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Brief communication

Maternal antibody-mediated dyslexia? Evidence for a pathogenic serum factor in a mother of two dyslexic children shown by transfer to mice using behavioural studies and magnetic resonance spectroscopy

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Abstract

The causes of dyslexia are unknown, but previous studies have suggested an immunological basis in some cases. We hypothesised that maternal antibodies, which cross the placenta and bind to fetal antigens, could be responsible, particularly when the dyslexia recurs in consecutive pregnancies. We injected serum samples from five mothers of two or more children with dyslexia into pregnant mice, and tested the offspring for behavioural abnormalities and cerebellar metabolites by magnetic resonance spectroscopy (MRS). Mice exposed in utero to serum factors from one woman with two dyslexic children, who had also had three spontaneous fetal losses, showed deficits in motor tests which correlated with cerebellar choline (Cho) and creatine (Cr) levels. These preliminary results are consistent with a role for maternal serum factors, probably antibodies, in causing some of the features of dyslexia.

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1. Introduction

Neurodevelopmental disorders have diverse aetiologies and many are thought to have a genetic basis. Dyslexia is a neurodevelopmental disorder associated with defects in temporal processing particularly involving the magnocellular neuronal system (Livingston et al., 1991; Stein, 2001). It shows genetic linkage (Cardon et al., 1994; Fisher et al., 1999), but it is also associated with an increased incidence of immunological abnormalities, in the children or their families (Galaburda, 1993; Adinolfi, 1993; Warren et al., 1990).

We previously identified a developmental disorder, arthrogryposis multiplex congenita, caused by maternal antibodies to the fetal isoform of the acetylcholine receptor (Riemersma et al., 1997), and showed that this condition

could be reproduced in mice by maternal-to-fetal transfer of the human maternal IgG antibodies (Jacobson et al., 1999). We hypothesised that other developmental disorders might be caused by maternal antibodies.

To test this hypothesis, we obtained sera from mothers of children with dyslexia and injected them into pregnant mice to see if they would affect development. As both functional and structural cerebellar changes have been described in dyslexia (Fawcett et al., 1996; Rae et al., 1998; Nicolson et al., 1999), we particularly focussed on alterations in cerebellar function.

2. Methods

2.1. Clinical material

Sera were from five women (Dys-M1–5) who have two to four children with dyslexia. Controls were five parous

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t1.1 Table 1
t1.2 Details of women studied and effect of maternal-to-fetal transfer on litter survival

Subject	Age	Sex, age of children at time of study	Dyslexic children	Relevant obstetric, personal or family history of subject	Surviving litters/number injected
t1.4 Dys-M1	51	m, 14; m, 11	m, 14; m, 11 dyslexic	Three spontaneous abortions, at 2–3 months	4/4
t1.5 Dys-M2		m, 11; m, 10; m, 8	m, 11; m, 10 dyslexic	Subject has dyslexia	1/2
t1.6 Dys-M3	49	f, 21; m, 18; f, 16; f, 16	m, 18; f, 16 dyslexic	None known	2/2
t1.7 Dys-M4	46	f, 21; f, 19; m, 17; f, 14	All dyslexia	None known	0/2
t1.8 Dys-M5	49	f, 20; f, 18; m, 15; f, 14	All dyslexia; m15 has no thyroid	None known	0/2
t1.9 Healthy-Ms (5)	35–42	7 m, ages 6–21; 3 f, ages 3–9	None known	Each had had one spontaneous abortion <3 months	7/10
t1.10 Controls (4)	21–26	None	None	None known	5/8

58 women (Healthy-M1–5) with two to three non-affected
59 children, and four nonparous healthy women (Control-1–
60 4). For details, see Table 1.

62 *2.2. Maternal-to-fetal mouse model*

63 MF1 mice (Harlan UK) were mated in-house. From
64 day E10 to E17, where E1 is the day following the ob-

65 servation of a vaginal plug, we injected 0.5 ml/day of
66 each serum sample into 1–2 pregnant mouse dams (see
67 [Jacobson et al., 1999](#)). In the second experiment, 1.0 ml
68 of fresh serum sample was injected daily from E14–E17.
69 The mice were either handled daily, or were exposed to a
70 stimulating environment during the week before testing to
71 familiarise them with handling and a different environ-
72 ment.

