Folinic Acid Treatment of Parents Positive for Folate Receptor Autoantibodies Could Reduce the Risk of Autism in Their Offspring

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Article

Folinic Acid Treatment of Parents Positive for Folate Receptor Autoantibodies Could Reduce the Risk of Autism in Their Offspring

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Abstract

Folate receptor-alpha (FRα) transports folate to germline cells and across the placental and blood-brain barrier to the brain. FRα autoantibodies (FRαAb) occur in ~70% of autistic children devoid of genetic defects, and ~1/3 of their asymptomatic parents. Folinic acid circumvents blocked folate transport. In children positive for FRαAb whose parents tested negative for FRαAb, high dose folinic acid treatment before 3 ½ years, results in improved outcome (88%). High autism prevalence with poor outcome (29%) is observed when mothers or both parents are positive for FRαAb. We postulated that folinic acid treatment of parents before and during pregnancy may reduce the risk of autism in further offspring. In a prospective open label pilot study, parents with positive FRαAb wishing to have further children, took daily levo-folinic acid (3.75 mg) or an equivalent dose of dl-folinic acid (7.5 mg) three months before conception and throughout pregnancy. Among five families where all mothers and three fathers tested positive for FRαAb, folinic acid prevented autism in all 7 children born. This contrasts significantly (Fisher's exact test p=0.011) with the high autism prevalence of 54 % in offspring from 28 untreated families where either only mothers (n=15) or both parents (n=13) had FRαAb. Therefore, folinic acid intervention before and throughout pregnancy, in parents positive for FRαAb, may reduce the risk of autism in their offspring.

Keywords: folate receptor autoimmunity; autism; folinic acid treatment; prevention of autism

1. Introduction

The rising autism rate world-wide is a serious medical concern with 1 in 31 children at the age of 8 years in the US, diagnosed with some form of autism spectrum disorder (ASD)(Center for Disease Control, USA). While causes remain elusive, multiple factors including genetic, immunologic, infectious, drug-related, metabolic and environmental, may contribute to the disorder [1–5]. Although most research on the origin of autism is focused on genetics and environmental factors, our recent studies suggest that an autoimmune disorder with autoantibodies (FRαAb) directed against the folate receptor alpha (FRα) may play a major role in the origin of infantile autism and ASD, where a genetic cause could not be identified [6–10]. Further supporting evidence on the role of folate deficiency during pregnancy ranging from at the time of conception and during early pregnancy contributing to fetal brain malformation and the pathogenesis of autism came from several additional studies [11–15]. We detected a high prevalence of serum FRαAb in children with cerebral folate deficiency (CFD), in whom exposure to the soluble folate receptor (sFR) from bovine milk, stimulates an immune response with antibody production of the IgG1 and IgG4 isotype [16]. These antibodies can cross-react with the human FRα protein because the bovine and the human FRα proteins possess 91% structural mimicry. On the choroid plexus, these antibodies can block folate transport by complexing with the membrane-anchored FRα protein either at the folate binding site (blocking type antibodies) or elsewhere (binding type antibodies), leading to complement cascade activation and

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inflammation. Consequently, both types of FRαAb can interfere with folate transport across the blood-brain barrier to the spinal fluid compartment and hence to the brain. Ultimately, this can lead to CFD and disturb normal brain development and in some instances regression of neurodevelopmental milestones [16].

