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Autism Spectrum Disorders and Gluten/Casein Free Diet Treatment: A Systematic Review (1990-2016)

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AbstractBackground: High number of patients suffering from autism spectrum disorders utilize dietary intervention methods, especially gluten-free/casein-free diet. In contrast with its' extensive usage no sufficient and consistent data exist to support its' efficiency and safety.

Objective: The main aim of this systematic review is to provide a general look to the efficiency and safety of gluten-free/ casein-free diet treatment for autism spectrum disorders.

Method: Studies used in this systematic review are gathered from 3 online databases **(**PubMed, Embase and the Cochrane Library). Inclusion criteria are established for study selection. Articles published in a peer-reviewed article between 1990 and 2016 in English about our topic of interest and conducted with patients under the age of eighteen (18) are selected and further analyzed ("Level of Evidence" and "Grade of Recommendation" criteria are utilized).

Results: Even though some studies with high values of "Level of Evidence" claim that gluten-free/casein-free diet is beneficial for patients suffering from autism spectrum disorders, studies with lower risk of bias demonstrate otherwise. Since studies such as case reports and cohort studies may contain bias associated with small sample size, absence of clear assessment methods, lack of randomization and short treatment period we suggest that there is no sufficient data to support gluten-free/casein-free diet treatment.

Conclusion: Due to controversial outcomes from studies that have different "Level of Evidence" we speculate that efficiency of gluten-free/casein-free diet might be related to individual genetic differences. Therefore, studies conducted with large study groups could not provide statistically significant data to support this treatment option. Further studies should be conducted, especially in the field of genetics, in order to test our hypothesis.

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Introduction

Autism spectrum disorder (ASD) is a class of neurodevelopmental disorder that is characterized by impairment in social behaviors and non-verbal communicative skills and troubles in the perception and maintenance of social relationship according to the *fifth edition of the* Diagnostic and Statistical Manual of Mental Disorders *(DSM-5)* [1]. Autism spectrum disorder includes autistic disorder, Asperger's disorder, childhood disintegrative disorder, and pervasive developmental disorder all of which were previously considered to be distinct diseases [1,2]. Although there is not a consensus on the definitive underlying cause of ASD, it has tight association with genetics, nutritional balances and pre- and perinatal disruptions. Studies have demonstrated that experiencing oxygen deprivation during birth process, being prematurely born and experiencing viral infections during pregnancy increase the risk of autism spectrum disorders. On average 0,6% of the population suffers from autism spectrum disorders which is independent from ethnic and cultural groups, however, prevalence of ASD is 3 times higher in males compared to females. One controversial explanation for the marked prevalence difference between genders is that prevalence of ASD is undervalued by physicians due to discrepancy in symptoms among genders. Patients suffering from ASD can have a wide range of intelligence test scores and even in rare instances they can have extra-ordinary talents is specific fields [2,3]

Diet treatment is a popular complementary treatment approach for ASD which includes gluten-free and casein-free diet (GFCF), increased omega 3 fatty acid intake and increased vitamin and mineral intake. GFCF diet is based on the opioid-excess theory that suggests gluteomorphine which is formed due to the metabolism of gluten and casomorphine that is formed due to the metabolism of casein can act as opiate-like chemicals and mimic their effect by binding to the specific receptors in central nervous system. By binding to those receptors casomorphine and gluteomorphine can enhance the activity of endogenous opioid system which is associated with the symptoms of ASD[4].

Omega 3 fatty acids are poly-unsaturated fatty acids some of which are essential fatty acids for human metabolism such as alpha-linolenic acid. Studies indicate that some of the fatty acids such as docosahexaenoic acid are necessary for proper development of the central nervous system and proven to have anti-inflammatory effects.Three out of four study conducted about the relationship between ASD and omega 3 fatty acids demonstrate that ASD patients have low levels of omega 3 fatty acids in blood compared to the controls. On the other hand, according to the systematic review conducted by Bent and Bertoglio there is not sufficient experimental evidence to support this treatment option even though some small studies claim otherwise [5]

In the case of increased vitamin and mineral intake diet there are studies indicating the significance of well-balanced diet in the treatment of ASD [6,7]. In a study conducted by Dr. Bernard Rimland in 1978 75% of the ASD patients (12 out of 16) had showed improvement in autistic symptoms when they had been treated with B6, C and magnesium supplements. 6 Moreover, in another study conducted with 60 autistic children symptoms of autism diminished when they had been treated with vitamin B6 and magnesium supplements [7]. Increased vitamin and mineral intake diet and increased omega 3 fatty acid intake diet will not be further discussed in the context of this systematic review.

According to a survey conducted in UK 29% of parents whose children suffer from ASD utilize GFCF diets that demonstrates the further need to conduct more comprehensive studies in this field [8] . In the context of this systematic review efficiency and safety of gluten-free/casein-free diet in the treatment of ASD will be discussed.

Materials and Method

Literature Search

We have searched our topic of interest via electronic databases (PubMed, Embase and the Cochrane Library) in August 2016 by utilizing Medical Subject Heading terms which are listed as follows: "autism", "autism spectrum disorders", "diet", "gluten-free", "casein-free", "gluten", "casein", "GFCF" and their combinations. Afterwards, references of the selected articles and systematic reviews were searched by two authors in order to eliminate the risk of overlooking (Study selection process can be viewed from Figure 1).

Study Selection

Abstracts of each study have been examined by two authors independently in order to determine their eligibility according to the specific inclusion criteria. The articles that do not fit the inclusion criteria are eliminated and the others are further examined for the purpose of this systematic review. Inclusion criteria for article selection are as follows:

1.The study should be conducted with patients that are diagnosed with ASD and treated with gluten-free and/or casein free diet

2.The article should be published in a peer-reviewed journal between 1990 and 2016 in English

Participants of the studies should be under the age of 18.

