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Research paper

Effect of antenatal magnesium sulphate on MRI biomarkers of white matter development at term equivalent age: The magnum study

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ABSTRACT

Background: Magnesium sulphate given to women immediately prior to very preterm birth protects the perinatal brain, so fewer babies die or develop cerebral palsy. How magnesium sulphate exerts these beneficial effects remains uncertain. The aim of the MagNUM Study was to assess the effect of exposure to antenatal magnesium sulphate on MRI measures of brain white matter microstructure at term equivalent age.

Methods: Nested cohort study within the randomised Magnesium sulphate at 30 to <34 weeks' Gestational age Neuroprotection Trial (MAGENTA). Mothers at risk of preterm birth at 30 to <34 weeks' gestation were randomised to receive either 4 g of magnesium sulphate heptahydrate [8 mmol magnesium ions], or saline placebo, infused over 30 min when preterm birth was planned or expected within 24 h. Participating babies underwent diffusion tensor MRI at term equivalent age. The main outcomes were fractional anisotropy across the white matter tract skeleton compared using Tract-based Spatial Statistics (TBSS), with adjustment for postmenstrual age at birth and at MRI, and MRI site. Researchers and families were blind to treatment group allocation during data collection and analyses.

Findings: Of the 109 participating babies the demographics of the 60 babies exposed to magnesium sulphate were similar to the 49 babies exposed to placebo. In babies whose mothers were allocated to magnesium sulphate, fractional anisotropy was higher within the corticospinal tracts and corona radiata, the superior and inferior longitudinal fasciculi, and the inferior fronto-occipital fasciculi compared to babies whose mothers were allocated placebo ($P < 0.05$).

Interpretation: In babies born preterm, antenatal magnesium sulphate exposure promotes development of white matter microstructure in pathways affecting both motor and cognitive function. This may be one mechanism for the neuroprotective effect of magnesium sulphate treatment prior to preterm birth.

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1. Introduction

Babies born preterm compared with those born at term have a higher chance of dying in the first few weeks of life. Those who survive have a greater risk of neurologic impairments, including cerebral palsy, cognitive dysfunction, educational difficulties and psychiatric disorders in adulthood, with increased educational and societal costs [1–3].

Antenatal magnesium sulphate is recommended for neuroprotection of the fetus in women at risk of very preterm birth [4], having been shown in individual participant data meta-analysis of relevant randomised trials to reduce the risk of death and cerebral palsy [5]. How magnesium exerts this protective effect, and whether magnesium protects parts of the brain that are important for learning and behaviour as well as those that control movement and posture, remains uncertain.

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Panel: Research in context

Evidence before this study

Antenatal magnesium sulphate is recommended in clinical practice guidelines worldwide for women at risk of very preterm birth for neuroprotection of their fetus. Our recent individual participant data meta-analysis, that included five, relevant, randomised trials with a total of 5493 women and 6131 babies, found antenatal magnesium sulphate given prior to preterm birth reduces the risk of cerebral palsy and death or cerebral palsy. What has remained uncertain is how magnesium exerts these neuroprotective effects, and whether magnesium protects parts of the brain that are important for learning and behavior as well as those that control movement and posture.

Added value of this study

MagNUM is a prospective, multicentre, cohort study, recruiting participants from five, tertiary, maternity hospitals in New Zealand and Australia, designed to compare brain white matter microstructure at term equivalent age between babies whose mothers were randomised to receive either antenatal magnesium sulphate or placebo.

We wished to assess whether magnesium sulphate would have neuroprotective effects on the white matter tracts that subserve motor and cognitive function. To our knowledge this is the first comparative study to explore neuroprotective mechanisms for antenatal magnesium sulphate using diffusion tensor magnetic resonance imaging.

Our results show that babies exposed to magnesium sulphate compared to babies not exposed had higher fractional anisotropy (FA) and lower radial diffusivity (RD) in key white matter tracts at term equivalent age consistent with greater fibre coherence and maturation, including improved myelination. Within the corticospinal tracts, reduced FA at term equivalent age in babies born preterm predicts motor delay and cerebral palsy. We also found higher FA and lower RD within association fibres that subserve cognitive processes.

Implications of all the available evidence

Our findings show that antenatal magnesium sulphate prior to preterm birth promotes white matter development in pathways important for motor and cognitive function. These data provide previously lacking evidence as to how magnesium sulphate may exert its clinical neuroprotective benefits, including reducing the risk of cerebral palsy.

Dysmaturation of developing white matter is an important neuropathological substrate of adverse neurological outcome after preterm birth [6]. It arises from upstream insults of hypoxia, ischaemia, and inflammation, which lead to primary injury or death of key cellular elements followed by secondary dysmaturation. The cells that are most susceptible are pre-myelinating oligodendrocytes, but axons within white matter, and subplate, thalamic and late migrating GABAergic neuronal populations are also affected. The end result of injurious and maldevelopmental processes affecting this range of cell types is a constellation of features, collectively termed the 'encephalopathy of prematurity', that are apparent on neonatal MRI as alterations in white and grey matter microstructure, impaired cortical folding and disturbances to regional brain growth [6].