In the absence of genetic defects, about 60-80% of children with infantile autism, test positive for FRαAb and show improvement following high dose folinic acid treatment [17–20]. Previous studies have also focused on the search for these FRαAb among parents [21]. FRα protein is also expressed in the gonads and placenta, where FRα mediates folate transport to germline cells and across the placenta to the embryo/fetus and the brain . The latter has been elegantly shown in a rat model of exposure to FRα antibodies [22]. Most parents are asymptomatic carriers with a prevalence of blocking FRαAb in healthy adult women estimated at 3-5% in Belgium, 4-7% in Spain, 9-13% in Ireland and 10-15% in the US population. However, binding antibodies are also prevalent in the both the patient population and in asymptomatic parents [21]. Thus, both types of these FRαAb in the child not only block folate transport to the brain after birth but in their parents may also impair folate transport to germline cells and across the placenta to the embryo/fetus as shown in the rat model. FRαAb can negatively affect fetal and postnatal brain development. Large population studies have pointed to families having an autistic child, to run a high recurrence risk of autism of about 20% in future pregnancies [23–26]. However, these studies did not consider the various known origins underlying autism, among them, different syndromes, genetic, metabolic, infectious causes, heavy metal exposure, drug-related causes and parental folate receptor autoantibodies contributing to fetal and cerebral folate deficiency. Therefore, family counseling justifies exploration of multiple genetic and other potential causes of autism to enable cause-specific intervention. Parental contribution to developmental disorders has been studied in gene-specific disorders but it remains controversial in autism. The role of folate on the other hand, is well established in pregnancy and fetal development [6,12–14]. The recent discovery of folate receptor autoantibodies identifies a whole new mechanism by which germline cells and the brain could be deprived of folate. Available testing for FRαAb and folinic acid treatment can provide specific intervention to restore folate. This same strategy could be extended to parents testing positive for FRαAb to provide adequate folate during reproduction and pregnany. Identifying FRαAb in parents and intervening before and during pregnancy can restore folate and prevent damage to brain development and consequently, functional deficits in the offspring.

Treatment of infantile autism using high-dose folinic acid supplements (0.5-2 mg/kg/day) has resulted in amelioration of core autistic features as well as significant improvement of delayed cognitive or verbal skills [27]. Although overall results in our studies indicate significant improvement, some children do not respond well. The reason for an incomplete response to high dose folinic acid treatment in these children often remains obscure with multiple underlying factors such as mitochondrial and metabolic dysfunction or the negative impact of other prenatal factors contributing to the pathology.

Data from our prospective study were used to determine the outcome after treating children for two years, depending on variables like the age at which folinic acid treatment was started and the different specific FRαAb profiles and titers present in the child and parents for each family. Preliminary findings suggested that FRαAb positive children from families where both parents tested negative, had the mildest form of autism, and responded best to high dose folinic acid initiated before the age of 3 ½ years. The treatment effect declined with advancing age, whereas there was a minimal but non-significant effect if high dose folinic acid was started after the age of 6 years (Figure 1A). Another finding was that the presence of FRαAb in mothers or both parents, potentially blocking folate transport to germline cells and to the fetus, increased the risk of severe or irreversible autism in their offspring (Figure 1B).

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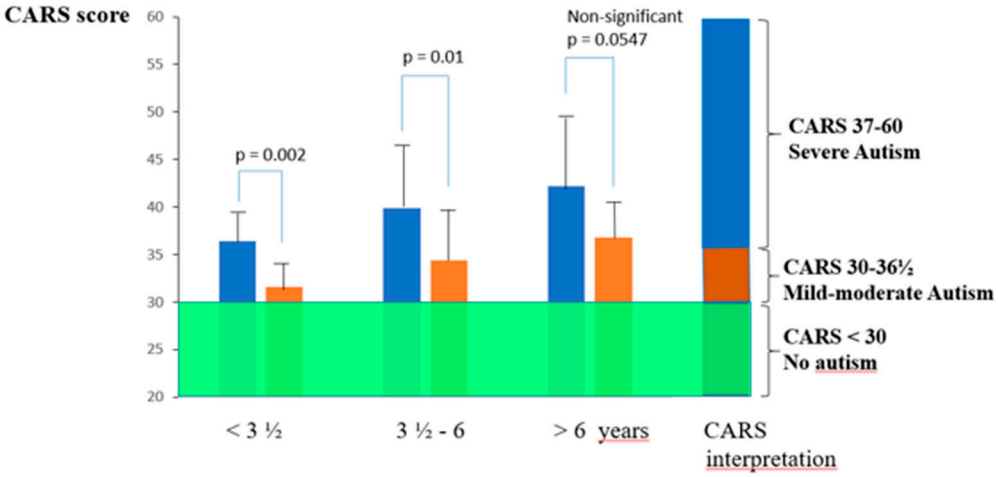
(**A**)



(**B**)