Selected studies for the purpose of this systematic review can be seen from Table 1.

Quality Assessment

After assessing the eligibility of studies to the inclusion criteria two authors have independently assessed the quality of each study according to the criteria established by Melnyk in the book entitled "Evidence-based practice in nursing & healthcare: a guide to best practice" (Brief explanation of the criteria used in this step can be seen from Table 2). Furthermore, studies are independently assessed by two authors in order to assign them a "Grade of Recommendation" according to the criteria of Centre for Evidence-based Medicine (CEBM) (The criteria used in this step of quality assessment can be seen from Table 3).

In addition to "Level of Evidence" and "Grade of Recommendation" we also evaluate and assess each study individually depending on the criteria as listed above:

1. Presence of control group in the study
2. Methods utilized to assess the conditions of participants involved in studies (parental reports, direct observations, standardized questions, blood or urine tests etc.)
3. Homogeneity of the participants involved in each study (age, gender, ethnicity, other medical conditions etc.)
4. *Size of the study group*
5. Consistency of the data extracted in each study (absence of statistically significant deviations)

Results

Thirty-three articles varying from case reports to systematic reviews are analyzed in terms of the duration of treatment, methods utilized to assess the conditions of the participants, number of participants, publication year and trustworthiness of the studies. Afterwards, studies are categorized depending on their "Level of Evidence" and results of each category will be discussed separately (Table 4).

Level of Evidence = 1

This category includes 9 different systematic reviews listed as follows: Millward et al, 2004, Christison and Ivany, 2006, Millward et al, 2008, Mulloy et al, 2010, Mulloy et al, 2011, Buie 2013, Hurwitz et al, 2013, Marı´-Bauset et al, 2014 and Elder et al 2015 (Table 4).

Systematic review conducted by Christison and Ivany in 2006 concludes that there is not sufficient data to support GFCF diet in ASD treatment by analyzing six uncontrolled clinical trials and a single-blind randomized controlled trial. We believe that the review conducted by Christison and Ivany is inadequate to reach a valid conclusion due to the absence of control group in most of the studies [23].

Hurwitz et al, 2013 reached the conclusion that elimination of gluten and casein from diet of an ASD patient would not cause significant improvement in ASD symptoms by analyzing 5 study (same conclusion with Christison and Ivany, 2006] [39]. Moreover, Hurwitz et al, 2013 states that 3 out of 5 study demonstrate that GFCF diet is not beneficial for ASD patients, two of which were reliable studies since they were blinded studies comparing participants' behavior and found consistent outcomes. On the other hand, other 2 studies claim that GFCF diet has positive impacts on ASD patients. However, these two studies do not reveal reliable results because of not having placebo control (high risk of bias][39].

Millward et al, 2004 states that the results about cognitive skills, linguistic ability and motor ability are unclear, whereas, there is an important beneficial effect in autistic traits associated with GFCF diet. Millward et al, 2004 touches upon the fact that there are wide confidence intervals in the outcomes since their review is

Table 1: Journals in which the selected studies were published and basic numerical data about the studies.

Article	Level of Evidence	Number of participants	Journal
Reichelt et al, 1990[11]	4	15	Applied Physiology, Nutrition and Metabolism
Knivsberg et al, 1990[12]	4	15	Brain Dysfunct
Sponheim, 1991[13]	2	3	Journal of Norwegian Medical Association
Lucarelli et al, 1995[14]	4	30	Panminerva Medica
Knivsberg et al, 1995[15]	4	Continium of Knivsberg et al, 1990	Scandinavian Journal of Educational Research
Whiteley et al, 1999[16]	4	22	Autism
Knivsberg et al, 1999[17]	6	1	Nutritional Neuroscience
Cade et al, 2000[18]	4	150	Nutritional Neuroscience
Knivsberg et al, 2002[19]	3	20	Nutritional Neuroscience
Millward et al, 2004[20]	1	Systematic review	The Cochrane Database Of Systematic Reviews
Elder et al, 2006[21]	2	15	Journal of Autism and Developmental Disorders
Irvin, 2006[22]	6	1	Journal of Positive Behavior Interventions
Christison and Ivany, 2006[23]	1	Systematic review	Journal of Developmental & Behavioral Pediatrics
Mageshwari US, Minitha SJ, 2006[24]	5	15	Indian Journal of Nutrition
Patel and Curtis, 2007[25]	5	10	The Journal of Alternative and Complementary Medicine
Seung et al, 2007[26]	2	13	The Journal of Medical Speech-Language
Millward et al, 2008[27]	1	Systematic review	The Cochrane Database Of Systematic Reviews
Nazni et al, 2008[28]	5	50	Iranian Journal of Pediatrics
Hsu et al, 2009[29]	6	1	Chang Gung Medical Journal
Hyman et al, 2010[30]	3	14	The Brown University Child and Adolescent Behavior Letter
Mulloy et al, 2010[31]	1	Systematic review	Research in Autism Spectrum Disorders
Whiteley et al, 2010[32]	2	72	Nutritional Neuroscience
Mulloy et al, 2011[33]	1	Systematic review	Research in Autism Spectrum Disorders
Buie, 2013[34]	1	Systematic review	Clinical Therapeutics
Johnson et al, 2011[35]	2	22	The Journal of Developmental and Physical Disabilities
Pennesi, 2012[36]	6	387 parents	Nutritional Neuroscience
Harris and Card, 2012[37]	6	13	Complementary Therapies in Medicine
Genuis et al, 2010[38]	6	1	Journal of Child Neurology
Hurwitz et al, 2013[39]	1	Systematic review	Journal of Early Intervention
Herbert and Buckley, 2013[40]	6	1	The Journal of Child Neurology
Marl´-Bauset et al, 2014[41]	1	Systematic review	Journal of Child Neurology
Elder et al, 2015[42]	1	Systematic review	Nutrition and Dietary Supplements
Hyman et al, 2016[43]	2	14	Journal of Autism and Developmental Disorders