Diffusion tensor magnetic resonance imaging (DTI) has provided valuable insights into the effects of maturational and injurious processes in the developing brain [7]. This is rooted in the premise that water movement is restricted by the presence of axons, neuronal cell bodies, glial cells and macromolecules, and this allows inference about brain water content, axonal density, axonal calibre, myelination, dendritic arborisation and synapse formation. Commonly used DTI parameters are fractional anisotropy (FA), which describes the directional dependence of random water motion, mean diffusivity (MD) a measure of the magnitude of water motion, axial diffusivity (AD), the largest eigenvalue of the diffusion tensor in each voxel, potentially indicative of water diffusion parallel to axons, and radial diffusivity (RD), the average of the two remaining eigenvalues, potentially indicative of water diffusion perpendicular to axons. A consistent finding is that FA increases and MD decreases with increasing maturation of the preterm brain [8], reflecting decreasing water content and increasing complexity of white matter. Lower FA and higher MD are seen in the white matter of preterm infants at term equivalent age compared with healthy infants born at term [9–11].

Tract-Based Spatial Statistics (TBSS) enables unbiased group-wise analysis of FA within the white matter derived from DTI data [9,12]. TBBS has been used to map microstructural alterations in neonatal white matter tracts associated with preterm birth [13], intrauterine inflammation [14], maternal opioid use [15], and early life nutrition [16], and has proven useful for investigating neuroprotective treatments in newborns including erythropoietin for preterm brain injury, and therapeutic hypothermia and inhaled xenon for hypoxic-ischaemic encephalopathy [17,18].

In this paper, we report TBSS results from the Magnesium for Neuroprotection: Understanding Mechanisms (MagNUM) Study which employed DTI to compare brain white matter microstructure at term equivalent age between babies whose mothers were randomised to receive antenatal magnesium sulphate and those randomised to receive placebo. Our hypothesis was that magnesium sulphate would have neuroprotective effects in the white matter tracts that subserve motor and cognitive function. Since brain white matter microstructure is also influenced by gestational age, sex, multiple pregnancy, breast milk intake and serious neonatal illness such as bronchopulmonary dysplasia and necrotising enterocolitis, we also explored the effect of these factors on our findings using subgroup and sensitivity analyses.

2. Methods

2.1. Study design and participants

The MagNUM Study was nested within the multicentre Magnesium Sulphate at 30 to <34 weeks' Gestational age Neuroprotection Trial (MAGENTA) comparing magnesium sulphate (magnesium sulphate heptahydrate: 8 mmol magnesium ions) with placebo (saline) in women at risk of imminent (within 24 h) preterm birth at 30 to <34 weeks' gestation for the prevention of death or cerebral palsy, the primary outcome [19] (Australian New Zealand Clinical Trials Registry ACTRN12611000491965). The central randomisation service stratified by collaborating centre, gestational age (30 to <32 weeks; 32 to <34 weeks' gestation) and number of fetuses (1 or 2).

Babies born to mothers enrolled in the MAGENTA Trial at Auckland City Hospital, Middlemore Hospital, and Christchurch Women's Hospital, New Zealand, and Women's and Children's Hospital and Flinders Medical Centre, Adelaide, Australia, were eligible for enrolment in the MagNUM Study. Exclusion criteria were known congenital or genetic disorders likely to affect brain structure, baby too unwell to have an MRI scan safely, or the family lived more than a one-hour drive from the MRI centre. Written informed consent was obtained from the caregiver of eligible babies. The MagNUM Study was approved by the New Zealand Northern B Health and Disability Ethics Committees LRS/12/06/021/AM02 and by the South Australian Human Research Ethics Committee HREC/16/WCHN/196.

2.2. Procedures

MRI was conducted at term equivalent age (38 to 42 weeks' post menstrual age) on a 3 Tesla Siemens *Skyra* system at the Auckland Centre for Advanced MRI and the South Australia Medical Research Institute in Adelaide, and a 3 Tesla General Electric *HDxt* system at Pacific Radiology in Christchurch, using a 32 channel adult head coil. Babies were scanned in a neonate MRI beanbag evacuated for stabilization during natural sleep following a feed and swaddling.

To ensure the validity and robustness of inter-site DTI comparisons, the MRI protocol was standardised to acquire whole brain diffusion-weighted and high resolution T1 - and T2 - weighted anatomical MRI data with the same number of baseline and diffusion encoding gradient directions, b-values, slice locations and voxel dimensions (Table 1). A detailed written protocol was provided to the radiographers at all sites.

2.3. Outcomes

The primary outcome was regional group differences in FA throughout the cerebral white matter skeleton measured using TBSS. Secondary outcomes were group differences in MD (average diffusion along the three main axes of the diffusion tensor), axial diffusivity (AD, parallel to the white matter tract) and radial diffusivity (RD, perpendicular to the main axis of the diffusion tensor). Based on computational modelling [20] and precedent from the TOBY-Xenon neonatal neuroprotective hypothermia randomised trial [18] a study of 60 infants in each treatment group was estimated to be able to detect a 10% difference in FA with 80% power and two-sided 5% significance.

