

Amikacin										
Netilmicin										
Gentamicin										
Vancomycin										
Levels NGV										
Furosemide										
Indomethacin										
Ibuprofen										
Inotropes										
Dexamethasone										
Hydrocortisone										
Methylprednisolone										
TSB ↑										
Exchange trans										
Blood transfusion										
Creatinine ↑										
Lactate ↑										
Lowest pH										
Vent – CV										
HFO										
Nitric										
CPAP										
02										
TPN										

Inotropes – dopamine, dobutamine, adrenaline, noradrenaline

Appendix 9: Subsidiary sites

Trust	Hospital	Local principal investigator
Ashford and St Peter's Hospitals NHS Foundation Trust	St Peter's Hospital	Dr Peter Reynolds
Barking, Havering and Redbridge University Hospitals NHS Trust	Queens Hospital King Georges Hospital	Dr Wilson Lopez Dr Wilson Lopez
Barnet and Chase Farm Hospitals NHS Trust	Barnet Hospital Chase Farm Hospital	Dr Tim Wickham Dr Tim Wickham
Barts Health NHS Trust	Whipps Cross University Hospital Royal London Hospital Newham University Hospital	Dr Nic Wilson Dr Divyen Shah Dr Vimala Gopinathan
Brighton and Sussex University Hospitals NHS Trust	Royal Sussex County Hospital Princess Royal Hospital	Dr Cathy Garland Dr Cathy Garland
Chelsea and Westminster NHS Foundation Trust	Chelsea and Westminster Hospital	Dr Sabita Uthaya
Colchester Hospital University NHS Foundation Trust	Colchester General Hospital	Dr Sarah Dalton
Croyden Health Services NHS Trust	Croyden University Hospital	Dr Arun Kumar
East Kent Hospitals University NHS Foundation Trust	William Harvey Hospital Queen Elizabeth, The Queen Mother Hospital	Dr Vimal Vasu Dr Vimal Vasu
Guy's and St Thomas' NHS Foundation Trust	St Thomas' Hospital	Dr Grenville Fox
Herts Community NHS Trust	Community trust	Dr Alpana Kulkarni
Homerton University Hospital NHS Foundation Trust	Homerton Hospital	Dr Narendra Aladangady

Imperial College Healthcare NHS Trust	Queen Charlotte's and Chelsea Hospital	Dr Aniko Deierl
	St Mary's Hospital	Dr Aniko Deierl
Kent Community Health NHS Trust	Community Trust	Dr Raj Nandi
Kings College Hospital NHS Foundation Trust	King's College Hospital	Dr Simon Hannam
Kingston Hospital NHS Trust	Kingston Hospital	Dr Jon Filkin
Maidstone and Tunbridge Wells NHS Trust	Maidstone General Hospital	Dr Hamudi Kisat
	Tunbridge Wells Hospital	Dr Hamudi Kisat
Medway NHS Foundation Trust	Medway Maritime Hospital	Dr Aung Soe
North East London Foundation Trust	Community Trust	Dr Iynga Vanniasegaram
Portsmouth Hospitals NHS Trust	Queen Alexandra Hospital	Dr Victor Osei-Lah Dr Tim Scorrer
St Georges Healthcare NHS Trust	St Georges Hospital	Dr Justin Richards
The Hillingdon Hospitals NHS Foundation Trust	Hillingdon Hospital	Dr Michelle Cruwys
The North West London Hospitals NHS Trust	Northwick Park Hospital	Dr Richard Nicholl
The Whittington Hospital NHS Trust	Whittington Hospital	Dr Nischal Rao
West Middlesex University Hospital NHS Trust	West Middlesex Hospital	Dr Didi Ratnasinghe

Appendix 10: Distribution of positive blood cultures between hearing impaired and normal hearing groups

	Hearing loss	Normal hearing (comparison group)	Total
Low pathogenicity commensals	26	50	76
High pathogenicity gram positives	8	11	19
Low pathogenicity gram negatives	2	2	4
High pathogenicity gram negatives	4	11	15
Viral	2	3	5
Fungal	2	1	3
Total	44	78	

Appendix 11: Protocol Publication BMC

Bitner-Glindzicz et al. BMC Pediatrics 2014, 14:66
http://www.biomedcentral.com/1471-2431/14/66

BMC Pediatrics

STUDY PROTOCOL Open Access

Gentamicin, genetic variation and deafness in preterm children

Maria Bitner-Glindzicz[1*], Shamima Rahman[1], Kathy Chant[2] and Neil Marlow[2]

Abstract

Background: Hearing loss in children born before 32 weeks of gestation is more prevalent than in full term infants. Aminoglycoside antibiotics are routinely used to treat bacterial infections in babies on neonatal intensive care units. However, this type of medication can have harmful effects on the auditory system. In order to avoid this blood levels should be maintained in the therapeutic range. However in individuals with a mitochondrial genetic variant (m.1555A > G), permanent hearing loss can occur even when drug levels are within normal limits. The aim of the study is to investigate the burden that the m.1555A > G mutation represents to deafness in very preterm infants.