Table 2: Quality Assessment Criteria for articles in this systematic review

Level of Evidence	Description
1	Evidence from a systematic review of all relevant randomized controlled trials (RCT's), or evidence-based clinical practice guidelines based on systematic reviews of RCT's
2	Evidence obtained from at least one well-designed Randomized Controlled Trial (RCT)
3	Evidence obtained from well-designed controlled trials without randomization, quasi-experimental
4	Evidence from well-designed case-control and cohort studies
5	Evidence from systematic reviews of descriptive and qualitative studies
6	Evidence from a single descriptive or qualitative study
7	Evidence from the opinion of authorities and/or reports of expert committees

("Evidence-based practice in nursing & healthcare: a guide to best practice" by Bernadette M. Melnyk and Ellen Fineout-Overholt. 2005, page 10.)

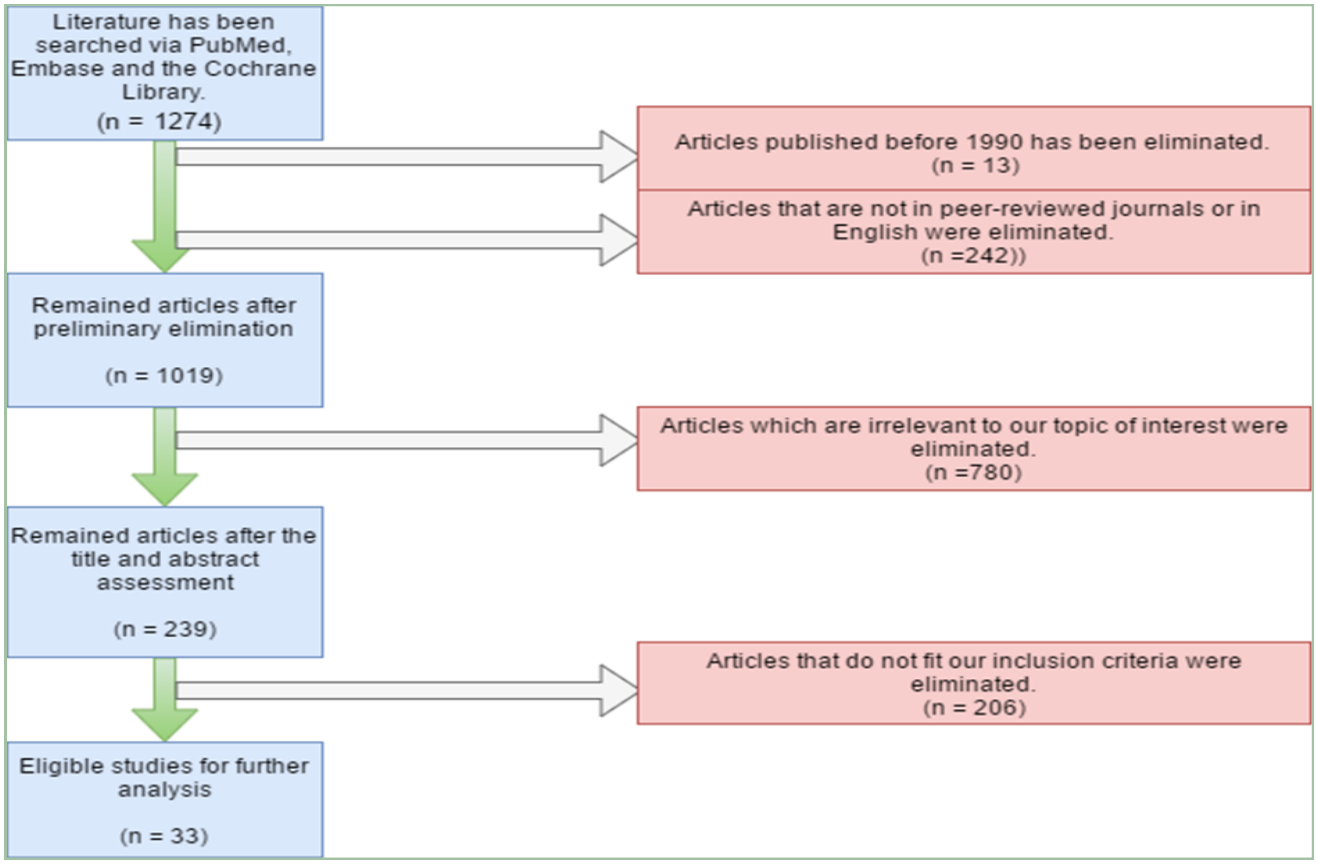

Figure 1: Flow chart of the process of study selection for the systematic review (Software used to design this flow chart is available from: https://www.draw.io/)

Table 3: Grade of Recommendation Criteria for quality assessment performed in this systematic review 10

Grade of Recommendation	Explanation
A	consistent level 1 studies
B	consistent level 2 or 3 studies ***or*** extrapolations from level 1 studies
C	level 4 studies ***or*** extrapolations from level 2 or 3 studies
D	level 5 evidence ***or*** troublingly inconsistent or inconclusive studies of any level

(Available from: http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/)

a small-scale study[20]. On the other hand, Millward et al, 2008 concludes that there is not statistically significant difference in any of the traits between the control group and treatment group [27]. Similarly, Marı´-Bauset et al, 2014, Mulloy et al, 2010 and Mulloy et al, 2011 argue that there is not sufficient data to support opioid-excess theory and, thus GFCF diet in treatment of ASD [31,33,41].There is a consensus on studies' about this topic being weak and limited . Moreover, all of these studies indicate that GFCF diet can be resorted by medical professionals if the patient experiences dramatic behavioral improvement associated with GFCF diet or if the patient has a confirmed allergy towards gluten and/or casein [20,27,31,33,41].

