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Therapies and therapeutic approaches in Congenital Disorders of Glycosylation

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Abstract Inborn errors in glycoconjugate biosynthesis termed 'Congenital Disorders of Glycosylation' (CDG) comprise a rapidly expanding group of metabolic diseases in man. Up till now more than 60 different inherited disorders in N- and O-glycosylation pathways have been identified. They affect the biosynthesis of glycan moieties linked to proteins as well as lipids. Due to failures in protein glycosylation, CDG patients suffer from multi systemic disorders, which mostly present with severe psychomotor and mental retardations, muscular impairment, ataxia, failure to thrive and developmental delay. Although improved biochemical and genetic investigations led to identification of a variety of new molecular defects in glycoconjugate biosynthesis, effective therapies for most types of the CDG are so far not available. Therefore, intensive investigations on treatment options for this group of diseases have been carried out in recent years.

Keywords Congenital Disorders of Glycosylation · N-glycosylation · O-glycosylation · Glycosylation deficiency · Therapy · Therapeutic approaches

Introduction

Carbohydrates linked to glycoproteins or glycolipids comprise a group of highly abundant molecules in nearly all organisms. The oligosaccharide moieties affect a variety of physicochemical properties like stability, solubility and polarity of the glycoconjugates, which are involved in a variety of intra and extra cellular processes like quality control, directed transport and biological activity. Nevertheless, sugar chains also influence pathologic processes such as tumour progression [1–3]. In contrast to other biological polymers like RNA, DNA or proteins, carbohydrates show the phenomenon of branching, which leads to an enormous diversity.

Hereditary diseases in the pathways of glycoconjugate biosynthesis lead to severe human deficiencies termed 'Congenital Disorders of Glycosylation' (CDG). Until now more than 60 different types of the CDG have been found with a progressively increasing number of patients who have been identified due to continuously improved diagnostics [4–6]. Nevertheless, the complexity of the affected metabolic pathways leads to the assumption, that the number of inherited molecular defects in glycoconjugate biosynthesis is much higher and a lot of CDG patients have not been identified so far.

CDG patients show a multisystemic clinical phenotype, which mostly presents with psychomotor and mental retardation, peripheral neuropathy, cerebellar atrophy, retinitis pigmentosa, cardiomyopathy, hepatopathy, blood clotting problems and muscular convulsion [7].

In recent years, newly developed biochemical and molecular biological methods improved effective diagnostic strategies in this group of diseases and led to, at least partially, effective therapies for MPI-CDG (formerly CDG-Ib), SLC35C1-CDG (formerly CDG-IIc) and ALG8-CDG (formerly CDG-Ih) in case of the N-glycosylation pathway and for PIG-M-CDG in GPI anchor biosynthesis. Nevertheless, therapeutic options for patients suffering from other CDG types are so far not available. Here we present an overview on the currently existing therapeutic methods and underline the progress in finding new treatment options for other CDG types.

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CDG types with treatment options

Deficiency of phosphomannose isomerase (MPI) lead to MPI-CDG

The molecular defect in MPI-CDG is caused by mutations in the cytosolic enzyme phosphomannose isomerase (MPI), which catalyzes the conversion of fructose-6-phosphate to mannose-6-phosphate (Fig. 1). Reduced MPI activity leads to a decreased level of GDP-mannose and its downstream metabolic product dolichol-phosphate-mannose. The severely reduced amount of both metabolic key substrates in dolichol-linked oligosaccharide assembly leads to a defect in the biosynthesis of full-length oligosaccharides, which ends up in a reduced transfer of sugar chains onto newly synthezised glycoproteins [8].

In contrast to most other CDG types, the patients present mostly without dysmorphic features, developmental delay and neurological manifestations. Rather, the characteristic clinical hallmarks of this disease are hepatomegaly combined with gastrointestinal problems as chronic diarrhea, recurrent vomiting and protein-losing enteropathy, mostly leading to failure to thrive. Until now, more than 30 patients affected by MPI-CDG are known [9, 10].

Therapy

Oral application of mannose elevates its serum concentration, which leads in turn to an increased import of mannose into the cytosol, where it is converted to mannose-6-phosphate in a MPI-independent manner (Fig. 1). Since MPI deficiency can be bypassed, remarkable biochemical and clinical improvements occur in the patients. In the best case normalization of hypoproteinemia, blood coagulation, protein-losing enteropathy, normalization of the serum transferrin isoelectric focusing pattern, hypoglycemia as well as diarrhea, reduction of hepatomegaly and normalization of growth is achieved [10]. Nevertheless, the improvement of clinical symptoms is patient-dependent. In some cases, symptoms like liver problems persisted or even worsened under mannose supplementation [11].