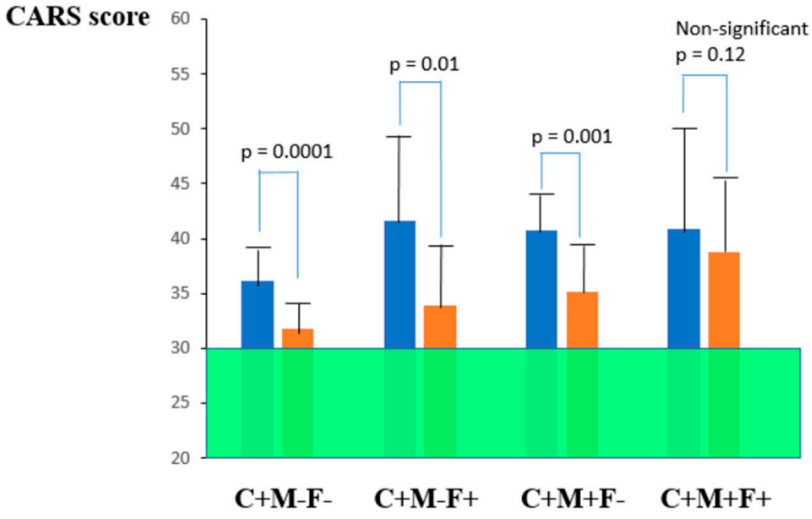


Figure 1. (A) shows CARS scores at baseline (blue bars) and after 2 years of folinic acid supplementation (orange bars) in autistic children with FRα antibodies and absent parental FRα antibodies. The decrease in CARS score shows response to treatment initiated before the age of 3 ½ years, between 3 ½ and 6 years and beyond the age of 6 years. Early treatment prior to 3 ½ years shows the best prognosis with no significant change beyond 6 years in children of parents who tested negative for FRαAb. (B) shows the CARS evolution for different families with a specific FRα antibody profile where children with positive FRα antibodies initiated folinic acid treatment before the age of 5 years. It showed prognosis worsening with antibodies in mothers or both parents. **C** stands for child, **M** for mother and **F** for father. Symbols + or – represent positive or negative for FRαAb.

The process of initial germline cell demethylation and particularly subsequent DNA re-methylation during fetal DNA programming in developing tissues and organs, depends on adequate one-carbon unit supplies mediated by adequate folate availability. Delivery of sufficient folate would be impaired by FRα autoantibodies in gonads and at the placental-fetal barriers. Considering the role of folate in the synthesis of nucleotides for RNA/DNA, methylation reactions for epigenetic control of imprinting and embryonic genes and numerous pathways involving intermediary metabolism, folate deprivation via dietary deficiency or by autoantibodies blocking folate transport to the fetus

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and the brain could have drastic effects on brain development and function. Therefore, we hypothesized that early intervention in parents could be beneficial in changing the outcome. For the reasons outlined above, parents testing positive for FRαAb, who wished to have further children, underwent extensive counseling to exclude any genetic abnormality. After negative results from full genetic analysis and after informed consent, moderate dose folinic acid intervention prior to conception and during pregnancy was initiated with the objective to reduce autism risk in future offspring. For comparison, we also determined the prevalence of autism and other neurodevelopmental disorders among children born from untreated families where either the mother, father, or both parents carried FRαAb

2. Materials and Methods

2.1. Study Design and Inclusion/Exclusion Criteria

After referral of children suspected of autism to the Autism Center Liège, extensive testing included the autism diagnostic observation schedule (ADOS), autism diagnostic interview (ADI-R), childhood autism rating scale (CARS), developmental and speech assessment, as well as observations at school and in the domestic environment [28]. Neurologic and pediatric examination was performed to exclude syndromal disorders due to specific gene mutations associated with autism. Brain MRI and EEG were performed on a routine basis. One important inclusion criterion was the finding of normal EEG and brain imaging results. Exclusion criteria included the presence of pre- or perinatal brain injuries associated with maternal infection during pregnancy or other maternal disease (drugs, alcohol, addictive agents and medication), birth injuries, prematurity or dysmaturity.

In addition, blood samples were collected to determine routine parameters including complete blood count, serum and red blood cell folate, vitamin B_{12}, plasma homocysteine, liver and renal function, creatine kinase, lactate, electrolytes, iron status, calcium, phosphorus, magnesium, TSH with T3 and T4, cholesterol, triglycerides, apolipoprotein-B, gliadin antibodies, vitamins A, D, E, gamma-tocopherol, coenzyme Q10, zinc, copper, selenium, manganese, and the heavy metals mercury, lead and aluminum. The reason for this extensive routine blood testing was because many children with infantile autism have selective eating habits often predisposing to nutritional deficiencies. Nutritional deficiencies were corrected in all children with regular monitoring during treatment.

