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(54) **Title:** METHODS AND PHARMACEUTICAL COMPOSITIONS OF THE TREATMENT OF AUTISTIC SYNDROME DIS-
ORDERS

(57) **Abstract:** The present invention relates to a method for the treatment of an autistic syndrome disorder comprising administering
to a subject in need thereof an effective amount of antibacterial agent.

**METHODS AND PHARMACEUTICAL COMPOSITIONS OF THE TREATMENT
OF AUTISTIC SYNDROME DISORDERS**

5 **FIELD OF THE INVENTION:**

The present invention relates to methods and pharmaceutical compositions for the treatment of Autistic Syndrome Disorders.

BACKGROUND OF THE INVENTION:

10 Infantile Autistic Syndrome Disorders (ASD) include a wide range of abnormalities including a genuine incapacity to organise affective relations, behavioural anomalies in reciprocal social interactions, verbal and non verbal communication, limited interest in the surrounding environment associated with stereotyped movements and repetitive plays (Kanner, 1943; Levy and Hyman, 1993; Levy and Hyman, 2005; Adrien et al., 2001; Blanc et
15 al., 2005; Bourreau et al., 2009). Research to date indicates that a genetic predisposition may play a role in the disease but one or more environmental factors must be in place for symptoms to occur including environmental contaminants and possibly maternal exposures during gestation (Persico and Bourgeron, 2006; Bourgeron, 2009; Patterson, 2002). It is suggested that genetic and environmental hazards will alter developmental programs leading
20 to cortical and/or sub-cortical malformations and the formation of misplaced/ misconnected neuronal ensembles. The first symptoms occur before 3 years of age with most likely an earlier origin. There is at present no efficient biological/ pharmaceutical treatment to ASD.

SUMMARY OF THE INVENTION:

25 The present invention relates to a method for the treatment of an autistic syndrome disorder comprising administering to a subject in need thereof with an effective amount of at least one antibacterial agent.

DETAILED DESCRIPTION OF THE INVENTION:

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The present invention relates to a method for the treatment of an autistic syndrome disorder comprising administering to a subject in need thereof with an effective amount of at least one antibacterial agent.

In a particular embodiment, the subject is diagnosed with autism. As used herein, the term “autism” denotes a family of disorders of neural development that is characterized by impaired social interaction and communication, restricted and repetitive behaviour accompanied with other deficits. These signs all begin before a child is three years old.

5 Autism affects information processing in the brain by altering how nerve cells and their synapses connect and organize; how this occurs is not well understood. The two other autism spectrum disorders (ASD) are Asperger syndrome, which lacks delays in cognitive development and language, atypical autism, diagnosed when full criteria for the other two disorders are not met, and PDD-NOS when pervasive developmental disorder are not

10 specified.

In a particular embodiment, the subject has been previously diagnosed with a latent bacterial infection. Typically said latent bacterial infection may be detected by detecting the presence of bacterial 16S sequence in a blood sample obtained from the subject (e.g. by RT-

15 PCR) or by performing the method as described in WO2007068831 or in US2012024701 in the blood sample, such as described in EXAMPLE 1 or 2.

As used herein the term “antibacterial agent” has its general meaning in the art. Antibacterial agents kill or inhibit the growth or function of bacteria. A large class of

20 antibacterial agents is antibiotics. Any kind of antibiotics may used according to the invention, but use of broadspectrum antibiotics are particularly desirable. A broad spectrum antibiotic for use in the invention is one that possesses activity against both grampositive and gram-negative organisms. Exemplary broad spectrum antibiotics for use in the invention include compounds falling within the following chemical classifications or categories:

25 aminoglycosides, macrolides, ketolides, quinolones, tetracyclines, sulfonamides, and beta-lactams (including the cephalosporins). In yet another embodiment, a broad spectrum antibiotic for use in the invention is one demonstrating a degree of anti-microbial activity comparable to that of any of the herein described aminoglycosides, macrolides, ketolides, quinolones, tetracyclines, sulfonamides, or beta-lactams, in particular, against species falling

30 within four or more different microbial genres selected from *Actinomyces*, *Bacillus*, *Bordetella*, *Borrelia*, *Campylobacter*, *Chlamydia*, *Clostridium*, *Corynebacterium*, *Cryptosporidium*, *Entamoeba*, *Enterobacter*, *Escherichia*, *Gardnerella*, *Haemophilus*, *Klebsiella*, *Legionella*, *Leishmania*, *Moraxella*, *Mycobacterium*, *Mycoplasma*, *Neisseria*,

Nocardia, Proteus, Providencia, Pseudomonas, Salmonella, Serpulina, Serratia, Shigella, Staphylococcus, Streptococcus, Suterella, Toxoplasmosis, Treponem, and Tubercle.