Mannose application of 0.1–0.2 g/ kg bodyweight/ every 4 h for 3–5x per day is recommended [8, 12] but has to be taken as guidance level only and must be determined for each patient individually by following the enhancement of biochemical (*e.g.* plasma mannose level, coagulation parameters, glycosylation state of serum transferrin) and clinical parameters (*e.g.* vomiting, gastrointestinal bleeding, diarrhoea) over some weeks. Provided that mannose is not overdosed [12, 13], it is generally well tolerated and therefore serves as a potent and easy to handle therapeutic agent.

Nevertheless, by implementation of a mannose therapy some points need special attention. In case of severely affected patients with low residual MPI activity, external mannose addition should be strictly justified to prevent an accumulation of mannose-6-phosphate in the cells. As has been learned from a knockout mouse model for MPI-CDG [14], due to the enzymatic blockage an excess of phosphorylated mannose would not flow off to glycolysis. Besides, mannose-6-phosphate would further inhibit glycolysis and lead as well to the so called 'honey-bee effect' as lethal consequence [15, 16].

Such an intracellular energy deficiency presumably based on a surplus of mannose-6-phosphate in the cells was recently described in case of the MPI-CDG index patient, who presented under oral mannose supplementation without

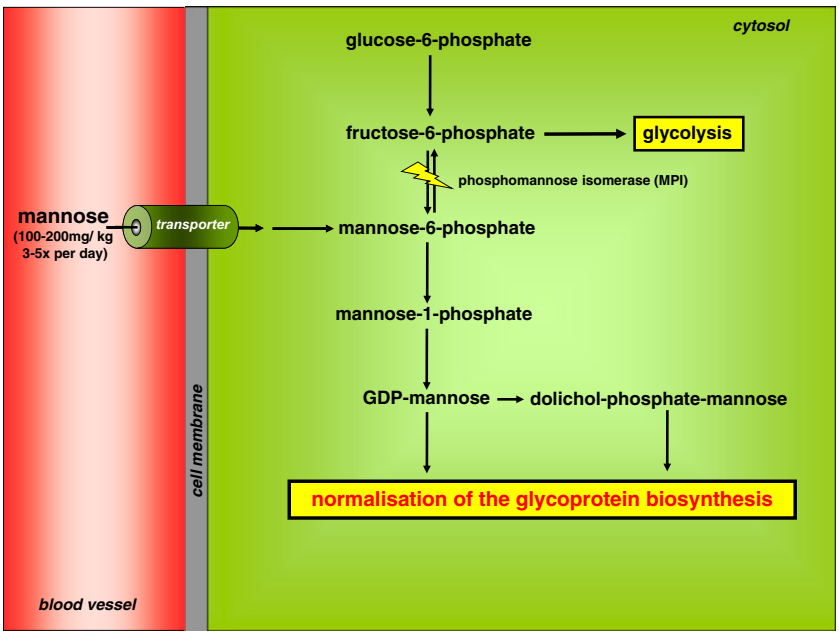

Fig. 1 *Mannose supplementation as therapy for MPI-CDG.* In MPI-CDG the conversion of fructose-6-phosphate to mannose-6-phosphate is affected due to reduced activity of phosphomannose isomerase (indicated by a yellow flash), leading to a severe reduction of GDP-mannose and dolichol-phosphate-mannose as essential donor substrates for dolichol-linked oligosaccharide biosynthesis. By oral mannose supplementation the severely reduced enzymatic activity of MPI in the patients can be bypassed, which leads to normalisation of glycoprotein biosynthesis

symptoms for many years [8, 17, 18]. Due to surgery, oral mannose treatment was switched to intravenous treatment while retaining identical sugar amounts (1 g/ kg bodyweight/ day). The reason is unclear, but intravenous mannose application led to life threatening central nervous system disturbance and transient hepatic dysfunction. It is noteworthy that the patient's symptoms could be overcome by continuous glucose supply to support his energy metabolism [18], proving the necessity of strictly monitoring MPI-CDG patients treated with mannose, especially in the event of hospitalization.

Furthermore, some MPI-CDG patients show intolerance against mannose, leading to progression of diarrhoea and abdominal pain, which prevents mannose supplementation. Initiating heparin therapy might help here to overcome at least their protein-losing enteropathy [19] which should be kept in mind when mannose fails.