Genetic testing was performed to exclude chromosomal disorders, fragile X-, Rett- and Angelman syndrome and copy number variations. Extensive genetic counseling was performed for each family having an autistic child using selected screening for autism associated genes or whole exome sequencing (WES). Children participating in the current study had no genetic defects or other known causes underlying autism.

For each autistic child and both parents, additional testing included determination of serum FRαAb of the binding and blocking type. For each family we recorded the family composition regarding the presence of autism, neurodevelopmental disorders and healthy children among all other siblings of the autistic child. These data were used to calculate the autism prevalence in different groups of families with a specific FRαAb profile, particularly among families where only the mother, only the father or both parents carried these antibodies.

From a total of 92 children who received a diagnosis of infantile autism and fulfilled the inclusion criteria, 15 children (16 %) were excluded because of the presence of genetic defects. In the remaining 77 children, the age was 4.5 ± 2.25 years (mean ± SD; range: 1.8-15.9 years); their gender distribution male:female was 4.5:1, and their CARS rating was 40.86 ± 6.49. For each autistic child fulfilling the exclusion criteria and testing positive for FRαAb, the CARS score at baseline was reassessed after two years following high dose folinic acid treatment (0.5-2 mg/kg/day; maximum 50 mg daily). The differences in CARS score were used as a tool to validate outcome. Families were divided in diverse subgroups according to age at which folinic acid was started and on the specific FRαAb profile in

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the child and its parents. The outcome for each specific subgroup of children after two years treatment was determined.

Families having an autistic child, who wished to have further children, were recruited since 2013 to participate in an open label prospective study to treat both parents for 3 months prior to conceiving and the mother throughout pregnancy. Based on the potential of autism recurrence risk with poor prognosis among families where mothers or both parents carried FRαAb, we designed a folinic acid intervention study. The untreated control group were families presenting during the same period of intervention and recruitment of parents but did not consent to participate in the study. These families served as our untreated group for statistical analysis.

2.2. Autism Risk Reduction in Future Offspring of Parents with FRα Antibodies

Low folate intake during early pregnancy and maternal FRαAb have been associated with an increased risk of childhood autism, neural tube defects and congenital malformations in the offspring [6–12]. Young parents whose child received the diagnosis of autism, and who wished to have further children, were evaluated for genetic counseling and risk assessment for further family planning. Thereupon extensive genetic counseling was performed with analysis of selected monogenetic disorders and in some families, whole exome sequencing. In the absence of any known genetic abnormality identified, we hypothesized that parents with FRαAb would be at a higher risk to have future children with congenital defects or severe autism, since parental antibodies could block folate supply to their germline cell lineages and exert epigenetic alterations.

Therefore, previous findings suggesting an important role of parental FRα antibodies with respect to an increased risk of autism and birth defects in future children had to be considered for the prevention treatment strategy using folate administration prior to conception and during pregnancy.

Parents with an autistic child, where the mothers or both parents were carriers of FRα Ab, who wished to have further children, could only enter the intervention study if inheritable genetic disorders had been excluded. The eligible parents had to take 3.75 mg levo-folinic acid or an equivalent dose of 7.5 mg Leucovorin (dl-folinic acid, containing equal amounts of active (levo) and inactive (dextro) forms) daily, initiated three months before conception and for the mother throughout pregnancy. The dose of folinic acid chosen was based on an earlier intervention trial with positive outcome [29] and though considered well above the physiologic requirement and conventional prenatal use, was deemed necessary to transport folate via an alternate pathway using the reduced folate carrier [30]. Following birth, all children were evaluated on a regular basis for at least 2 ½ years .

Statistical analysis used the Fisher's exact probability test to assess stastical significance of the number of autistic and healthy children born to the group of parents treated with folinic acid, compared to the number of autistic children born during the same period to the group of untreated parents where either the mother or both parents had FRα antibodies.