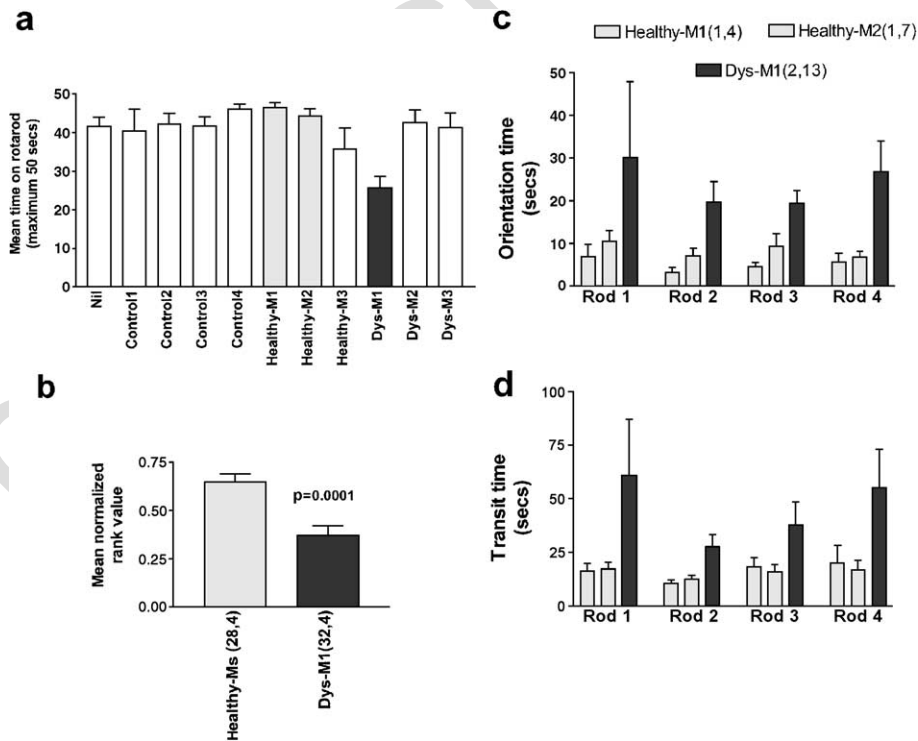


Fig. 1. Behavioural testing in young adult mice after in utero exposure to human maternal serum factors. The pregnant mice were either untreated (nil) or injected with serum from nonparous women (Control), mothers of healthy children (Healthy-M) or mothers of dyslexic children (Dys-M) as described in Table 1. Two accelerating rotarod apparatuses were used: initially an in-house model, and then the 7650 Ugo Basile model. The results in each set of test and control mice were ranked and normalised for the number of mice tested. The results represent (a) the raw data showing speed at falling off the in-house accelerating rotarod; (b) the mean ranked normalised values for the Healthy-M-treated and Dys-M1-treated mice from the two sets of independent experiments. Figures in brackets refer to the number of mice, number of litters tested. The multiple static rods apparatus consisted of a series of five horizontal wooden rods, 60 cm long, fixed to a solid base at one end only (see [Guenther et al., 2001](#)). The mice were placed at the exposed end of each rod in turn, facing away from the base. The times taken to turn 180° to face the base (orientation time, c) and to traverse the 60 cm length of the rod to the base (transit time, d) were measured, with an upper limit of 300 s. Mean (+S.E.M.) results from the first experiment involving mice treated with Dys-M1 or two Healthy-Ms are shown.

73 2.3. Behavioural tests

75 Standard tests for assessing development were applied
76 (Crawley, 2000) during the first 21 days. Performance on
77 the accelerating rotarod was tested from 6 weeks of age, and
78 on the multiple static rods from 10 weeks of age (Guenther
79 et al., 2001). The observer was always blind to the nature of
80 the injected material.

82 2.4. Magnetic resonance studies

83 Representative mice were investigated by MRS. The
84 mice were anaesthetised with an i.p. injection of a 1:1:2
85 solution of Hypnorm/Hypnovel/sterile water at a dose of 10
86 ml/kg, wrapped in a water-heated blanket to maintain body
87 temperature, positioned within an Alderman-Grant resonator
88 and placed in a 300-MHz Varian microimaging system
89 (Varian, Palo Alto, CA). The methods used are described
90 in Fig. 2 and in Frahm et al. (1989).

91 3. Results

93 3.1. Mouse development and behaviour

94 Most of the pups were born spontaneously at E18–E19,
95 but there were a number of failed pregnancies including those
96 in each of the four dams injected with Dys-M4 and Dys-M5
97 sera. There were no obvious differences between the remain-
98 ing Dys-M sera-treated, the Healthy-M sera-treated and
99 Control sera-treated offspring (for simplicity to be called
100 Dys-M, Healthy-M and Control mice) on any of the imme-
101 diate postnatal developmental tests (for example, righting
102 reflex, open field exploration: data not shown).

103 Strikingly, however, the two Dys-M1 litters at 6 weeks
104 showed impairment on the accelerating rotarod, which tests
105 coordination on a moving substrate, as shown by the speed
106 at which the mice fell off the rotarod. These results were
107 reproduced on several occasions, and then replicated with
108 new samples of Healthy-M and Dys-M sera injected into a
109 fresh batch of pregnant mice (two for each), and tested on
110 the commercial Ugo Basile accelerating rotarod apparatus at
111 11 weeks of age. The mean rank values for all experiments
112 are shown in Fig. 1b.