2.4. Statistical analysis

Analyses followed a statistical analysis plan prepared prior to any data analyses. Pre-specified reasons for participants to be excluded from TBSS analyses were if there was significant brain injury defined as parenchymal injury or brain abnormality identified on structural MRI by clinical reviewers and considered likely to confound image registration; if there was no diffusion MRI, scanner error, or excessive motion; if more than 10 diffusion weighted MRI volumes had slice dropout, if registration failed. Application of these exclusions criteria was done blind to treatment group allocation to reduce the risk of selection bias in deriving the MagNUM Study per-protocol population. Researchers were blind to treatment group allocation until analyses were completed.

DTI data were analysed using the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain Software Library, version 5.0.10. (https://fsl.fmrib.ox.ac.uk/fsl) [21] and DTI-ToolKit (v2.3.1 www.dti-tk.sourceforge.net) (DTI-TK) [22]. Data were corrected for phase encoding distortions, eddy-induced distortions and motion using the topup-eddy algorithm [21], using T2 structural volumes rigidly registered to b0 maps and assuming a bandwidth of zero (no phase-encoding) [23]. A diffusion tensor model was fitted to each voxel and the eigenvalues and eigenvectors were used to convert the corrected diffusion weighted images into diffusion tensor volumes (DTI-TK).

Tensor-based image registration (DTI-TK) was used to produce a population specific diffusion tensor template. From this template the mean FA volume was derived and thinned by perpendicular non-maximum suppression to create the mean white matter tract skeleton, thresholded at FA > 0.15 to exclude peripheral tracts [9]. All participants' diffusion tensor volumes were registered to the diffusion tensor template and FA, MD, AD and RD were extracted and projected onto the white matter tract skeleton.

TBSS [12] was used to compare voxel-wise statistics across the white matter skeleton between treatment groups, using a general linear model adjusting for post menstrual age at birth and at MRI, and scan site. Significance was set as $P < 0.05$, following family-wise error rate correction and threshold-free cluster enhancement.

Pre-specified sensitivity analyses included only babies who were exposed to at least some of the treatment allocated at randomisation to the MAGENTA Trial (magnesium sulphate or saline placebo); scanned at the largest MRI site; singletons; born before 34 weeks' gestation; without bronchopulmonary dysplasia or necrotising enterocolitis; and exclusively received breast milk at the time of scanning. Pre-specified subgroup analyses assessed gestation at trial entry (30 to <32 weeks and 32 to <34 weeks), and boys and girls separately, and were not adjusted for scan site. For the sensitivity and subgroup analyses, where smaller sample sizes were expected to limit power, a pre-specified sequential analysis was undertaken. Magnesium sulphate and placebo groups were initially compared using TBSS analysis. If no significant differences were detected, a region-of-interest analysis was undertaken to determine if the direction of effect was consistent with the primary analysis. Voxels with significant between group differences in the primary analysis were used as the region of interest. Regions of interest were defined separately for FA, MD, AD and RD.

2.5. Role of the funding sources

The funders of the study had no role in study design, data collection, analysis, interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

Of 191 babies eligible for the MagNUM Study, 159 babies were recruited for MRI at term equivalent age and, after exclusions, DTI

Table 1
Summary table of DTI parameters used in the scanners at the study sites.

	Siemens Skyra (Auckland and Adelaide)	GE HDxT (Christchurch)
T2 weighted MRI	Sampling perfection with application optimized contrasts using a different flip angle evolution (SPACE)	GE SPACE ("CUBE")
Repetition time	3200 ms	2500 ms
Echo time	405 ms	100 ms
Voxels	0•9 × 0•9 × 1 mm	1 × 1 × 1 mm
Field of view	180 mm	180 mm
Diffusion weighted MRI		
Repetition time	7300 ms	7300 ms
Echo time	106 ms	97 ms
Voxels	2 mm^3	2 mm^3
Field of view	256 mm	256 mm
Diffusion weighted directions	64	64
b-value	750	750
b0 images	11	11
Phase-encoding	Right to left	Right to left

data from 109 babies (60 in the magnesium sulphate group andetus 49 in the placebo group) were included (Fig. 1). The per-protocol MagNUM Study population of mothers and babies were similar in the magnesium sulphate and placebo groups in their demographics at entry into the MAGENTA Trial and after birth (table 2).

Babies whose mothers were randomised to antenatal magnesium sulphate compared with babies whose mothers were randomised to placebo had significantly higher FA including in the superior portions of the corticospinal tracts and the inferior fronto-occipital fasciculi bilaterally, and in the right corona radiata, the right superior longitudinal fasciculus, the right precentral and postcentral gyri, the right posterior limb of the internal capsule, and the left inferior longitudinal fasciculus (Fig. 2).

The magnesium sulphate group had reduced RD in the superior corona radiata (magnesium sulphate group mean ± standard deviation; 1.18 ± 0.08, placebo group 1.23 ± 0.09) and the right precentral gyrus. There were no significant differences in MD or AD between the treatment groups in any region and there were no regions where FA was higher or RD lower in the placebo than in the magnesium sulphate group (Fig. 2).