The first type of broad spectrum antibiotic for use in the invention, are tetracyclines. Tetracyclines belongs to a class that shares a four-membered ring structure composed of four
5 fused 6-membered (hexacyclic) rings. The tetracyclines exhibit their activity by inhibiting the binding of the aminoacyl tRNA to the 30S ribosomal subunit in susceptible bacteria. Tetracyclines for use in the invention include chlortetracycline, demeclocycline, doxycycline, minocycline, oxytetracycline, chlortetracycline, methacycline, mecocycline, tigecycline, limecycline, and tetracycline. The tetracyclines are effective against many known organisms
10 including a-hemolytic streptococci, nonhemolytic streptococci, gramnegative bacilli, rickettsiae, spirochetes, *Mycoplasma*, and *Chlamydia*.

Another type of broad spectrum antibiotics for use in the invention is the aminoglycosides. Aminoglycosides are compounds derived from species of *Streptomyces* or *Micomonospora* bacteria and are primarily used to treat infections caused by gram-negative
15 bacteria. Drugs belonging to this class all possess the same basic chemical structure, i.e., a central hexose or diaminohexose molecule to which two or more amino sugars are attached by a glycosidic bond. The aminoglycosides are bactericidal antibiotics that bind to the 30S ribosome and inhibit bacterial protein synthesis. They are active primarily against aerobic gram-negative bacilli and staphylococci. Aminoglycoside antibiotics for use in the invention
20 include amikacin (Amikin®), gentamicin (Garamycin®), kanamycin (Kantrex®), neomycin (Mycifradin®), netilmicin (Netromycin®), paromomycin (Humatin®), streptomycin, and tobramycin (TOBI Solution®, TobraDex®).

Yet another type of broad spectrum antibiotic for use in the invention is a macrolide. The macrolides are a group of polyketide antibiotic drugs whose activity stems from the
25 presence of a macrolide ring (a large 14-, 15-, or 16-membered lactone ring) to which one or more deoxy sugars, usually cladinose and desosamine, are attached. Macrolides are primarily bacteriostatic and bind to the 50S subunit of the ribosome, thereby inhibiting bacterial synthesis. Macrolides are active against aerobic and anaerobic gram positive cocci (with the exception of enterococci) and against gram-negative anaerobes. Macrolides for use in the
30 invention include azithromycin (Zithromax®), clarithromycin (Biaxin®), dirithromycin (Dynabac®), erythromycin, clindamycin, josamycin, roxithromycin and lincomycin.

Also suitable for use in the present invention are the ketolides, another type of broad spectrum antibiotic. The ketolides belong to a new class of semi-synthetic 14-membered ring macrolides in which the erythromycin macrolactone ring structure and the D-desosamine

sugar attached at position 5 are retained, however, replacing the L-cladinose5 moiety and hydroxyl group at position 3 is a 3-keto functional group. The ketolides bind to the 23S rRNA, and their mechanism of action is similar to that of macrolides (Zhan, G. G., et al., *Drugs*, 2001; 61(4):443-98). The ketolides exhibit good activity against gram-positive aerobes and some gram-negative aerobes, and possess excellent activity against *Streptococcus* spp. including *mefA* and *ermB*-producing *Streptococcus pneumoniae*, and *Haemophilus influenzae*. Representative ketolides for use in the invention include telithromycin (formerly known as HMR-3647), HMR 3004, HMR 3647, cethromycin, EDP-420, and ABT-773.

