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*Autism* 2006; 10; 189
DOI: 10.1177/1362361306062024

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IgA antibodies in Rett syndrome

K.L. REICHELT  The National Hospital, Oslo, Norway
O. SKJELDAL  The National Hospital, Oslo, Norway

ABSTRACT  The level of IgA antibodies to gluten and gliadin proteins found in grains and to casein found in milk, as well as the level of IgG to gluten and gliadin, have been examined in 23 girls with Rett syndrome and 53 controls. Highly statistically significant increases were found for the Rett population compared to the controls. The reason for this remains unknown, but because IgA antibodies reflect the uptake of proteins and/or epitopes of proteins from the gut, this may be indicative of increased protein uptake.

ADDRESS  Correspondence should be addressed to: K.L. REICHELT, Institute of Pediatric Research, University of Oslo, Rikshospitalet, N-0027 Oslo, Norway. e-mail: K.L.Reichelt@klinmed.uio.no

KEYWORDS  casein; gliadin; gluten; IgA antibodies; Rett syndrome

Introduction

Rett syndrome is a neuro-developmental disorder and one of the leading causes of mental retardation in girls, affecting 1 per 10,000. Although there is recent evidence of abnormal early development in Rett syndrome (Burford et al., 2003), most children with this disorder appear to the parents to develop normally until 6–18 months of age, after which time they undergo a period of neurological regression. Although special neurological symptoms may be detected in some from early infancy (Wright et al., 2003), these are usually not noticed by the parents until later. Typical changes are loss of purposeful hand use (dyspraxia), deceleration of head circumference, peculiar stereotyped hand movements, transient autistic behaviour, respiratory abnormalities, bruxism and seizures. The period of rapid deterioration is often followed by a stagnation phase which lasts into adulthood. The phenotype can vary, with some girls having a serious form of the disease and some having a milder clinical picture.

In males the disorder is apparently mostly fatal, but a few cases have been described. Approximately 84 percent of the Rett patients have a genetic lesion in the X-linked MECP-2 encoding methyl-CpG-binding protein 2 that binds specifically to methylated DNA and acts as a general
transcription repressor, which affects a series of genes in an epigenetic way (Amir et al., 1999; Chen et al., 2001).

IgA antibodies are formed in the mucosa of the gut and secreted to the gut, protecting against foreign proteins, and are a measure of protein uptake from the gut. These antibodies are distributed to all mucus membranes in the body and are for instance increased in celiac disease. IgG antibodies are the circulating antibodies against foreign proteins.

Increased levels of IgA antibodies to gluten, gliadin and casein have been found in pervasive developmental disorders (PDD: Cade et al., 2000; Lucarelli et al., 1995; Reichelt et al., 1990). Rett syndrome is also classified as one of the PDD syndromes and some of these patients initially show transient autistic symptoms. The purpose of this article is to examine whether girls with Rett show increased uptake of proteins from the gut (measured as IgA antibody levels in serum) against dietary proteins.

**Participants and methods**

The clinical group consisted of 23 females all diagnosed with Rett syndrome, based on well established criteria (Hagberg, 1995; Hagberg and Skjeldal, 1994). Their age range was from 1 to 36 years with a median of 11 years (mean = 13). Controls were all healthy females of age range 1 to 55 years with median of 12 years (mean 16) and N = 53. The Rett participants were volunteered by their parents, to whom the purpose of the examination had been explained.