113 When subsequently tested as adults, Dys-M mice from
114 the first experiment showed a striking reduced performance
115 on the static rods (longer times to orientate and to transit
116 the rod) compared to mice from Healthy-M litters (Fig.
117 1c,d). There was a strong positive correlation between the
118 ranked values for orientation and transit times for all mice
119 ($r^2=0.83$, $p<0.0001$). At 6 months of age, these mice
120 were tested (litters coded) on a six-arm radial maze
121 reference memory test (as in Deacon et al., in press).
122 The mean results were almost identical between the Dys-
123 M1 and Healthy-M litters, indicating no obvious cognitive
124 impairment.

3.2. Magnetic resonance spectroscopy

126

The mice from the first experiment were tested using
magnetic resonance imaging and proton magnetic resonance

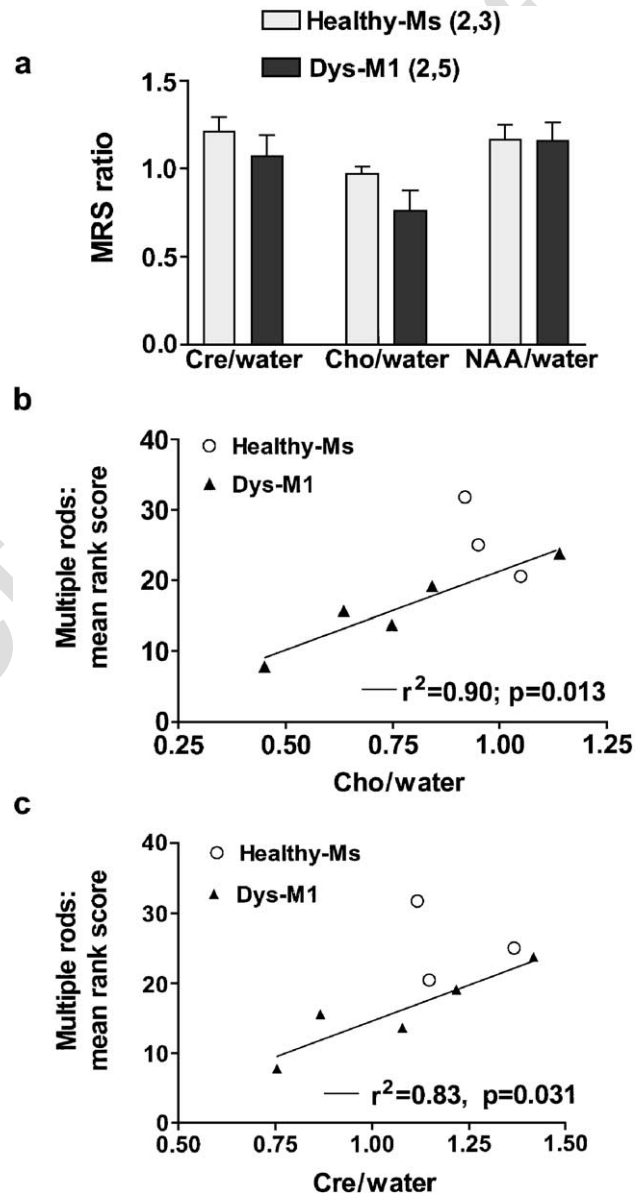
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Fig. 2. Magnetic resonance spectroscopy in adult mice after in utero exposure to human maternal serum factors. (a) Mean values of metabolites in $2.5 \times 3.0 \times 5.5 \text{ mm}^2$ voxels, centred over the cerebella, were related to the water signal in arbitrary units. Ranked individual performance on the multiple static rod test of Dys-M1-treated mice plotted against (b) Cre/water or (c) Cho/water. The correlation coefficients are based on the five Dys-M1 results only. Multislice spin-echo images were acquired in transverse, coronal and sagittal views to determine tissue anatomy (TR=2.8 s, TE=55 ms, 1 mm contiguous slices, 150 μm in plane resolution). Spectra were acquired using STEAM signal localisation (Frahm et al., 1989) from a $2.5 \times 3.0 \times 5.5 \text{ mm}^3$ voxel centred in the cerebellum (TR=3 s, TE=40 ms, TM=50 ms, 256 averages). Spectral peaks were quantified relative to the water peak in an unsuppressed spectrum, using constant normalisation factors, and expressed as a ratio.