Yet another type of broad spectrum antibiotic for use in the invention is the quinolone class. Structurally, the quinolones possess a 1,4 dihydro-4-oxo-quinolinyl moiety bearing an essential carboxyl group at position 3. Functionally, the quinolones inhibit prokaryotic type II topoisomerases, namely DNA gyrase and, in a few cases, topoisomerase IV, through direct binding to the bacterial chromosome. Quinolones for use in the invention span first, second, third and fourth generation quinolones, including fluoroquinolones. Such compounds include nalidixic acid, cinoxacin, oxolinic acid, flumequine, pipemidic acid, rosoxacin, norfloxacin, lomefloxacin, ofloxacin, enrofloxacin, ciprofloxacin, enoxacin, amifloxacin, fleroxacin, gatifloxacin, gemifloxacin, clinafloxacin, sitafloxacin, pefloxacin, rufloxacin, sparfloxacin, temafloxacin, tosufloxacin, grepafloxacin, levofloxacin, moxifloxacin, and trovafloxacin. Additional quinolones suitable for use in the invention include those described in Hooper, D., and Rubinstein, E., *"Quinolone Antimicrobial Agents, 4th Edition"*, American Society of Microbiology Press, Washington D.C. (2004).

A broad spectrum antibiotic for use in the invention may also be a sulfonamide. Drugs belonging to the sulfonamide class all possess a sulfonamide moiety, $-\text{SO}_2\text{NH}_2$, or a substituted sulfonamide moiety, where one of the hydrogens on the nitrogen is replaced by an organic substituent. Illustrative N-substituents include substituted or unsubstituted thiazole, pyrimidine, isoxazole, and other functional groups. Sulfonamide antibiotics all share a common structural feature, i.e., they are all benzene sulfonamides, meaning that the sulfonamide functionality is directly attached to a benzene ring. The structure of sulfonamide antibiotics is similar to p-aminobenzoic acid (PABA), a compound that is needed in bacteria as a substrate for the enzyme, dihydropteroate synthetase, for the synthesis of tetrahydro-folic acid. The sulfonamides function as antibiotics by interfering with the metabolic processes in bacteria that require PABA, thereby inhibiting bacterial growth and activity. Sulfonamide antibiotics for use in the invention include the following: mafenide, phthalylsulfathiazole, succinylsulfathiazole, sulfacetamide, sulfadiazine, sulfadoxine,

sulfamazone, sulfamethazine, sulfamethoxazole, sulfametopirazine, sulfametoxypridazine, sulfametrol, sulfamonomethoxine, sulfamylon, sulfanilamide, sulfaquinoxaline, sulfasalazine, sulfathiazole, sulfisoxazole, sulfisoxazole diolamine, and sulfaguanidine.

Also suitable for use in the invention are the broad spectrum antibiotics classified structurally as beta-lactams. All members of this broad spectrum antibiotic class possess a beta-lactam ring and a carboxyl group, resulting in 55 similarities in both their pharmacokinetics and mechanism of action. The majority of clinically useful beta-lactams belong to either the penicillin group or the cephalosporin group, including cefamycins and oxacephems. The beta-lactams also include the carbapenems and monobactams. Generally speaking, beta-lactams inhibit bacterial cell wall synthesis. More specifically, these antibiotics cause 'nicks' in the peptidoglycan net of the cell wall that allow the bacterial protoplasm to flow from its protective net into the surrounding hypotonic medium. Fluid then accumulates in the naked protoplast (a cell devoid of its wall), and it eventually bursts, leading to death of the organism. Mechanistically, beta-lactams act by inhibiting D-alanyl-D-alanine transpeptidase activity by forming stable esters with the carboxyl of the open lactam ring attached to the hydroxyl group of the enzyme target site. Beta-lactams are extremely effective and typically are of low toxicity. As a group, these drugs are active against many grampositive, gram-negative and anaerobic organisms. Drugs falling into this category include 2-(3-alanyl)clavam, 2-hydroxymethylclavam, 7-methoxycephalosporin, epithienamycin, acetyl-thienamycin, amoxicillin, apalcillin, aspoxicillin, azidocillin, azlocillin, aztreonam, bacampicillin, blapenem, carbenicillin, carfecillin, carindacillin, carpetimycin A and B, cefacetril, cefaclor, cefadroxil, cefalexin, cefaloglycin, cefaloridine, cefalotin, cefamandole, cefapirin, cefatrizine, cefazedone, cefazolin, cefbuperazone, cefcapene, cefdinir, cefditoren, cefepime, cefetamet, cefixime, cefinenoxime, cefinetazole, cefminox, cefmolexin, cefodizime, cefonicid, cefoperazone, ceforamide, cefoselis, cefotaxime, cefotetan, cefotiam, cefoxitin, cefozopran, cefpiramide, cefpirome, cefpodoxime, cefprozil, cefquinome, cefradine, cefroxadine, cefsulodin, ceftazidime, cefteram, ceftazole, ceftibuten, ceftizoxime, ceftriaxone, cefuroxime, cephalosporin C, cephamycinA, cephamycinC, cephalothin, chitinovorin A, chitinovorin B, chitinovorin C, ciclacillin, clavulanate salt, clavulanic acid, clometocillin, cloxacillin, cycloserine, deoxy pluracidomycin B and C, dicloxacillin, dihydro pluracidomycin C, epicillin, epithienamycin D, E, and F, ertapenem, faropenem, flomoxef, flucloxacillin, hetacillin, imipenem, lenampicillin, loracarbef, mecillinam, meropenem, metampicillin, meticillin (also referred to as methicillin), mezlocillin, moxalactam, nafcillin, northienamycin, oxacillin, panipenem, penamecillin, penicillin G, N, and V, phenethicillin,