Even though Buie 2013 reaches the same conclusion as all other systematic reviews, Buie 2013 emphasizes the possibility that inconsistent and insufficient data regarding to GFCF diet might be related to genetic differences and possible gene expression differences between individuals suffering from ASD [34].The emphasis of Buie 2013 about the individualistic differences and individual-based treatment strategies should not be overlooked.

Level of Evidence = 2

This category includes six studies all of which are well-conducted randomized controlled trials aim to reach out a conclusion about the efficiency of GFCF diet in ASD treatment (Table 4).

Sponheim, 1991 has both conducted double-blind gluten/placebo studies (3 patients) and open study (4 patients), thus become a fundamental study in this category. Sponheim, 1991 argues that GF diet is not beneficial for ASD patients, also in some instances it becomes a negative factor that causes further social isolation of patient [13]. Elder et al, 2006 conducted randomized, double-blind study with 15 patient (age 2-16) and argues that there is no connection between GFCF diet and ASD symptoms despite the presence of improvement in some patients (statistically insignificant). One must consider the fact that results of Elder et al, 2006 are preliminary and based on a short-term treatment, thus not complete [21].

Hyman et al, 2016 is a double-blind, placebo-controlled study conducted with 14 children (age 3-5] which demonstrates no statistically significant benefit associated with GFCF diet treatment for patients suffering from ASD (Similar results were established by Hyman et al, 2010 in a randomized, double-blind study] [30,43]. Depending of the size of study group (72 patients) and longer treatment period (24 months) Whiteley et al, 2010 is an attention-grabbing study. It promotes the same conclusion reached by other studies in this group, though, Whiteley et al, 2010 does not provide conclusive evidence due to lack of placebo control [32]. Even though these studies utilize distinct assessment methods to evaluate patiens' conditions they reveal supportive outcomes [21,26,30,43].

Level of Evidence = 3

Despite the fact that two studies in this category, Knivsberg et al, 2002 and Johnson et al, 2011, are designed differently we believe that they are equal

Table 4: Plot summary of the selected studies.

Article	Level of Evidence	Grade of Recommendation	Assessment	Results	Duration	Number of Participants
Millward et al, 2008	1	A	Systematic review	No sufficient data to recommend GFCF diet treatment	-	-
Millward et al, 2004	1	A	Systematic review	There is not enough empirical data to support GFCF diet in the treatment of ASD.	-	-
Christison and Ivany, 2006	1	B	Systematic review	Inadequate evidence to support GFCF diet in the treatment of ASD	-	-
Mulloy et al, 2010	1	A	Systematic review	No sufficient data to recommend GFCF diet treatment	-	-
Mulloy et al, 2011	1	A	Systematic review	Significant improvements in some ASD subgroups whereas no significant improvement in others	-	-
Buie, 2013	1	A	Systematic review	Although there are small subgroups in which there is a correlation between gluten and ASD, there is not sufficent information to confirm this theory	-	-
Hurwitz et al, 2013	1	A	Systematic review	No significant improvement and high family investment	-	-
Marl´-Bauset et al, 2014	1	A	Systematic review	No sufficient data to recommend GFCF diet treatment	-	-
Elder et al, 2015	1	A	Systematic review	No sufficient data to recommend GFCF diet treatment	-	-
Sponheim, 1991	2	B	Visual Analogue Scale and Real Life Rating Scale	No significant improvement	6 months	7
Elder et al, 2006	2	B	Urinary peptide levels and standardized assessment questions	No significant improvement	6 weeks	15
Seung et al, 2007	2	B	Analysis of video recordings and verbal and non-verbal communication assessment	No significant improvement	6 weeks	13
Whiteley et al, 2010	2	B	Urinary peptide levels and standardized assessment questions	Significant improvements in some ASD subgroups whereas no significant improvement in others	2 years	72
Hyman et al, 2016	2	B	Behaviour assessment, autism symptoms	No statistically significant differences were noted between treatment groups.	4-6 weeks	14
Hyman et al, 2010	2	C	Direct observations and video recording	No significant improvement in autistic symptoms	12 weeks	14
Knivsberg et al, 2002	3	C	Behaviour assessment, autism symptoms (additional statistical tests)	Significant improvement in the diet group even though small improvements are observed also in the control group	1 year	20
Johnson al, 2011	3	C	Child Behavior Checklist and direct behavioral assessment	No statistically significant differences were noted between treatment groups.	3 months	22