Method: This is a case control study of children born at less than 32 completed weeks of gestation with confirmed hearing loss. Children in the control group will be matched for sex, gestational age and neonatal intensive care unit on which they were treated, and will have normal hearing. Saliva samples will be taken from children in both groups; DNA will be extracted and tested for the mutation. Retrospective pharmacological data and clinical history will be abstracted from the medical notes. Risk associated with gentamicin, m.1555A > G and other co-morbid risk factors will be evaluated using conditional logistic regression.

Discussion: If there is an increased burden of hearing loss with m.1555A > G and aminoglycoside use, consideration will be given to genetic testing during pregnancy, postnatal testing prior to drug administration, or the use of an alternative first line antibiotic. Detailed perinatal data collection will also allow greater definition of the causal pathway of acquired hearing loss in very preterm children.

Keywords: Gentamicin, Aminoglycosides, Deafness, Hearing loss, Mitochondrial, m.1555A > G, Preterm, Prematurity

Background

Advances in neonatal intensive care have resulted in substantial improvements in outcome, in particular the survival of very preterm infants [1]. However, among the surviving population there is a high level of disability [2,3]. The prevalence of deafness is around 1-2% in preterm or low birth weight babies, and is often co-morbid with other disabilities such as cerebral palsy, and intellectual and visual impairment [4]. Hearing loss in this group of infants is up to ten times more prevalent than in term babies [4]. Deafness has long term implications for communication skills, educational achievement, and overall quality of life, even in mild cases of unilateral and bilateral hearing loss [5]. The cause of acquired deafness in this group is likely to be multifactorial although several high risk factors have been suggested. These include very low birth weight, severe jaundice [6], hypoxia, medication [7] and infection [8].

Infection is a common occurrence during the neonatal period due to the underdeveloped immune system of the preterm infant, and can rapidly become life threatening. Aminoglycoside antibiotics are frequently used as a first line of treatment for suspected or proven bacterial sepsis. High efficacy, low levels of resistance and low cost make this group of medications an effective and recommended option [9]. Aminoglycoside exposure is known to have nephrotoxic and ototoxic side effects, hence regular monitoring of blood concentrations. However reassurance has been taken from the repeated observations that aminoglycoside toxicity does not appear to be a frequent occurrence in this population.

A genetic predisposition renders some individuals more susceptible to rapidly progressive, permanent hearing loss

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even when aminoglycoside levels are maintained within the therapeutic range. The most common predisposing mutation is the mitochondrial m.1555A > G variant, a maternally inherited genetic trait. Although the exact process of aminoglycoside induced deafness is unknown it is thought that the m.1555A > G mutation enables aminoglycosides to bind to the mitochondrial ribosomes more readily than the normal genetic sequence. This is thought to result in abnormal translation of mitochondrial proteins necessary for energy production. Hair cells in the inner ear are metabolically highly active and consequently have large numbers of mitochondria. In addition, aminoglycosides easily enter the hair cells but take a long time to be cleared [10].

In susceptible individuals, a single dose of aminoglycoside may cause hearing loss. Early indications were that penetrance of deafness following aminoglycoside exposure was close to 100% in the presence of m.1555A > G. For example, in a study of European families with a history of maternally transmitted deafness and presence of the m.1555A > G mutation, all individuals who received aminoglycosides became deaf [11]. Furthermore, there was a lower mean age of onset of deafness in the group exposed to these antibiotics implicating aminoglycosides as a catalyst for rapidly progressive hearing loss. However, it is possible that such research is biased. Individuals that are given aminoglycosides with no ill effect are unlikely to be genetically screened for the mutation. More recently in identical twins born at 24 weeks gestation both with m.1555A > G and treated with aminoglycosides, only one of the twins has hearing loss. The deaf twin received multiple courses of aminoglycosides whilst the twin with normal hearing received a single course (unpublished data). The hearing twin showed no clinical indication for genetic screening, but was tested with the other twin. It is possible that single courses may be tolerated in some cases.