2.3. Criteria for Recruitment and Ethical Considerations

All participating parents who had previously a child with autism, and who wished to have more children, were fully informed about the recurrence risk of autism or other developmental defects in future children based on published literature. They were also informed how the presence folate receptor autoantibodies in parents could increase the risk of folate deficiency in the fetus. Before pregnancy, parents agreed to extensive genetic analysis (trio WES analysis) in their autistic child to detect the possibility of genetic causes as the basis of autism. Parents agreed that genetic analysis should be normal before being eligible to participate in the preventive study using folinic acid supplement. They were also informed about the higher folate dose to be administered to overcome the effect of FRαAb and provide adequate folate to the developing fetus. After informed consent, parents who agreed to participate to the prevention study using folinic acid before and during pregnancy were enrolled in the study. Study protocols were approved by the University Hospital Liège Ethics Committee since 18 February 2013 (Protocol FOL040113).

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2.4. Serum FRα Antibody Assessment

The assay for both FRα autoantibodies of the blocking and binding type have been described previously. Blocking FRα autoantibodies were expressed as pmoles of folic acid blocked from binding to FRα per ml serum and binding FRα autoantibodies were expressed as pmoles of IgG antibody per ml of serum [31]. Repeated serum samples were taken from all autistic children and their parents before initiation of this study.

3. Results

3.1. Distribution of Folate Receptor Autoantibodies in Autistic Children and Parents

FRαAb were detected in 62 out of 77 children (81%), among 32 of 77 mothers (41%) and 28 out of 77 fathers (36%). For the 77 children, the FRαAb profile for each trio (child, mother, father) showed no FRαAb in 10% of families, while in 31% of families only the child tested positive. In the remaining families either the father, mother or both parents had FRαAb (Table1). Our observations among these families with an autistic child where genetic defects have been excluded, suggested a high prevalence of FRαAb and heritability of this autoimmune disorder.

Table 1. FRα autoantibody distribution in children and parents*

FRα antibody profile in Trio	Number of families (%)	
C+M-F-	24	(31%)
C+M+F+	13	(17%)
C+M+F-	13	(17%)
C+M-F+	12	(16%)
C-M-F-	8	(10%)
C-M+F-	5	(7%)
C-M+F+	1	(1%)
C-M-F+	1	(1%)
Total patients	**77**	**(100%)**

* FRα autoantibody distribution in families showing positive testing in 62 out of 77 children (C: 81%), among 32 of 77 mothers (M:41%) and 27 out of 77 fathers (F:35%). FRα antibody profiles for each trio (Child, Mother, Father) are listed.

For the analysis of family distribution of FRαAb and autism, we excluded one child families. Among 15 families where only the mother had FRαAb, the prevalence of autism was 61% (19 out of 31 children). In addition to these 19 autistic children, 2 of the 12 healthy children in these families had ADHD.

Among 7 families where only the father had FRαAb, 8 out of 16 children suffered from autism (50%) and 8 children were healthy.

In 13 families where both parents tested positive, the calculated prevalence of autism was 46% (13 of 28 children). However, from the total of 15 non-autistic children, 11 children were healthy, whereas 2 children had developmental speech delay, 1 child had intellectual disability and 1 child had ADHD.

3.2. Outcome Following Folinic Acid Supplement to Parents Before and During Pregnancy

We recruited five families into the intervention study after informed consent by mothers and fathers with FRαAb, having one autistic child previously who wished to have further children. In four families parents were healthy and had a child with autism and FRαAb. In one family both parents with no previous children, suffered from Asperger autism and had FRαAb. Using the treatment protocol of folinic acid supplementation among five families (all mothers and 3 fathers

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testing positive for FRαAb), a total of seven healthy children born (4 girls and 3 boys) were followed for a period of at least 2 ½ years (Table 2). None of these children developed autism or other neurodevelopmental disorders.

Table 2. Selection criteria and outcome after folinic acid treatment

	Autistic child	Mother	Father	Siblings after Folinic acid Treatment
Family I	+	+	-	2 healthy sisters and 1 brother
Family II	+	+	+	1 healthy sister
Family III	+	+	-	1 healthy brother
Family IV	+	+	+	1 healthy brother
Family V		+	+	1 healthy girl

Folinic acid administration prior to conception and throughout pregnancy resulted in seven healthy normal children. The presence or absence of antibody is indicated by + or – symbols.