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Continuation of table: 4

Article	Level of Evidence	Grade of Recommendation	Assessment	Results	Duration	Number of Participants
Reichelt et al, 1990	4	C	Peptiduria test and retrospective comparison	Improvement in patients treated with GF diet over 50%.	3 months	15
Knivsberg et al, 1990	4	C	Urinary peptide levels and standardized assessment questions	High levels of improvement in the GFCF diet group compared to the control group	1 year	15
Lucarelli et al, 1995	4	C	immunological signs of food allergy, levels of IgA antigen specific antibodies	Improvement in behaviour and decrease in spesific IgA suggesting relationship between casein and autism.	2 months	36
Knivsberg et al, 1995	4	C	Urinary peptide levels and standardized assessment questions	Significant improvement in behavior and social skills	1 year	Continium of Knivsberg et al, 1990
Whiteley et al, 1999	4	C	Urinary peptide levels and standardized assessment	Significant improvement in behavior and social skills	5 months	22
Cade et al, 2000	4	C	Behavioral assessment and urinary peptide levels	Decrease in autism symptoms	3 months	150
Mageshwari US, Minitha SJ, 2006	5	D	Direct observation	Improvements in autistic symptoms	3 months	15
Patel and Curtis, 2007	5	D	Behavioral assessment and urinary heavy metal levels	Behavioral improvement and lower levels of heavy metal in urine	3-6 months	10
Nazni et al, 2008	5	D	Day-to-day direct observation	GFCF diet is beneficial for treating behavioral symptoms of ASD	2 months	50
Knivsberg et al, 1999	6	D	Standardized assessment questions	Improvement in social skills	2 year	1
Irvin, 2006	6	D	Direct observation	No significant behavioral improvement	4 days	1
Pennesi, 2012	6	D	Parent reports and standardized assessment questions	Significant improvement in behaviour and social skills	-	387 parents
Harris and Card, 2012	6	D	Parental reports and standardized assessment questions	No statistically significant association between ASD and GFCF diet	-	13
Herbert and Buckley, 2013	6	D	Childhood Autism Rating Scale, direct observation	Improvement in language, auditory sensitivity and autistic symptoms	14 months	1
Hsu et al, 2009	6	D	Direct observation	Improvement in social interactions, eye contact and autistic symptoms	2.5 months	1
Genuis, 2010	6	D	Parent reports, direct observations and standardized assessment questions	Significant improvement in symptoms and resolution of gastrointestinal problems	6 months	1

in terms of trustworthiness and "Level of Evidence" (Table 4).

Positive correlation between GFCF diets and ASD treatment has been demonstrated for the first time in this systematic review by Knivsberg et al, 2002. Even though the study group is not large (20 participants) use of two-tailed Mann–Whitney U-test, p-test and mean value analysis reduces the risk of bias. The comparison between control group and diet group indicates significant improvement in the diet group even though small improvements are observed also in the control group.These statistically insignificant improvements in the control group might be related to the fluctuations in autism symptoms as argued by the authors [19].On the other hand, Johnson et al, 2011, is a non-randomized, single blinded study which also rejects the relation between gluten and casein free diet and autistic behaviors [35].

Level of Evidence = 4

Lucarelli et al, 1995 is a double blind study which examines the effect of gluten-free and casein-free diet on patients individually. Lucarelli et al, 1995 concludes that significant improvement is associated with GF and CF diets. Additionally, Lucarelli et al, 1995 indicates that casein challenge is related to the worsening of autistic symptoms which becomes a supportive evidence to the interest of this study [14].

Similar results are established by Knivsberg et al, 1990 and Knivsberg et al, 1995 both of which utilize urinary peptide level analysis and standard assessment questionaries [12,15]. Also, Cade et al, 2000 demonstrates that schizophrenia or autism is associated with exorphins formed due to incomplete digestion of gluten and casein in the intestine by immunoglobulin analysis as claimed by opioid-excess theory [18].

On the other hand, controversial findings are established by Whiteley et al, 1999 which argues that GFCF is associated with behavioral and social improvements whereas no significant decrease in specific urinary compounds are observed expected by opioid-excess theory. Therefore, outcomes of this study point out the possibility that possible success of eliminating gluten and casein from diet might be related to a different reason, rather than opioid-excess theory [16].

Level of Evidence = 5

Patel and Curtis, 2007 is a qualitative study performed with a small study group comprised of 10 participants. This study indicates significant improvement in social interaction, concentration, writing, language, and behavior in all of the participants and decrease in urinary heavy metal levels (lead) in every participant [25]. Nazni et al, 2008 investigates the efficiency of gluten-free diet and casein-free diet separately and concludes that they are both beneficial for autistic patients (Higher improvements are observed in gluten-free diet compared to casein-free diet). Other study in this category conducted by Mageshwari and Minitha, 2006 provide supportive evidence to the conclusion reached by Patel and Curtis, 2007 and Nazni et al, 2008 and demonstrates improvements in behavioral symptoms of autism [24,28].

Level of Evidence = 6

This category consists of five case reports and two studies based on parental reports. Even though Irvin, 2006 does not suggest any association between ASD and gluten-free diet, Knivsberg et al, 1999, Hsu et al, 2009 and Genius, 2010 claim otherwise [17,22,29, 38]. Moreover, Herbert and Buckley, 2013 shows improvements in autistic symptoms, language skills and auditory sensitivity. 40 These studies will not be further discussed since they are case reports which may include high levels of bias associated with small study groups and unreliable assessment methods.

Adverse Effects

After completing the discussion about the efficiency of GFCF diet for patients suffering from ASD one should consider adverse effects associated with the implementation of GFCF diet. Despite the fact that GFCF diet is a relatively new treatment method for ASD numerous studies exist about its' safety, as well as its' efficiency. Although 2 studies, Cornish et al, 2002 and Johnson et al, 2011, report no significant side-effects associated with GFCF diet in any kind of macro or micronutrient, most of the studies report lower bone density and calcium intake [35,44,45,46,47,48]. In addition to lower calcium intake and bone density Neumayer et al, 2012 also reports lower vitamin D and lactose intake from gut [49].