3.1. Sensitivity analyses

When only babies whose mother received at least some of the allocated treatment were included (n = 107; 59 magnesium sulphate, 48 placebo) the findings were similar to the primary analysis (Fig. 3a).

When including only babies who were scanned at the largest MRI site (n = 66; 33 magnesium sulphate, 33 placebo) there were no significant differences between groups on whole skeleton TBSS analysis, but on region-of-interest analysis, the magnesium sulphate group had marginally higher FA (mean ± standard deviation; 0.25± 0.02) than the placebo group (0.23±0.02, P = 0.025) but RD did not differ (magnesium sulphate group $1.21\pm0.08 \times 10^{-3}$ mm^2 s^{-1}, placebo group $1.24\pm0.07 \times 10^{-3}$ mm^2 s^{-1}, (P = 0.07)). When including only babies born before 34 weeks' gestation (n = 106; 60 magnesium sulphate, 46 placebo) the findings were similar to the primary analysis (Fig. 3b).

When only singletons were included (n = 79; 41 magnesium sulphate, 38 placebo), there were no significant differences between groups on whole skeleton TBSS analysis. On region-of-interest analysis, the magnesium sulphate group had slightly higher FA (0.25±0.02)

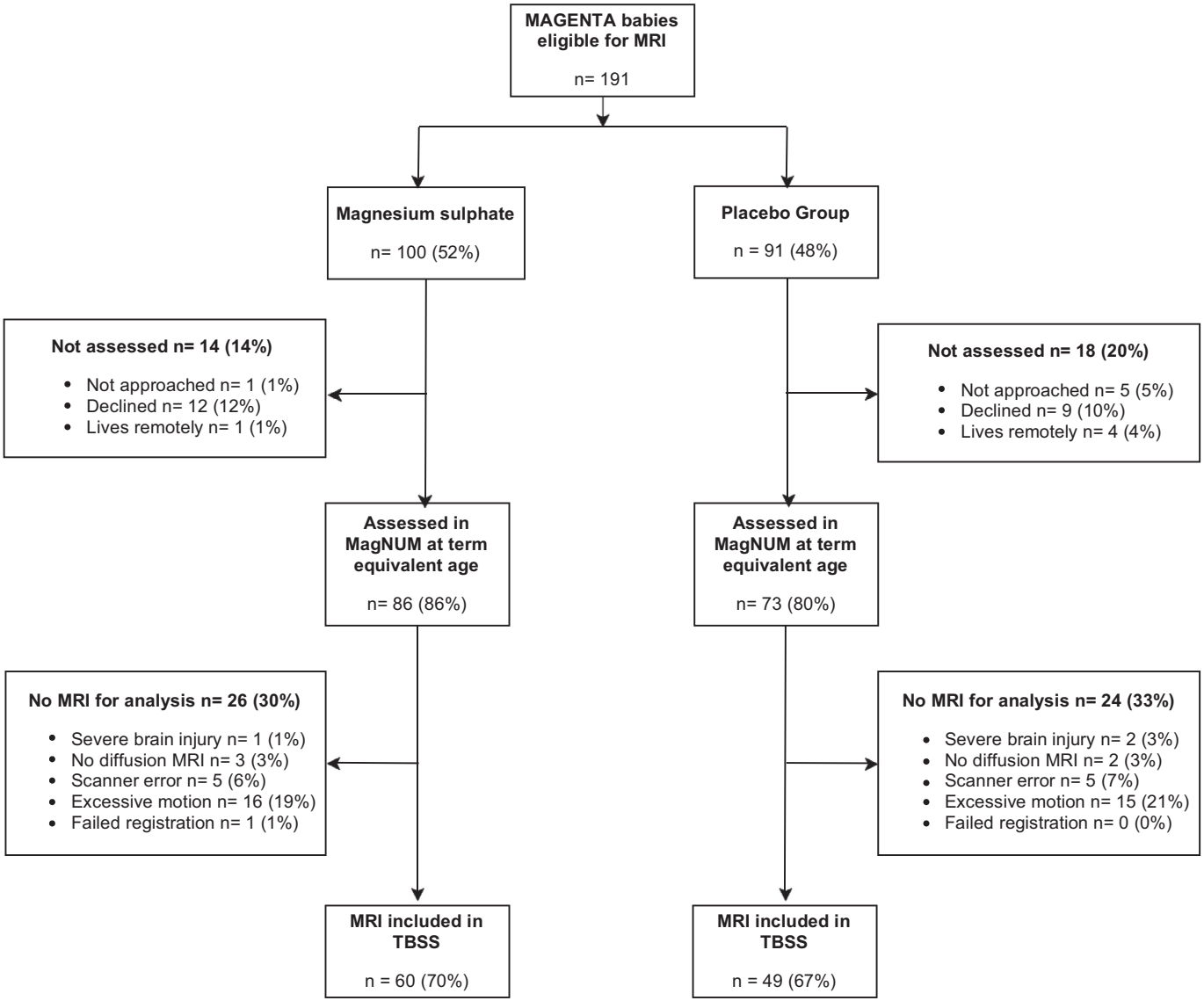

Fig. 1. MagNUM Study recruitment and inclusion in Tract-Based Spatial Statistics (TBSS) analysis.