Folinic acid administration prior to conception and throughout pregnancy resulted in seven healthy normal children. The presence or absence of antibody is indicated by + or – symbols.

During this period, among 28 families where all mothers and 13 fathers had tested positive for FRαAb but did not receive folinic acid supplement, 32 children were born with moderate to severe autism and 27 children without autism. In the latter non-autistic group of 27 children, one child suffered from an intellectual deficit, two children had speech delay and ADHD was found in three children. Our results support the hypothesis that folinic acid intervention prior to conception and during pregnancy represents a readily applicable strategy to reduce significantly the high recurrence risk of autism in children among parents testing positive for FRαAb. In the absence of folinic acid intervention, the prevalence of autism varied between 46-61 % respectively among families where only the mother or both parents tested positive for FRαAb. Although the number of families receiving folinic acid was small, the preventive effect reached statistical significance (Fisher's exact test p-value = 0.011) compared to the group of families who presented to the clinic during the same period but did not receive folinic acid intervention during subsequent pregnancies.

4. Discussion

While the cause(s) of autism remain elusive, it is generally accepted that both maternal and paternal influences in the form of genetic, epigenetic and environmental effects can dictate fetal outcome [3,9–11]. Over the past two decades, our investigation into folate receptor autoimmune disorder has established that it is highly prevalent in families with ~70% of the children diagnosed with ASD testing positive for FRαAb [21]. In patients tested for CSF folate, FRαAb titer correlates inversely with decreased CSF folate. In both open-label and double-blind studies, intervention with high dose leucovorin has shown significant improvement in core ASD deficits [7,17,27]. Since folate is a critical determinant of fertility, pregnancy outcome and brain development, restoring folate transport disrupted by FRαAb should be a first approach to implement for a positive outcome.

This preliminary study attempted to evaluate folinic acid intervention among families where mothers or both parents tested positive for FRαAb. The premise of this pilot study was to determine if supraphysiological folate in a form that can be transported from mother to fetus despite the presence of autoantibodies that can block folate via the folate receptor, can restore folate status and influence pregnancy outcome. It should be stressed that genetic abnormalities as the root cause of autism were ruled out in all cases. We were encouraged by two previous studies that showed positive outcome. The first, a single case of multiple pregnancy losses and high titer of FRαAb, placed on a milk-free diet and treated with folinic acid for 6 months resulted in undectable FRαAb, conceived

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naturally, carried to term and delivered a normal healthy boy [32]. The second case study from Italy reported the outcome in two mothers having had autistic children previously, who tested positive for FRαAb and were treated before and during pregnancy with 7.5mg of dl-folinic acid. The two healthy boys born were followed for 3 years without any signs of ASD [29].

Figure 2 summarizes the pathogenesis of autism under different conditions present in each individual family, where it is shown that there is a cumulative effect by the prenatal presence of FRαAb in fathers, mothers or both parents together with postnatally acquired FRαAb in the child after birth.

Our results about the impact of FRα autoimmunity on autism prevalence and the autism recurrence risk in families having a previous child with autism, are hitherto not well studied, particularly because genetic factors and gene defects have been generally accepted to represent the major causes of autism along with many ill-defined environmental factors, by mainstream autism researchers [33–41]. A recent large study indicated a 20% recurrence risk of autism among younger siblings in families having had an autistic child previously [23]. This was also confirmed by another study that compared the risk in unaffected families to affected families having a child with autism spectrum disorder or childhood autism. This study estimated a 8.4-fold increase in the risk following an older sibling with autism spectrum disorder and a 17.4-fold increase in the risk following an older sibling with childhood autism [24]. However, neither epigenetic factors nor FRα autoimmunity were considered in those large population studies [42–48]. In contrast to the estimated recurrence risk around 20% in these large population studies, the autism prevalence and recurrence risk for families without genetic defects where either one or both parents are positive for FRαAb, runs much higher from 46% up to 61%. For these reasons, in addition to genetic counseling, our findings warrant a search for FRαAb in families having an autistic child, since the real risk for autism recurrence in these families is not 20 %, as generally accepted, but much higher.