Another aspect of gluten free diet, effects on microbiome in human gut, is investigated in Bonder et

al, 2016. According to their 4 weeks of study gluten-free diet results in moderate but significant changes in human gut microbiome, especially for 8 bacteria species. For instance, remarkable decrease in Veillonellaceae, known for lactate fermentation, and Ruminococcus bromii, known for starch degradation is reported (50] Nevertheless, there is an obvious need to conduct more comprehensive research about the safety of GFCF diet since none of the previous studies were conducted with large study groups.

Conclusion

The main purpose behind categorizing studies in this systematic review depending on their "Level of Evidence" is to examine and assess the outcomes of each study as well as its' trustworthiness. By categorizing studies depending on their "Level of Evidence" we emphasize the contrast between outcomes of studies with high trustworthiness and low trustworthiness (high risk of bias).

In general studies conducted with a small study group (have high values of "Level of Evidence") illustrate significant improvements in autistic symptoms when participants are treated with GFCF diets, whereas, studies conducted with a large study group or systematic reviews argue that association between ASD and GFCF diet is statistically insignificant (Figure 2). Even though some of the participant in those studies with high trustworthiness have showed improvement in some autistic symptoms, due to the size of study group they become statistically insignificant. Thus, we speculate that the efficiency of GFCF diet and validity of opioid-excess theory might be related to individual genetic differences. Therefore, rather than rejecting opioid-excess theory and use of GFCF diet as a treatment option for ASD patients we claim that GFCF diet should remain as a treatment option for ASD and physicians should consider its' implementation depending on the patient. Further studies, especially in the field of genetics, is needed in order to prove our hypothesis about the relation between genetic characteristics and the efficiency of GFCF diet in ASD patients.

Author Contributions

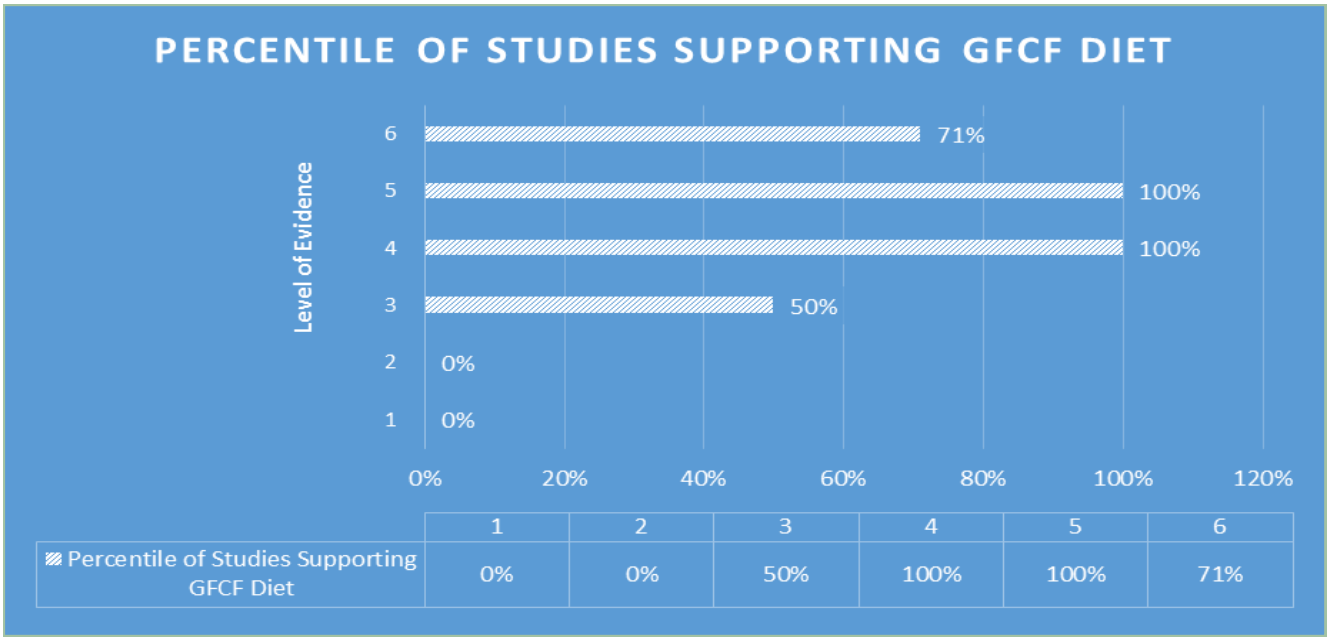

Figure 2: Percentile of studies at each "Level of Evidence" that support gluten-free and/or casein-free diet in the treatment of autism spectrum disorders

M.Ç.: Design, Literature Search, Study Selection, Study Assessment, Article Writing; S.Ç.: Literature Search, Study Selection, Study Assessment, Article Writing.

*No conflict of interest is reported

References

1. American Psychiatric Association, & American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders: DSM-5. Washington, DC: American Psychiatric Association.