Table 2
Characteristics of mothers and babies in the MagNUM Study.

Characteristics	Magnesium sulphate	Placebo	p value
Mothers	*n* = 52	*n* = 45	
Age (years)	32•1 (5•3)	30•9 (7•1)	0•36
Nulliparous	28 (53•8)	27 (60•0)	0•54
Ethnicity			0•43
. Caucasian	24 (46•2)	17 (37•8)	
. Asian	12 (23•1)	7 (15•6)	
. Aboriginal or Torres Strait Islander	1 (1•9)	0 (0•0)	
. Polynesian	3 (5•8)	2 (4•4)	
. Māori	5 (9•6)	7 (15•6)	
. Other	7 (13•5)	12 (26•7)	
BMI (kg/m^2)	26•9 (7•2)	26•4 (5•2)	0•70
Gestation at trial entry (weeks)	31•9 (1•0)	31•9 (1•2)	0•70
Main reason at risk of preterm birth:			
. Antepartum haemorrhage	6 (11•5)	5 (11•1)	0•95
. Preterm prelabour rupture of membranes	16 (30•8)	16 (35•6)	0•62
. Preterm labour	24 (46•2)	19 (42•2)	0•70
. Pre-eclampsia	9 (17•3)	8 (17•8)	0•95
. Fetal compromise	11 (21•2)	5 (11•1)	0•18
. Other	11 (21•2)	10 (22•2)	0•90
Received treatment allocated	51 (98•1)	44 (97•8)	0•92
Babies	*n* = 60	*n* = 49	
Gestation at birth (weeks)	31•9 (0•9)	32•2 (1•6)	0•28
Twins			0•53
. Singleton	41 (68•3)	38 (77•6)	
. Twin 1	10 (16•7)	5 (10•2)	
. Twin 2	9 (15•0)	6 (12•2)	
MRI site			0•15
. Auckland	33 (55•0)	33 (67•3)	
. Christchurch	16 (26•7)	13 (26•5)	
. Adelaide	11 (18•3)	3 (6•1)	
Post-menstrual age at MRI (weeks)	40•1 (1•5)	40•2 (1•3)	0•75
Birth weight (g)	1663 (362)	1821 (536)	0•08
Birth weight (z score)	−0•04 (1•04)	0•25 (1•09)	0•16
Bronchopulmonary dysplasia	6 (10•0)	2 (4•1)	0•24
Necrotising enterocolitis	0	0	
Exclusive breast milk feeding at time of MRI	39 (65•0)	36 (73•5)	0•41
Sex (Female)	27 (45•0)	23 (46•9)	0•84

Data are mean (standard deviation) or n (%). Bronchopulmonary dysplasia was defined as oxygen requirement at 36 week's post-menstrual age. Z scores were calculated using WHO standards.38.

than the placebo group (0.24±0.03, (P = 0.034)) but there was no significant difference in RD (1.18±0.09 × 10^{-3} mm^2 s^{-1} vs 1.21± 0.09 × 10^{-3} mm^2 s^{-1}, (P = 0.10)).

When only babies without bronchopulmonary dysplasia were included (n = 101; 54 magnesium sulphate, 47 placebo), the findings were similar to the primary analysis but there were no significant differences in the superior longitudinal fasciculus or the inferior fronto-occipital fasciculus. However, the magnesium sulphate group had lower MD in the precentral gyrus, and more extensive clusters with significantly lower RD in both the left and right corona radiata (Fig. 3c). There were no differences between groups in AD.

When only babies who were exclusively receiving breast milk at the time of MRI were included (n = 75; 39 magnesium sulphate, 36 placebo), FA results were similar to the primary analysis, but with more differences between groups in the left hemisphere. The magnesium sulphate group had higher FA in the left superior longitudinal fasciculus, the left and right optic radiations, the left and right corticospinal tracts (including the left posterior limb of the internal capsule), and precentral gyri (Fig. 3d). There were no significant differences between groups in MD, AD or RD.