piperacillin, povampicillin, pivcefalexin, povmecillinam, prvmecillinam, pluracidomycin B, C, and D, propicillin, sarmoxicillin, sulbactam, sultamicillin, talampicillin, temocillin, terconazole, thienamycin, andticarcillin.

5 By a "an effective amount" is meant a sufficient amount of the antibacterial agent to for treating autism at a reasonable benefit/risk ratio applicable to any medical treatment.

It will be understood that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient
10 will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific
15 polypeptide employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Preferably, the compositions contain 0.01, 0.05, 0.1,
20 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, preferably from 1 mg to about 100 mg of the active ingredient. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day,
25 especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

Combination of antibacterial agents (e.g. antibiotics) is encompassed by the present invention.

30 In a particular embodiment, the subjected undergoes a sustained administration with the antibacterial agent. Typically, the subject is administered with the antibacterial agent for 1, 2, 3, 4 or 5 weeks.

In a particular embodiment, the subject may also be administered with antifungal agents or anti-parasitic agents.

In a particular embodiment, the subject is administered with the antibacterial agent optionally in combination with anti-fungal or or anti-parasitic agents following the typical 5 regiment : for 3 weeks per month during the 3 first months of treatment, then 15 days per month during the following three months, then 15 days every 2 months during the following 6 months and finally 3 or 4 courses of 10 days treatment the following years.

10 The antibacterial agent may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form pharmaceutical compositions.

In the pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local or rectal administration, the 15 active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, 20 intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms.

Preferably, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, 25 potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and 30 sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

Solutions comprising compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The antibacterial agent of the invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active polypeptides in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

5 For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the
10 present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

15 The antibacterial agent of the invention may be formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose or so. Multiple doses can also be administered.

In addition to the compounds of the invention formulated for parenteral
20 administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration ; liposomal formulations ; time release capsules ; and any other form currently used.

The invention will be further illustrated by the following figures and examples.
25 However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

Example 1:

30 The technology as described in WO2007068831 or in US2012024701 allows the detection of aqueous structures induced by certain DNA molecules that emit low frequency electromagnetic signals. These DNA sequences "sensors" are present in most bacteria potentially pathogenic in humans and induce nanostructures present in blood plasma or in certain dilutions of DNA extracted from plasma or blood cells. By performing said method

the inventors demonstrate that detection of a latent bacterial infection (but not viral infection) can be made for 70 to 90% of autistic children who were included in the study. Interestingly, in a blind study, the sole autistic child that were considered as negative for the presence of a latent bacterial infection was treated with antibiotics in a long-term manner. In another
5 example, a child who had signals saw them reduced after treatment and clinical improvement. This correlation between disappearance of the signals of bacterial and clinical improvement on antibiotics shows that the infection is not a simple consequence but is one of the causes of autism and signal detection can serve as a biomarker in clinical trials.