The Avon Longitudinal Study of Parents and Children cohort (ALSPAC) showed the prevalence of the mutation to be 1 in 520 children [12]. Audiological screening was normal at the age of 9 years, there were no histories of admissions to neonatal units and exposure to aminoglycosides was thought to be unlikely. Thus, there were no clinically distinguishing features between those with the mutation and those without, and therefore no reason under normal circumstances for genetic testing. Nevertheless, it is also possible that there had been exposure to ototoxic medication but hearing had been preserved, which would indicate that the penetrance of aminoglycoside-mediated ototoxicity in m.1555A > G is less than previous research has suggested. These findings demonstrate the need for further research in this domain.

The primary aim of this study is to evaluate the contribution of the m.1555A > G mutation to acquired deafness in babies born at a gestational age of less than 32 weeks, who are likely to have received multiple doses of aminoglycoside antibiotics. Further to this, the study aims to calculate the relative odds of carrying the genetic variant and being in the deaf group versus the hearing group. Risk associated with gentamicin, m.1555A > G and other co-morbid risk factors will be evaluated using conditional logistic regression to determine potential gene-drug interactions in the context of other ototoxic risks.

Method/Design

Study design

Case control study, with 5 controls for each index case.

Definition of index cases

Any child born at 31 weeks and 6 days gestation or less with confirmed hearing loss (>40dBHL). Children with clear genetic syndromic causes for hearing loss will be excluded.

Index population

This will include all children with hearing loss who were born at less than 31 weeks and 6 days gestation between January 2009 and December 2013. All children will have been treated in a Neonatal Intensive Care Unit (NICU) within the Greater London region. Participants will be identified through electronic databases, hearing assessment centres, neonatal follow up services and advertisements promoting the study.

Control population

Each child recruited with hearing loss will be matched with five children with normal hearing. Control children will be identified through the Standardised Electronic Neonatal Database (SEND) which contains demographic and clinical information about infants admitted to neonatal units across London. Matching will be based upon gestational age (same number of completed weeks of gestation), sex (male/female) and neonatal unit on which they received their neonatal intensive care over the first two weeks after birth. Control children with partially complete or missing pharmacological records will be excluded on the premise that other matched control children will be identifiable with more comprehensive records.

Procedure

Index children will be invited to participate in the study via a letter sent by the audiological paediatrician responsible for their care. This will enclose information about the study and parents who require further information or would like to participate will be asked to return a short form to indicate their willingness to speak to the research team. The research nurse will telephone the parent(s), explain the study further and arrange a convenient time to

meet parents if they wish to participate. Parents who identify themselves to the research team via newsletters or advertisements will be spoken to directly by the research team. Written informed consent will be obtained for genetic testing of a saliva sample, and to extract data from neonatal and audiological clinical notes. During a subsequent home visit saliva samples will be taken if testing for m.1555A > G has not been undertaken as part of clinical care. Saliva samples will be collected using an Oragene collection kit and transported to the laboratory for testing. DNA is extracted using standard procedures and analysed by polymerase chain reaction and restriction enzyme digestion, followed by sequence confirmation by bi-directional Sanger sequencing in mutation-positive cases.

Matched controls will be identified though the national neonatal research dataset. Parents of control children identified will be invited to participate by letter sent by the clinician responsible for their neonatal care. Those wishing to participate in the study will contact the research team as above.

Children identified to have the mutation will be recalled and the presence of m.1555A > G reconfirmed by analysis of DNA extracted from a second sample. Families confirmed to have the mutation will be offered genetic counselling. Since mitochondrial DNA is maternally inherited, there are additional implications for extended maternal family members, who will also be offered genetic counselling.

Clinical data will be extracted from the neonatal and audiological medical notes for all children using an agreed proforma to record details of demographics, clinical events and therapeutic history, with serum levels of drugs as available. Data will be collected by day after birth to identify coincidence of risk factors. Pharmacy records will be examined to confirm aminoglycoside exposure, including dose, duration, number of courses and blood level concentrations. Audiological records will be reviewed to define the severity and pattern of hearing loss.

Data will be encoded for data analysis using double entry into an automated error detection system in SPSS (IBM Inc) in its latest version. Subsequently data will be checked for outliers, prior to analysis using Stata (Version 13).