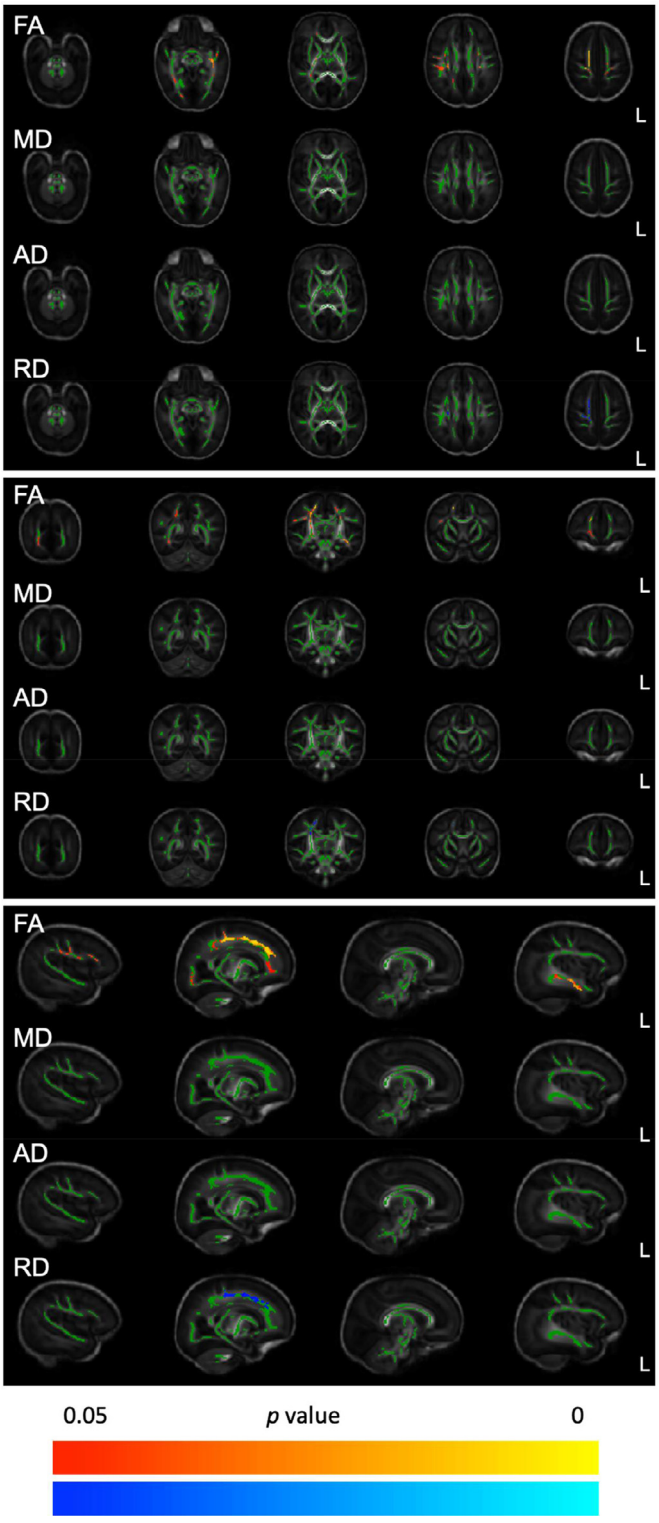

Fig. 2. Tract-based spatial statistics comparing the brain white matter skeletons at term equivalent age of babies exposed in utero to magnesium sulphate or placebo.

Footnote: A group-specific template underlies each axial (top four rows) coronal (middle four rows) and sagittal (bottom four rows) slices, with the white matter tract skeleton shown in green. Regions where the magnesium sulphate group (n = 60) had a significantly higher diffusion metric than the placebo group (n = 49) are shown in red-yellow (family-wise error corrected, P < 0•05), while regions where the magnesium sulphate group had significantly lower measures than the placebo group are in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