2. Dombrowski, S. (2014). Autism spectrum disorder. Salem Press Encyclopedia Of Health [serial online]. Available from: Research Starters, Ipswich, MA. Accessed September 13, 2016.
3. Akshoomoff, N. (2006). Autism spectrum disorders. Hove: Psychology Press, 2006.
4. Hunter, L., O'Hare, A., Herron, W., Fisher, L., Jones, G. (2003). Opioid peptides and dipeptidyl peptidase in autism. Developmental Medicine And Child Neurology [serial online]; 45(2), 121-128. Available from: MEDLINE, Ipswich, MA. Accessed September 13, 2016.
5. Bent, S., Bertoglio, K., Hendren, R. (2009). Omega-3 fatty acids for autistic spectrum disorder: a systematic review. Journal Of Autism & Developmental Disorders [serial online], 39(8), 1145-1154. Available from: CINAHL Plus with Full Text, Ipswich, MA. Accessed September 13, 2016.
6. Rimland, B., Callaway, E., Dreyfus, P. (1978). The effects of high doses of vitamin B6 on autistic children: a double-blind crossover study. American Journal of Psychiatry, 135, 472–75.
7. Martineau, J., Barthelemy, C., Garreau, B., Lelord, G. (1985). Vitamin B6, magnesium, and combined B6-Mg: therapeutic effects in childhood autism. Biol. Psychiatry, 20, 467–78.
8. Winburn, E., Charlton, J., McConachie, H., McColl, E., Parr, J., O'Hare, A., & ... Couteur, A. (2014). Parents' and Child Health Professionals' Attitudes Towards Dietary Interventions for Children with Autism Spectrum Disorders. Journal Of Autism & Developmental Disorders, 44(4), 747-757. doi:10.1007/s10803-013-1922-8
9. Melnyk, B. M., & Fineout-Overholt, E. (2005). Evidence-based practice in nursing & healthcare: a guide to best practice. Philadelphia: Lippincott Williams & Wilkins, 2005.
10. Oxford Centre for Evidence-based Medicine - Levels of Evidence (March 2009) - CEBM. (n.d.). Retrieved from http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/.
11. Reichelt, K. L., Ekrem, J., Scott, H. (1990). Gluten, milk proteins and autism: dietary intervention effects on behavior and peptide secretion. J Appl Nutr, 42, 1-11.
12. Knivsberg A.M., Wiig K., Lind G., Nødland M., Reichelt K. L. (1990). Dietary intervention in autistic syndromes. Brain Dysfunct, 3, 315–317
13. Sponheim, E. (1991). Gluten free diet in infantile autism. Journal of Norwegian Medical Association, 111, 704-707.
14. Lucarelli, S., Frediani, T., Zingoni, A. M., Ferruzzi, F., Giardini, O., Quintieri, F. (1995). Food allergy and infantile autism. Panminerva Med., 37, 137–141.
15. Knivsberg, A. M., Reichelt, K. L., Nødland, M., Høien, T. (1995). Autistic syndromes and diet: a follow-up study. Scan. J. Educ. Res, 39, 223–236.
16. Whiteley, P., Rodgers, J., Savery, D., & Shattock, P. (1999). A gluten-free diet as an intervention for autism and associated spectrum disorders: Preliminary findings. Autism, 3, 45–66.
17. Knivsberg, A., Reichelt, K., & Nodland, M. (1999). Dietary intervention for a seven year old girl with autistic behavior. Nutritional Neuroscience, 2, 435–439.
18. Cade, R., Privette, M., Fregly, M., Rowland, N., Sun, Z. J., Zele, V., et al. (2000). Autism and schizophrenia: intestinal disorders. Nutritional. Neuroscience, 3, 57–72.
19. Knivsberg, A., Reichelt, K., Høien, T., Nødland, M. (2002). A Randomized Controlled Study of Dietary Intervention in Autistic Syndromes. Nutritional Neuroscience [serial online], 5(4), 251-261. Available from: Academic Search Complete, Ipswich, MA. Accessed September 10, 2016.
20. Millward, C., Ferriter, M., Calver, S., Connell-Jones, G. (2004). Gluten- and casein-free diets for autistic spectrum disorder. The Cochrane Database Of Systematic Reviews [serial online], (2), CD003498. Available from: MEDLINE, Ipswich, MA. Accessed September 10, 2016.
21. Harrison-Elder, J., Shankar, M., Shuster, J., Theriaque, D., Burns, S., Sherrill, L. (2006). The Gluten-Free, Casein-Free Diet In Autism: Results of A Preliminary Double Blind Clinical Trial. Journal Of Autism & Developmental Disorders [serial online], 36 (3), 413-420. Available from: Academic Search Complete, Ipswich, MA. Accessed September 10, 2016.

22. Irvin, D. (2006). Using analog assessment procedures for determining the effects of a gluten-free and casein-free diet on rate of problem behaviors for an adolescent with autism. Behavioral Interventions [serial online], 21(4), 281-286. Available from: Academic Search Complete, Ipswich, MA. Accessed September 10, 2016.

23. Christison, G., Ivany, K. (2006). Elimination diets in autism spectrum disorders: any wheat amidst the chaff?. Journal Of Developmental And Behavioral Pediatrics: JDBP [serial online], 27(2 Suppl), 162-171. Available from: MEDLINE, Ipswich, MA. Accessed September 10, 2016.

24. Mageshwari, U. S., Minitha, S.J. (2006). Impact of dietary exclusion of casein and gluten on selected autistic children. Ind J Nutr Diet, 43, 183–191.

25. Patel, K., Curtis, L. (2007). A comprehensive approach to treating autism and attention-deficit hyperactivity disorder: A pre-pilot study. Journal of Alternative and Complementary Medicine, 13, 1091–1097.

26. Seung, H., Rogalski, Y., Shankar, M., & Elder, J. (2007). The gluten- and casein-free diet and autism: Communication outcomes from a preliminary double -blind clinical trial. Journal of Medical Speech-Language Pathology, 15, 337–345.

27. Millward, C., Ferriter, M., Calver, S., Connell-Jones, G. (2008). Gluten- and casein-free diets for autistic spectrum disorder. The Cochrane Database of Systematic Reviews [serial online], (2), CD003498.

28. Peerkhan, N., Edward-Gnanaraj, W., Veerappan, N. (2008). Impact of Casein and Gluten Free Dietary Intervention on Selected Autistic Children. Iranian Journal Of Pediatrics [serial online], (3), 244.