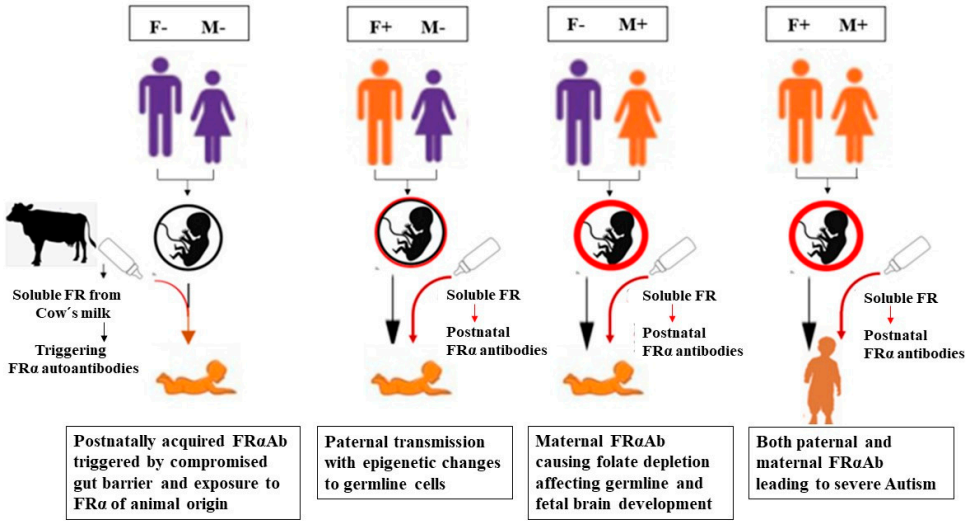

Figure 2. Pathogenesis of pre-and postnatal exposure to FRαAb. This diagrammatic representation shows the different FRαAb profiles in parents and children. On the left, when parents test negative and FRαAb are postnatally acquired by the child, this situation supports a favorable prognosis if diagnosis and folinic acid treatment is started early. From left to right the different trio´s with either positive FRαAb in one or in both parents, leading to increased negative prenatal influences and folate deficiency in germline cells and in the developing embryo/fetus leading to increased severity of autism with decreased response to folinic acid treatment.

Together these results support our hypothesis that for mothers or both parents testing positive for FRαAb, intervention with folinic acid prior to conception and throughout pregnancy is warranted

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to reduce the risk of autism in their offspring. Further trials on prevention are mandatory based on the high prevalence of FRαAb in families with ASD. A positive outcome in a larger clinical trial is likely to provide a readily usable strategy to reduce the ever-increasing autism prevalence in the population. While food fortification has raised the folate status in the population and combined with prenatal folate supplementation has had some positive effect on pregnancy outcome, the dose and folate form appears insufficient to overcome the effect of FRAb on folate transport [6]. Since epigenetic alterations in germline lineages and folate transport to the developing fetal brain may be affected, treatment with the correct form of folate at higher doses should be initiated prior to conceiving and throughout pregnancy. It is becoming increasingly evident that paternal influences through imprinting and other genes play a role in fetal outcome [34,42–48]. In this situation, exposure of the fetus to the maternal environment that includes genetic, epigenetic, inflammation and immune disturbances along with environmental exposure and maternal overall health and nutrition during the whole period of gestation, could play a pivotal role in health outcome of the offspring [47–55] Therefore, treating both parents prior to pregnancy seems reasonable. This pilot study suffers from many shortfalls such as the small number of families and open label without randomization, but paves the way for a larger controlled study. While using a placebo group may be considered unethical, comparing the treatment outcome to the outcome in the general population may be a reasonable approach to prove the efficacy of folinic acid intervention.

It is evident that available treatments for autism have at best only extended to how to manage affected children. It has been more than a century since identifying autism as a neurodevelopmental disorder with no understanding of what causes the disorder and how to prevent it. Considering the increasing rate of autism in our population, any approach to reduce this rate would be a significant milestone in reversing the trajectory. Positive outcome in these small number of intervention trials is extremely encouraging to wards understanding neurodevelopmental deficits leading to autism. We hope that our observations would encourage agencies to fund larger clinical trials. Even a small reduction in autism rate would be a major contribution towards decreasing autism prevalence.

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Data Availability Statement: All data are available in the main text.

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Conflicts of Interest: Authors declare that they have no competing interests.

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