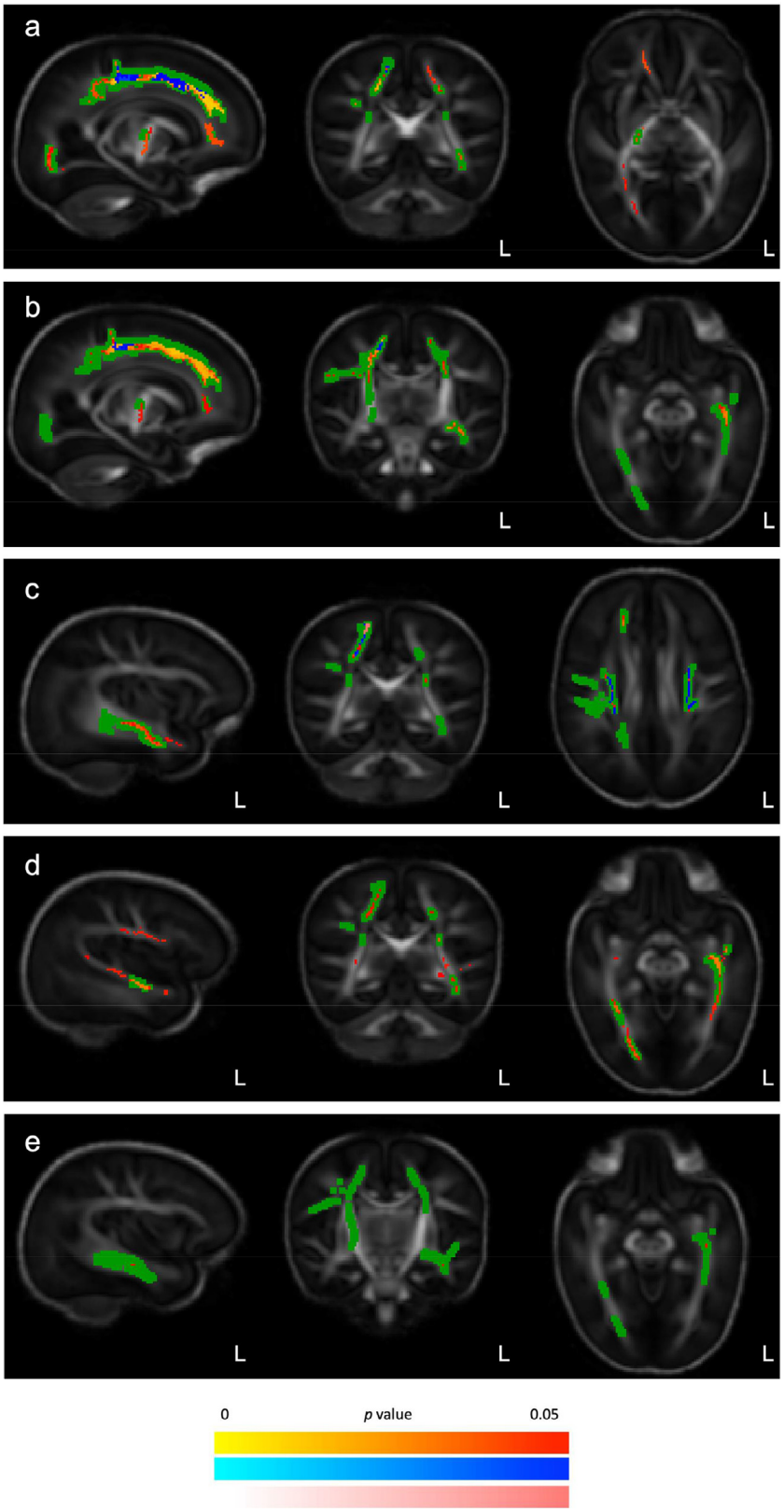

Fig. 3. Sensitivity and subgroup tract-based spatial statistics comparing the white matter skeletons of babies at term equivalent age of babies exposed in utero to magnesium sulphate or placebo

Footnote: A group-specific template underlies each axial (right) coronal (middle) and sagittal (left) slices, with significant primary analysis differences between the magnesium and placebo groups indicated in green. Regions where babies who received magnesium sulphate had a significantly (family-wise error corrected, ($P < 0•05$)) higher FA (red-yellow), lower RD (blue) or lower MD (pink) than babies who received placebo are layered over the template.

a: Babies whose mother received at least some of their allocated treatment;

b: Babies born <34 weeks' gestation;

3.2. Subgroup analyses

In the subgroup of babies randomised at 30 to <32 weeks' gestation (n = 56; 30 magnesium sulphate, 26 placebo) there were no significant differences between groups in any regions of the white matter skeleton. On region-of-interest analysis, there were also no differences between groups in FA or RD. In the subgroup of babies randomised at 32 to <34 weeks' gestation (n = 53; 30 magnesium sulphate, 23 placebo), the magnesium sulphate group had higher FA in a cluster of the left inferior longitudinal fasciculus (Fig. 3e). There were no significant differences between groups in MD, AD or RD.

When babies of each sex were analysed separately (50 girls; 27 magnesium sulphate, 23 placebo, and 59 boys; 33 magnesium sulphate, 26 placebo) there were no significant differences between treatment groups on TBSS analysis. On region-of-interest analysis, boys in the magnesium sulphate group had higher FA than those in the placebo group (0.25±0.02 vs 0.24±0.03, (P = 0.01)) and lower RD (1.19±0.08 vs 1.24±0.09 × 10^{-3} mm^2 s^{-1}, (P = 0.03)). For girls, there were no differences in the region-of-interest analyses for FA or for RD.

4. Discussion

Antenatal magnesium sulphate administered to mothers at risk of imminent preterm birth from 30 to <34 weeks' gestation protected white matter development in their babies. At term equivalent age, key white matter tracts of babies exposed to antenatal magnesium sulphate compared with those babies not exposed had higher FA and lower RD, indicating greater fibre coherence and maturation along the axis of greatest water molecule diffusion. Furthermore, the reduction in RD is consistent with a protective effect resulting in improved myelination. Effects in corticospinal tracts are of importance because reduced FA of the corticospinal tract at term equivalent age in babies born preterm independently predicts motor delay and cerebral palsy [24]. The effects of magnesium sulphate reached the white matter underlying the cortex in the pre- and post-central gyri which are the primary sensory and motor cortices. Our findings are consistent with the neuroprotective benefits, including lower rates of cerebral palsy, observed after exposure to antenatal magnesium sulphate before 30 weeks' gestation [25].

The effects of antenatal magnesium sulphate on FA and RD were not confined to motor pathways and extended to the superior longitudinal fasciculus, inferior longitudinal fasciculus and the inferior fronto-occipital fasciculus. These association fibres subserve cognitive processes including regulation of motor behaviour, visually guided behaviors, language, and working memory, and all are implicated in the cerebral phenotype of preterm brain injury [23,26,27]. Furthermore, alterations in DTI parameters have been associated with cognitive deficits in children and adults born preterm [28,29], Our findings therefore suggest that children in the magnesium sulphate group may have improved cognitive as well as motor outcomes. Participants in the MAGENTA Tria [19], are currently being assessed at two years of age for the primary outcome, which will enable further assessment of the neuroprotective effect of magnesium sulphate and outcome across a broad range of developmental domains.