129 spectroscopy (MRS) that can detect the relative concentra-
130 tions of water, *N*-acetylaspartate (NAA, a marker of viable
131 neurones), creatine (Cr, which includes phosphocreatine)
132 and choline-containing compounds (Cho, involved in mem-
133 brane and neurotransmitter metabolism). There were only
134 modest reductions in the concentrations of Cho and Cre
135 relative to water in the Dys-M1 mice (Fig. 2a), and no
136 changes in NAA. However, remarkably, there were signifi-
137 cant positive correlations between their Dys-M1 rank scores
138 on the multiple static rods and the Cho/water ($p=0.013$;
139 Fig. 2b) or Cre/water ($p=0.031$; Fig. 2c) ratios.

141 3.3. Clinical features of Dys-M1

142 Dys-M1 was 51 at the time of first sampling and had two
143 dyslexic boys of 13 and 10 years. She had had three
144 spontaneous miscarriages between the ages of 31 and 38
145 (including one twin pregnancy). Dys-M2 also has dyslexia,
146 which suggests that a dominant genetic factor is likely to be
147 responsible for the dyslexia in two of her three children.
148 Dys-M3 has no family history of dyslexia and two of her
149 four children are affected. Dys-M4 and Dys-M5 also had no
150 family history but all children were affected, consistent with
151 a maternal immune factor; however, the mice injected with
152 their sera failed to give birth.

153 4. Discussion

154 We demonstrate for the first time that reproducible
155 changes in tests of motor activity and coordination can be
156 induced in mouse offspring by injecting the mouse dam with
157 serum from a woman whose children have dyslexia. These
158 effects may be due to placental transfer of maternally
159 derived agents, perhaps antibodies that can adversely affect
160 neuronal development. Remarkably, the behavioural deficits
161 in the mice correlated with MRS measurements on the
162 affected mouse cerebella, suggesting that changes either in
163 cerebellar metabolism or cellular composition may be asso-
164 ciated with the behavioural abnormalities.

165 Passive transfer of disorders by injection of mice with
166 IgG has been a very valuable tool for confirming the
167 pathogenic nature of serum antibodies to autoantigens such
168 as to acetylcholine receptors in autoimmune myasthenia
169 gravis (Drachman, 1994) or the fetal acetylcholine receptor
170 in arthrogryposis (Jacobson et al., 1999). However, we are
171 not aware of any previous studies attempting to demonstrate
172 a role for human maternal serum antibodies in causing
173 central nervous system disorders.

174 The Dys-M1 serum markedly affected motor coordina-
175 tion on the accelerating rotarod, and also on the static rods
176 suggesting involvement of the cerebellum. Cerebellar abnor-
177 malities have been reported in dyslexia including reduced
178 size in the magnocellular neurones (Livingston et al., 1991),
179 and impaired cerebellar function (Fawcett et al., 1996;
180 Nicolson et al., 1999). Moreover, adult dyslexics have

181 abnormalities in cerebellar metabolite concentrations (Rae
182 et al., 1998). Interpretation of MRS findings is difficult, but
183 we believe that abnormal cerebellar cell type distribution is
184 the most likely explanation for our preliminary findings.

185 Maternal antibodies are most likely the pathogenic
186 factor. Tests for antibodies to neuronal proteins in Dys-
187 M1's serum gave equivocal results (PD and AV, unpub-
188 lished results), and we now need to test a larger number of
189 mothers of dyslexic children. However, Dys-M1 had had
190 several spontaneous abortions which can be caused by
191 antiphospholipid antibodies, and the fetal loss associated
192 with injection of two of the other Dys-M sera, from
193 women who had all children affected, is also consistent
194 with an immune abnormality. Thus, there may be a
195 subpopulation of mothers of dyslexic children who have
196 antibodies or other factors in their serum that are likely to
197 cause fetal loss, but which might cause neurodevelopmen-
198 tal abnormalities if the fetus survives. Nevertheless, we do
199 not expect maternal antibodies to be causative in all
200 children who suffer from dyslexia; they would most likely
201 be found predominantly in those mothers who have several
202 affected offspring (as tested here), while many other cases
203 may have a genetic or other basis.

204 It is also possible that dyslexia is dependent on inter-
205 action between genetic and autoimmune factors. Dyslexia
206 shows linkage to a site on chromosome 6 close to the MHC
207 genes that control immune responses (Cardon et al., 1994;
208 Fisher et al., 1999). It has been proposed that development
209 of the magnocellular cells may be influenced by these
210 polymorphic MHC genes (Stein and Talcott, 1999). In fact,
211 recent evidence suggests that development of the connec-
212 tions within this cell population is dependent on expression
213 of certain class I MHC antigens (Huh et al., 2000).

214 This study raises many questions concerning the patho-
215 genic targets of the putative antibodies, their site of action,
216 how and when the antibodies act, and the nature of the
217 permanent changes they induce. We believe, however, that
218 these preliminary results illustrate the feasibility of using the
219 mouse maternal-to-fetal transfer model and a battery of
220 behavioural and other noninvasive tests, to study the role of
221 maternal antibodies, and other serum factors, in causing
222 developmental disorders.

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