Blood was drawn by venepuncture and centrifuged and serum was sent to the immunological laboratory (Nycomed Pharma) and assayed for IgA antibodies against gliadin, gluten, lactalbumin, lactoglobulin, casein and ovalbumin. In those cases where IgA antibodies were above the upper cutoff for celiac disease, the celiac typical endomycium (later anti-transglutaminase antibodies) were also measured. IgG antibodies to gluten and gliadin and total IgA were also measured by using enzyme-linked immunosorbent assay (ELISA) as described by Scott et al. (1985a; 1985b). Costar EIA Microplates (no. 3490) were coated with antigens at the following concentrations: casein and lactalbumin 0.001 g/l; gliadin and gluten 0.01 g/l; lactoglobulin 0.05 g/l; and ovalbumin 0.1 g/l. Serum samples were tested at 1:400 dilution for IgA activity by the ELISA method (Scott et al., 1984). Optical density (OD) was read at 405 nm. Every microplate included three serum samples tested with each antigen. On the basis of reproducibility, tests showed a day-to-day variation of less than 0.1 OD units (Scott et al., 1985a; 1985b). Recently Fürsts Laboratory in Oslo have bought this laboratory but they use the same techniques, equipment, staff and consultants.
Urine peptides were measured as described by Solaas et al. (2002). Briefly, urine equivalent to 250 nanomoles creatinine was separated by High Pressure Liquid Chromatography (HPLC) and the peptides that are retained on a C-18 reverse phase column at pH 2, and eluted by acetonitril gradient after hippuric acid, are measured at 215 nm as the area under the curve. As a control for purity, the ratio 215 to 280 was routinely measured (Reichelt and Knivsberg, 2003). That the peaks are indeed peptides has been extensively discussed (Reichelt et al., 1998).

Statistics
Because the distribution of IgA antibodies in the normal population is very skewed towards the lower levels, the non-paired and non-parametric Mann–Whitney U-test was used. The statistical characteristics U and U’ are given as well as the p-value.

Results
Table 1 shows the overall results of the two groups for IgA. The Rett population is different from the normal controls for gluten, gliadin and casein, as seen in Figure 1 for gliadin and in Figure 2 for gluten. The statistics give a p-value of 0.001 for gluten and gliadin and 0.005 for casein.

Lactalbumin and lactoglobulin were not different from the controls. Thus for lactoglobulin the Mann–Whitney statistic had values \( U = 310 \) and \( U' = 345 \) and \( p = 0.785 \) (not significant). For lactalbumin \( U = 249 \) and \( U' = 411 \) and \( p = 0.124 \) (not significant).

Two of the girls with Rett but none of the controls had no demonstrable specific IgA levels without a general IgA deficiency, for which we have no explanation. None had endomycium or anti-transglutaminase increases, which are special markers for celiac disease; therefore the Rett

<table>
<thead>
<tr>
<th>Table 1 IgA antibodies in girls with Rett and controls</th>
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<tbody>
<tr>
<td>Gliadin</td>
</tr>
<tr>
<td>(N = 23)</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>95% CI:</td>
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<tr>
<td>min.</td>
</tr>
<tr>
<td>max.</td>
</tr>
</tbody>
</table>

The values are expressed as optical density units from ELISA as described in Scott et al. (1985a; 1985b). Two-tailed \( p \) for gliadin in Rett against controls was < 0.0001, as is the value for gluten. Two-tailed \( p \)-value for casein in Rett against controls was \( = 0.0005 \).
Figure 1  The level of IgA antibodies against gliadin in serum in Rett syndrome (series 1) and 53 controls (series 2). The Mann–Whitney U-statistic = 249 and U' = 969.5, and the two-tailed p-value was < 0.0001 (highly significant)
girls did not have celiac disease. Total IgA antibody level in the serum of the Rett group had a range of 0.25 to 3.91 and the given control values are 0.7 to 3.7. The total IgA levels were not statistically different for girls with Rett compared to controls.

Total IgG levels were also measured for gluten and gliadin (see Table 2). Here we found IgG levels for gliadin with $U = 165$ and $U' = 1077$ and a two-tailed $p$-value < 0.0001. For gluten we found $U = 158$ and $U' = 1138$ and again a two-tailed $p$-value < 0.0001 (highly significant).

Eight girls had their antibody levels tested again after 2 years. No statistical differences were found. For example, IgA to gluten in one participant was 0.42, and at retest was 0.39. Another participant was measured at 0.94 and at 0.97 on the retest. A third participant came out with 0.15 and a retest value of 0.20.

IgE (or typically allergy related antibodies) levels in 11 of the 23 girls with Rett were measured by ELISA immune assay at the same laboratory, and none were above the upper limit (average level expressed as percentage of upper IgE limit of the controls = 10.7 ± 8.9 percent, $N = 11$).