29. Chia-Lin, H., Delmar, C.Y. L., Chia-Lin, C., Chin-Man, W., Alice, M.K. W. (2009). The Effects of A Gluten and Casein-free Diet in Children with Autism: A Case Report. Chang Gung Medical Journal [serial online], (04), 459.

30. Hyman, S., Stewart, P., Smith, T. (2010). The gluten - and casein-free diet in children with autism. The Brown University Child and Adolescent Behavior Letter [serial online], 3. Available from: Academic OneFile, Ipswich, MA. Accessed September 10, 2016.

31. Mulloy, A., Lang, R., O'Reilly, M., Sigafoos, J., Lancioni, G., Rispoli, M. (2010). Review: Gluten-free and casein-free diets in the treatment of autism spectrum disorders: A systematic review. Research In Autism Spectrum Disorders [serial online], 4, 328-339.

32. Whiteley, P., Shattock, P., Carr, K., Hooper, M., Todd, L. (2010). How could a gluten-and casein-free diet ameliorate symptoms associated with autism spectrum conditions?. Autism Insights [serial online], 2010, 39. Available from: Academic OneFile, Ipswich, MA. Accessed September 10, 2016.

33. Mulloy, A., Lang, R., O'Reilly, M., Sigafoos, J., Lancioni, G., Rispoli, M. (2011). Addendum to "gluten-free and casein-free diets in treatment of autism spectrum disorders: a systematic review". Res Autism Spectrum Disorders, 5(1), 86–88.

34. Buie, T. (2013). The Relationship of Autism and Gluten. Clinical Therapeutics [serial online], (5), 578. Available from: Academic OneFile, Ipswich, MA. Accessed September 10, 2016.

35. Johnson, C., Handen, B., Zimmer, M., Sacco, K., Turner, K. (2011). Effects of Gluten Free / Casein Free Diet in Young Children with Autism: A Pilot Study. Journal Of Developmental And Physical Disabilities [serial online], (3), 213. Available from: Academic OneFile, Ipswich, MA. Accessed September 10, 2016.

36. Pennesi, C., Klein, L. (2012). Effectiveness of the gluten-free, casein-free diet for children diagnosed with autism spectrum disorder: Based on parental report. Nutritional Neuroscience [serial online], 15 (2), 85-91.

37. Harris, C., Card, B. (2012). A pilot study to evaluate nutritional influences on gastrointestinal symptoms and behavior patterns in children with Autism Spectrum Disorder. Complementary Therapies In Medicine [serial online], 20(6), 437-440. Available from: MEDLINE, Ipswich, MA. Accessed September 10, 2016.

38. Genuis, S., Bouchard, T. (2010). Celiac Disease Presenting as Autism. Journal Of Child Neurology [serial online], 25(1), 114-119.

39. Hurwitz, S. (2013). The Gluten-Free, Casein-Free Diet and Autism: Limited Return on Family Investment. Journal Of Early Intervention [serial online], 35(1), 3-19.

40. Herbert, M., Buckley, J. (2013). Autism and Dietary Therapy: Case Report and Review of the Literature. Journal Of Child Neurology [serial online], 28(8), 975. Available from: Publisher Provided Full Text Searching File, Ipswich, MA. Accessed September 10, 2016.

41. Marí-Bauset, S., Zazpe, I., Mari-Sanchis, A., Llopis-González, A., Morales-Suárez-Varela, M. (2014). Evidence of the gluten-free and casein-free diet in autism spectrum disorders: a systematic review. Journal Of Child Neurology [serial online]. December, 29(12), 1718-1727.

42. Elder, J., Kreider, C., Schaefer, N., de Laosa, M. (2005). A review of gluten- and casein-free diets for treatment of autism: 2005-2015. Nutrition And Dietary Supplements [serial online], 2015, 87.

43. Hyman, S., Stewart, P., Smith, T., et al. (2016). The gluten-free/casein-free diet: A double-blind challenge trial in children with autism. Journal Of Autism And Developmental Disorders [serial online], 1, 205. Available from: Academic OneFile, Ipswich, MA. Accessed September 10, 2016.

44. Cornish, E. (2002). Gluten and casein free diets in autism: a study of the effects on food choice and nutrition. J. Hum. Nutr. Diet, 15, 261–269.

45. Konstantynowicz, J., Nguyen, T.V., Kaczmarski, M., et al. (2007). Fractures during growth: Potential role of a milk-free diet. Osteoporos Int, 18, 1601-1607.

46. Hediger, M. L., England, L. J., Molloy, C. A., Yu, K. F., Manning-Courtney, P., Mills J. L. (2008). Reduced bone cortical thickness in boys with autism or autism spectrum disorder. J. Autism Dev. Disord. 38, 848–856 10.1007/s10803-007-0453-6.

47. Monti, PM., Barnett, N.P., Colby, SM., Gwaltney, C.J., Spirito, A., Rohsenow, D.J., Woolard, R. (2007). Motivational interviewing versus feedback only in emergency care for young adult problem drinking. Addiction, 102, 1234–1243.

48. Black, K. E., Murray, J. A., & David, C. S. (2002). HLA-DQ determines the response to exogenous wheat proteins: a model of gluten sensitivity in transgenic knockout mice. J. Immunol, 169, 5595–5600 (2002).

49. Neumeyer, A.M., Gates, A., Ferrone, C., et al. (2013). Bone density in peripu-bertal boys with autism spectrum disorders. J Autism Dev Disord, 43, 1623-1629.

50. Bonder, M., Tigchelaar, E., Zhernakova A., et al. (2016). The influence of a short-term gluten-free diet on the human gut microbiome. Genome Medicine [serial online], 8