Deficiency of the Golgi GDP-fucose transporter (SLC35C1) lead to SLC35C1-CDG

The clinical picture of SLC35C1 was first described in 1992 as 'Leukocyte Adhesion Deficiency type II' (LAD II) according to the most striking symptom of the disease [20]. The clinical characteristics include severe mental retardation prevalently associated with microcephaly, cerebral atrophy and convulsions and profound leukocytosis with recurrent infections. Physical stigmata include a short stature and a distinctive facial appearance, characterised by *e.g.* a flat face with a depressed nasal bridge, antiverted nostrils and long eyelashes. Biochemical features imply, among others, a defective selectin ligand formation caused by lack of sialyl Lewis X and the presence of the Bombay blood phenotype due to loss of the fucosylated antigen H on erythrocytes. The molecular cause for SLC35C1-CDG has been identified in mutations in the Golgi GDP-fucose transporter (SLC35C1; [21, 22]), leading to a severely reduced import of GDP-fucose into Golgi vesicles [23] and subsequently to a generalized hypofucosylation of N-linked glycoproteins. Up to date seven SLC35C1 patients are described [20, 24–27].

Therapy

SLC35C1-CDG represents the second CDG deficiency with partial success in treatment by application of the monosaccharide sugar L-fucose. The effect of fucose supplementation is not completely understood but it is hypothesized that the increase of the GDP-fucose pool in the cytosol leads to a higher import of GDP-fucose into the Golgi as well (Fig. 2). In contrast to MPI-CDG, where the biochemical defect can smartly be bypassed by mannose supplementation, the therapeutic outcome for SLC35C1-CDG patients is highly addicted to their affected transporter itself. Depending on the mutation(s) and the associated residual activity of SLC35C1, oral treatment with L-fucose either leads to improvement of biochemical and clinical symptoms or not [25, 28–32].

In case of the two patients with positive response to fucose, oral therapy was started at 25 or 33 mg/ kg bodyweight 5x per day and increased within eight months to 492 or 1000 mg/ kg bodyweight 5x per day, respectively [28, 32]. Fucose supplementation led to re-expression of selectin ligands on neutrophils and correction of the hypofucosylation of serum glycoproteins within 2 weeks. Subsequently, the number of peripheral neutrophils reduced to normal levels and infections or episodes of fever of unknown origin disappeared [28, 30–32]. Nevertheless, both treated patients generated fucosylated antigens on neutrophiles, leading to an autoimmune response in one of them [32, 33]. This exhibits the necessity of closely monitoring distinct biochemical markers as *e.g.* levels of hemoglobin, reticulocyte and haptoglobin or expression of fucosylated structures on the surfaces of erythrocytes in parallel to initiating a fucose therapy to prevent the risk of hemolysis [32].

Deficiency of the ALG8-glucosyltransferase (ALG8) lead to ALG8-CDG

Different from the abovementioned CDG types, which could be treated in part due to the application of monosaccharides, a comparable therapy is thus far not available for ALG8-CDG. Nevertheless, as some of the clinical symptoms in case of the ALG8 index patient could be enhanced, it should be cited here. In contrast to the mostly severely affected ALG8 patients, the female index patient presented without dysmorphic symptoms, hypotonia, gastrointestinal disorders, hepatomegaly, coagulopathy, edema and cardiorespiratory problems, but with massive diarrhoea and moderate hepatomegaly. Due to her severe digestive complications a low fat diet in association with essential fatty acid supplementation was initiated, which gradually led to correction of diarrhoea and protein-losing enteropathy [34].

As heparin used in MPI-CDG to treat diarrhoea and protein-losing enteropathy in patients with mannose intolerance, the low fat diet in association with essential fatty acid supplementation used in ALG8-CDG does not display a therapy for the glycosylation deficiency itself. Nevertheless, since the intestinal problems were successfully overcome, the symptomatical treatment indicated here might also be useful for patients of other CDG types.

Deficiency of phosphatidylinositol-glycan biosynthesis class M protein (PIG-M) leads to PIG-M-CDG

Glycosylphosphatidylinisotol (GPI) anchors are synthesized at the cytosolic and the luminal part of the endoplasmic reticulum and link proteins to the cell surface at the outside