10 **Example 2: Autism: the gut-blood-brain connection: Improved Methods for the Detection and Diagnostic of Abnormal Bacteremia in Autistic Patients**

Within a decade, autism and its related disorders have become a major health problem worldwide. In most developed and even in developing countries, their incidence has been
15 growing to more than 1% of the total child population.

The reason for this continuous increase is unclear, but cannot be ascribed to genetic changes suddenly affecting the new generations. Rather, the increased exposure to changing environmental factors may be involved.

There is mounting indication that these environmental changes occurring at the
20 intestinal level may allow the abnormal passage of bacteria or bacterial products in the blood circulation which could then reach the brain. There is also evidence that the blood-brain barrier can become more permeable, due also to environmental changes.

Recently, the group of Williams and Lipkin has described a significant increase of a particular genus belonging to a Gram negative family (*Alcaligenaceae*), the bacteria
25 *Sutterella*, in ileal biopsies of autistic children suffering from gastro-intestinal disturbance, as opposed to non-autistic children suffering of the same affection.

The present example describes the abnormal presence of bacterial DNA in the blood of the majority of autistic children studied, and in particular of bacterial DNA identical or
30 close to that of the *Sutterella* genus.

This bacterial DNA is reduced by a long term antibiotic treatment of children which improves at the same time their clinical condition (example 3).

The detection of bacterial DNA is done by the use of two technologies:

a) One has already been described in several patent applications (WO2007068831 or in US2012024701).

5

In short, it consists in measuring the intensity of the electromagnetic signals emitted by some high water dilutions of DNA extracted from the plasma of such patients.

This DNA may originate from bacterial or viral DNA sequences. Filtration of the DNA solution by 100 nM porosity filters allows one to detect structures derived from
10 bacterial DNA.

Filtration at 20 nM porosity allows one to detect small structures derived from DNA of small DNA viruses and HIV DNA.

In the case of autistic patients, we have found that a majority of those who do possess in their plasma some DNA sequences inducing nanostructures able to emit EMS. Since
15 filtration at 100 nM was required, these nanostructures are presumed to be of bacterial origin.

This technology, in its present state, does not yet permit us to distinguish between bacterial species since the signals are similar.

However there are indications that the signals also contain the specific information for transmitting particular DNA sequences. This phenomenon has been reproduced in several
20 independent laboratories.

b) the classical technology, Polymerase Chain Reaction (PCR) to identify the species of bacteria involved.

In a first approach, we used primers able to detect all types of Gram positive bacteria
25 which yielded a majority of positive signals in a cohort of 22 autistic children but not in the same number of healthy children of matching age.

We also designed primers to recognize the group of Gram negative bacteria, based also on the 16 S ribosomal DNA. However our controls with pure sterile water were always positive due to the presence of small fragments contaminating bacterial DNA in various
30 samples of that water, whatever its treatment.

Finally we used primers specific for the Sutterella genus and have clear-cut results : a large majority of the plasma of autistic children yielded a specific DNA band of the required size (260 bp) and sequencing of the bands confirm that they belong to two closely related

families (*Alcaligenaceae* and *Burkholderiaceae*). Less frequently, we could detect *Borrelia* sequences, *the agent of Lyme disease*, by primers specific for its 16 S ribosomal DNA.

5 Table A: Distribution of EMS and Sutterella PCR in Autistic Children and healthy controls (French - Italian cohort)

	n	EMS		PCR Sutterella	
		+	-	+	-
Autistic children	78	68 (87%)	10 (13%)	65 (83%)	13 (17%)
Controls	28	1 (3.5%)	27(96.5%)	3 (10%)	25 (89%)

Legend: EMS = Electromagnetic Signals

N = Number of patients

PCR = Polymerase Chain Reaction.

10

Example 3: Clinical study

Study: 97 children were included in the study: children diagnosed with autism (n=73), atypical autism (n=10), Dravet syndrome (n=4), Rett syndrome (n=2), Asperger syndrome
 15 (n=3), epilepsy with mental retardation (n=3) and Gilles de la Tourette syndrome (n=2). 88% of the children were aged between 2,5 years old and 12 years old (min = 15 months old and max = 29 years old). Said children were administered with broad spectrum antibiotics for 3 weeks: for children older than 8 years old with macrolides and children older less than 8 years with tetracyclins. Furthermore the children were administered with an antifungal agent
 20 (Triflucan) and with anti-parasitic agents (Fluvermal and Flagyl). Nutritional and immunological deficiencies were also corrected.