Sample size

The annual birth-rate in London is approximately 120 000, (600 000 in 5 years). It is estimated that 1% of these babies have a birth weight of less than 1501 g [13]. This gives a likely population of babies at less than 32 weeks gestational age of 3 000–6 000 over 5 years. The prevalence of hearing loss within this group is 1-2% [4], predicting 30–120 children with hearing loss over the 5 year study period. From a cohort of 3 000–6 000 children, we anticipate that 6–12 children will have the m.1555A > G mutation, given a mutation frequency of 1 in 526 (95% CI 1 in 357 to 1 in 770) [14]. For each index child, we will recruit up to 5 matched controls.

Thus the total study population should include between 180–720 children, 30–120 index and 150–600 controls.

Statistical analysis

The burden of disease will be calculated as the proportion (and 95% CI) of children with hearing loss that have the mutation, using the method of Wilson. The odds ratio of children with the m.1555A > G mutation having hearing loss will be calculated using Fisher's exact test. The analysis for the burden of disease and the relative risk of deafness for those with the mutation will initially include all cases. This will be repeated excluding children who received less than one dose of aminoglycoside medication, and repeated again excluding those who received fewer than two doses.

The antecedents and associates of hearing loss will be determined using conditional logistic regression to account for matching. Factors will be evaluated individually and as combinations occurring simultaneously to account for additive risk.

Ethical issues

The study has been approved by the National Research Ethics Service (NRES Committee London – Central, ref: 12/LO/0005) and is registered under the National Institute for Health Research (NIHR) Portfolio scheme. The study is funded by a financial grant from Action on Hearing Loss; the funding source has had no input into the design or execution of the research study.

Discussion

We aim to investigate the contribution of m.1555A > G to deafness in preterm infants where aminoglycosides are likely to have been used and to evaluate this role within the occurrence of multiple risk factors for hearing loss identified during neonatal intensive care. We hypothesise that the mutation will make a significant contribution to deafness even when aminoglycoside levels are maintained within the therapeutic range. From our clinical experience, we suspect that in some cases, single courses of aminoglycosides may be tolerated but multiple courses may not be, possibly due to the accumulation of the medication in the hair cells of the inner ear, which can take several months to clear. Multiple courses of aminoglycosides during the neonatal period would thus inhibit the clearance process.

Alongside such a potential interaction, to date multiple studies have failed to provide consistent evidence for specific risk factors. Intriguingly one small study has reported that it is the coincidence of risk factors that may determine the development of hearing loss [4]. A range

of factors, such as other ototoxic drugs, hyperbilirubinaemia, acidosis and sepsis, will be evaluated as part of this study and these risks assessed for co-occurrence. The conditional case control analysis will maximise the potential sensitivity of this large study to determine the presence of such associations.

Should m.1555A > G be implicated in the aetiology of neonatal hearing loss in very preterm babies, then alternative strategies may be necessary to manage preterm neonatal sepsis in affected babies.

Alternative first line antibiotics could be used instead of aminoglycosides. Using more broad spectrum agents runs the risk of increasing patterns of bacterial resistance in the relatively closed microbiological setting of neonatal intensive care. Further, owing to low cost and the synergistic effects of aminoglycosides, their use is preferred by many services for babies at risk of life threatening infection, considering that the risk of aminoglycoside associated hearing loss is as yet unsubstantiated in newborn babies.

A further alternative would be universal genetic screening (of pregnant women) or focussed genetic screening of babies prior to treatment, which is at present impracticable [15]. Due to the nature of illness experienced by many babies being treated on the neonatal intensive care unit, aminoglycoside administration may be required before the results of genetic screening are available. Screening women during pregnancy would overcome this since the mutation is maternally inherited but this is expensive and would require clear evidence that this interaction is a key step in the causal pathway. This would, however, enable early recognition of the potential for hearing impairment and lead to the use of an alternative medication for these infants.

Mitogent is designed to provide critical answers concerning the aetiology of acquired hearing impairment in very preterm babies and specifically to identify the role of aminoglycoside toxicity in individuals with m.1555A > G.

Abbreviations

ALSPAC: Avon longitudinal study of parents and children cohort; dBHL: Decibels hearing level; NICU: Neonatal intensive care unit; SEND: Standardised electronic neonatal database; CI: Confidence interval; NIHR: National institute for health research.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KC wrote the first draft and produced the final version. NM, SR and MB-G conceived the idea and obtained funding. Each has reviewed this paper and approved the final version.

Authors' information

KC is the research fellow conducting the study; NM is a professor of neonatal medicine and has a major interest in perinatal influences on long term outcomes; SR is a Reader and Consultant in metabolic and mitochondrial medicine; MB-G is a professor of clinical and molecular genetics specialising in genetic hearing loss.

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