No correlation of age and antibody levels was shown.

Plotting peptide levels in urine, obtained as published by Solaas et al.
against IgA antibody levels towards gluten, a negative correlation of peptide levels to IgA antibodies against gliadin (Figure 1) was found. The same was found for gliadin IgA and IgG against peptide levels (not shown). However, the few excessively high peptide containing samples would dominate the correlation.

**Discussion**

Our data indicate that as a group the girls with Rett syndrome show higher IgA antibody levels in serum against gluten, gliadin and casein proteins compared to controls. They do not show this for lactalbumin, lactoglobulin and ovalbumin. Because endomycium antibodies or trans-glutaminase antibodies were not increased, this is not a celiac condition. It is, however, indicative of increased uptake of some proteins from the gut in Rett syndrome. The increased IgG antibody levels may also be seen as a possible byproduct of increased uptake with concomitant tissue damage. Increased pterin levels in Rett syndrome (Messahel et al., 2000) have been proposed as a marker of immune system activation in Rett. This could also explain the antibody increases but not why the antibody increase is selective.

Healthy persons also absorb intact proteins from the gastro-intestinal tract (Husby et al., 1985; Kilshaw and Cant, 1984). Furthermore the uptake of active enzymes has been demonstrated (Gardner, 1995; Ross et al., 1973; Schoutsen and De Jung, 1984) as has the uptake of the proteinase/peptidase known as botulinum toxin. This fact is reinforced by finding intact antigens from ingested proteins in mothers’ milk (Axelsson et al., 1986; Stuart et al., 1984; Troncone et al., 1987). It is normal to have IgG antibodies against most food proteins and usually also IgA antibodies.

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**Table 2** IgG antibody levels in serum of girls with Rett and controls

<table>
<thead>
<tr>
<th>Gliadin IgG</th>
<th>Gluten IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rett (N = 23)</td>
<td>Controls (N = 54)</td>
</tr>
<tr>
<td>Rett (N = 24)</td>
<td>Controls (N = 54)</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Median</th>
<th>95% CI:</th>
<th>Mean</th>
<th>Median</th>
<th>95% CI:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rett</td>
<td>1.11</td>
<td>1.06</td>
<td>min. 0.93</td>
<td>1.17</td>
<td>1.12</td>
<td>min. 0.93</td>
</tr>
<tr>
<td>Controls</td>
<td>0.46</td>
<td>0.39</td>
<td>max. 1.29</td>
<td>0.43</td>
<td>0.39</td>
<td>max. 1.40</td>
</tr>
</tbody>
</table>

All numbers are in OD units from ELISA. The two-tailed $p < 0.0001$ is found for gluten in Rett girls compared to controls. Two-tailed $p < 0.0001$ is also found for gliadin in Rett girls compared to controls.
but with a different profile and a lower average. Furthermore peptides are taken up post-prandially (Chabance et al., 1998), and protein epitopes that induce antibodies are usually peptidic in nature. Ingested proteins may induce antibodies, for instance against myelin oligodendrocyte glycoprotein in experimental autoimmune encephalomyelitis (Steffler et al., 2000). However, because lactoglobulin and lactalbumin and ovalbumin did not show this increase, the increased uptake must be somewhat selective.

What increased uptake means is more difficult to explain. It could be part of a general membrane leakiness, because we have earlier found increased protein levels in urine from girls with Rett syndrome (Reichelt et al., 2001) and in more than 80 percent of examined cases hyperpeptiduria is often quite severe (Solaas et al., 2002). The severe stress of the disorder may also increase gut permeability for casein, gliadin and gluten precursor proteins; this may possibly be relevant as an explanation also for Rett girls, but is so far speculative.

Recently, ataxia with polyneuritis and cerebellar volume decrease and especially Purkinje cell loss has been characterized (Hadjivassiliou et al., 1998; 2002) with anti-gliadin and anti-gluten antibodies but without celiac disease. Only further research can elucidate these intricacies. However, an increased gut uptake of some proteins may perhaps be related to the developmental inhibition which is found in Rett syndrome.

References