Results: The treatment was interrupted for 17% of the children due to side effects. Slow or jagged progression was observed for 28% of the children. Rapide and regular
 25 progression was observed for 55% of the children (Tables 1 and 2). More particularly, in the first month, improvement in physical signs can be noticed. In a second time, behavioural symptoms are improved in a progressive manner. In a third time, mental progression can resume its course to where it was interrupted (psychomotricity, learning, communication, and language and graphics). Administration of antibiotics, regular at the beginning, may become

less frequent when improvement are observed and finally may be interrupted with an annual control. In some cases, the clinical improvement obtained is durable and persists after cessation of treatment.

<i>Table 1</i>	<i>Rapid progression</i>	<i>Slow progression</i>	<i>Insufficient progression</i>	<i>Treatment interrupted</i>
Autism (n=73)	41 (56%)	19 (26%)	8 (11%)	5 (7%)
Atypical (n=10)	7	3		
DRAVET (EMSN) (n=4)	2	2		
RETT (n=2)	1	1		
epilepsy with mental retardation (n=3)	1	1	1	
Asperger (n=3)			3	
Gilles de la Tourette syndrome (n=2)	1	1		
TOTAL = 97	53 (55%)	27 (28%)	12 (12%)	5 (5%)

5

<i>Table 2</i>	<i>Rapid progression</i>	<i>Slow progression</i>	<i>Insufficient progression</i>	<i>Treatment interrupted</i>
Autistic children ≤ 7 years old (n=45)	32 (71%)	6 (13%)	3 (7%)	4 (9%)
Autistic children > 7 years old (n=28)	9 (32%)	13 (46%)	5 (18%)	1 (4%)

REFERENCES:

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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5 Regulation of cognitive activity and early communication development in young autistic, mentally retarded, and young normal children. *Dev Psychobiol* 39:124-136.

Blanc R, Adrien JL, Roux S, Barthelemy C (2005) Dysregulation of pretend play and communication development in children with autism. *Autism* 9:229-245.

Bourgeron T (2009) A synaptic trek to autism. *Curr Opin Neurobiol* 19:231-234.

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CLAIMS:

- 5 1. A method for the treatment of an autistic syndrome disorder comprising administering to a subject in need thereof with an effective amount of at least one antibacterial agent.
2. The method according to claim 1 wherein the subject was previously diagnosed with a latent bacterial infection.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/055834

A. CLASSIFICATION OF SUBJECT MATTER
 INV. A61K31/00 A61P25/00
 ADD.
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
 Minimum documentation searched (classification system followed by classification symbols)
 A61K A61P
 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/93904 A1 (US DEPT VETERANS AFFAIRS [US]; FINEGOLD SIDNEY M [US]) 13 December 2001 (2001-12-13) page 8, line 5 - line 30; claim 12; example 1	1,2
X	----- CORNELIA BRENDEL ET AL: "Readthrough of nonsense mutations in Rett syndrome: evaluation of novel aminoglycosides and generation of a new mouse model", JOURNAL OF MOLECULAR MEDICINE, SPRINGER, BERLIN, DE, vol. 89, no. 4, 1 December 2010 (2010-12-01), pages 389-398, XP019889972, ISSN: 1432-1440, DOI: 10.1007/S00109-010-0704-4 the whole document -----	1
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Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>
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Date of the actual completion of the international search 11 April 2013	Date of mailing of the international search report 08/05/2013
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Loher, Florian
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/055834

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/069399 A1 (GANT THOMAS G [US] ET AL) 18 March 2010 (2010-03-18) claim 59 -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2013/055834

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
WO 0193904	A1	13-12-2001	AU 6974201 A	17-12-2001
			US 2002013270 A1	31-01-2002
			US 2004167062 A1	26-08-2004
			WO 0193904 A1	13-12-2001

US 2010069399	A1	18-03-2010	NONE	