As this was a nested study within a multicentre randomised trial, we used three different MRI facilities to maximise recruitment. To ameliorate the possible confounding effects of scanner variation, image acquisition sequences were matched as closely as possible, scan site was included as a covariate in primary analysis, and we carried out a sensitivity analysis using data only from the largest site (60% of all MRI scans). There were no significant differences between magnesium sulfate and placebo groups on TBSS analysis, and in the region-of-interest analysis the direction of effect was maintained. These results were consistent with the primary analysis.

There were several notable findings from the sensitivity analyses. When only singletons were included (73% of the cohort) there were no differences between groups on TBBS analysis, and the direction of effect was maintained on region-of-interest analysis. This is consistent with previous findings which indicate that the clinical effects of antenatal magnesium sulphate for neuroprotection are similar in singleton and multiple pregnancies [25].

Breastmilk intake can affect early brain development and later neurodevelopment after preterm birth, and was therefore a potential confounder. In very preterm babies, the proportion of early enteral intake from breastmilk is associated with neonatal white matter connectivity [16,30]. In the subgroup of babies who were exclusively receiving breastmilk at the time of scanning (74% of the cohort), overall findings were similar to those of the primary analysis, although higher FA was seen in the optic radiations, associated with visual function, in the magnesium sulphate exposed group.

In sensitivity analyses, we also investigated a group without bronchopulmonary dysplasia because of its consistent association with atypical brain development [9], and found that the beneficial effects of antenatal magnesium sulphate were apparent in babies without this comorbidity of preterm birth.

Magnesium sulphate is recommended for fetal neuroprotection in women at risk of preterm birth although the optimal gestational age for its use remains uncertain [4,19],[31–34]. We found that in babies randomised at 30 to <32 weeks (51% of the cohort) there were no differences between magnesium exposed and placebo groups in any white matter region, whereas in the babies randomised at 32 <34 weeks (49% of the cohort) there was higher FA in the magnesium exposed group, although this was restricted to the left inferior longitudinal fasciculus. This finding was unexpected and most likely explained by a type 2 error due to small subgroups.

Male sex is associated with diffuse white matter injury after preterm birth [35]. In extremely preterm babies who underwent MRI at term equivalent age, boys were reported to have delayed myelination [36], and lower FA in the splenium of the corpus callosum [37] compared with girls. Although we found no differences in whole skeleton analysis between the magnesium sulphate and placebo groups in girls or boys separately, we did observe a modest increase in FA and lower RD in region-of-interest analysis, which was significant only for boys. This raises the possibility that boys receive a greater neuroprotective benefit from magnesium sulphate exposure than girls, and this warrants further investigation.

A major strength of the MagNUM Study is that it was nested within a randomised controlled trial, so the changes observed after magnesium sulphate exposure are likely to be causal. Follow-up of the trial participants will allow assessment of the relationships between the MRI changes at term equivalent age and later developmental outcomes. A possible weakness was the sample size, which reduced power to detect small effects in subgroups of interest including those for gestational age. However, in all subgroups the direction of the differences in FA and RD were consistent with those of the primary analysis.

In babies born preterm, antenatal magnesium sulphate exposure promotes development of white matter microstructure in pathways affecting both motor and cognitive functions. This may be one mechanism for the neuroprotective effect of magnesium sulphate treatment prior to preterm birth.

c: Babies who did not have bronchopulmonary dysplasia or necrotising enterocolitis;

d: Babies who were exclusively fed breast milk at the time of MRI;

e: Babies who were 32 to <34 weeks' gestation at trial entry.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Contributors

CAC, JEH conceived the study and developed the study design with BT, JPB, MEB and JA. TP, JA, GD, JEH, CAC were involved in data acquisition. TP and BT analysed the data. TP and CAC wrote the initial draft of the manuscript and all authors contributed to interpretation of the data, critical revision of the manuscript and approved the final version.

Declaration of Competing Interests

We declare no competing interests.

The MagNUM Study Group

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Collaboration by sites: (total number of babies recruited)

Auckland (102): *Centre for Advanced MRI, University of Auckland* Anna Lydon, Gerard Deib; *Auckland City Hospital* Jane Alsweiler, David Perry; *Middlemore Hospital* David Hou, Jen Schroder.

Christchurch (42): *Pacific Radiology Group* Ross Keenan, Scott Wells, Tracy Melzer; *Christchurch Women's Hospital* Nicola Austin, Nicola Ellis, Trish Graham, Jo Gullam, Di Leishman.

Adelaide (15): *South Australia Medical Research Institute, Jones and Partners Radiology* Andrew Dwyer, Angela Walls; *Women's and Children's Hospital* Pat Ashwood, Andrew McPhee; *Flinders Medical Centre* Scott Morris.

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