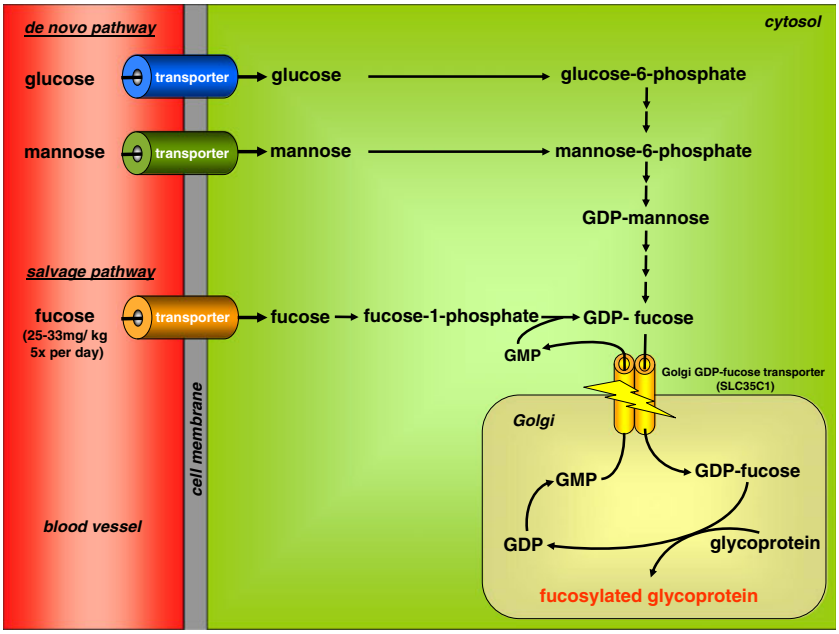

Fig. 2 *Fucose supplementation as therapy for SLC35C1-CDG.* Cytosolic GDP-fucose is derived from the *de novo* pathway by use of glucose or mannose as substrate and from the *salvage* pathway by utilization of fucose. For fucosylation processes in the Golgi, GDP-fucose is transported from the cytosol into the Golgi by antiport with GMP, which is catalyzed by the transporter SLC35C1. Due to a severely reduced transporter activity in SLC35C1-CDG (indicated by a yellow flash) insufficient amounts of fucose residues are transferred to glycoconjugates leading to a general hypofucosylation. It is assumed that due to an oral fucose supplementation fucose residues enter the cell over the salvage pathway leading subsequently to an enhanced synthesis of GDP-fucose. In those cases in which the SLC35C1 transporter possesses a distinct residual activity, the elevated allocation of the donor substrate results in an increased transport of GDP-fucose into the Golgi, whereby an improvement of biochemical and clinical symptoms is achieved

of the plasma membran. GPI anchored proteins, which are found in all eukaryotic cells, fulfill functions necessary for *e.g.* enzymatic activity, antigenic properties and adhesion or receptor-mediated signal transduction. Deficiency of the PIG-M/ PIG-X mannosyltransferase complex caused by mutations in the promotor region of the catalytic subunit PIG-M leads to severely reduced transfer of mannose residues from dolichol-phosphate-mannose in α1-4 orientation to the structure glucosamine-(acyl) phosphatidyl inositol, subsequently to loss of GPI biosynthesis and finally to PIG-M-CDG. Clinical characteristics of PIG-M-CDG patients comprise portal and hepatic vein thrombosis by the age of 2 years, persistent absence seizures and positive Ham test, presumably due to absence from the erythrocyte surface of the GPI-linked complement inhibitor CD59 [35].

Treatment with sodium phenylbutyrate was initiated in a PIG-M-CDG patient, who was wheelchair-bound and showed global hypotonia, drooling, drowsiness and frequent seizures, at a dose of 20 mg/ kg bodyweight 3x per day and raised to 30 mg/ kg bodyweight 3x per day after 2 month. Due to phenylbutyrate supplementation *PIG-M* transcription and therewith cell-surface GPI expression increased significantly, leading to a general improvement of the clinical symptoms, *e.g.* ability to walk again, interact and feed itself after the treatment. Besides, the seizures disappeared within two weeks [36, 37].

If sodium phenylbutyrate can serve as potent therapeutic in case of patients with different mutations in the *PIG-M* promotor structure or in the coding region remains questionable. Nevertheless, the clinical improvement achieved for this patient is striking and thereby appreciated.

Therapeutic approaches in CDG

The partially very successful therapies for the abovementioned CDG defects have embolden to search also for therapeutic approaches for other types of the CDG. Huge progress is achieved for the most common CDG type, PMM2-CDG (formerly CDG-Ia), which affects about 80 % of the patients worldwide and as well for the dystroglycanopathies from the group of congenital muscular dystrophies.

A promising concept for a PMM2-CDG therapy that was initially pursued was to counter the increased K_m requirements of mutated PMM2 by raising the intracellular substrate levels of mannose-6-phosphate by either oral or intravenous mannose supplementation. This would lead to higher levels of mannose-1-phosphate when metabolized by attenuated PMM2, increases in production of GDP-mannose and dolichol-phosphate-mannose, and subsequent normalization of glycosylation (Fig. 3). Although in PMM2-CDG

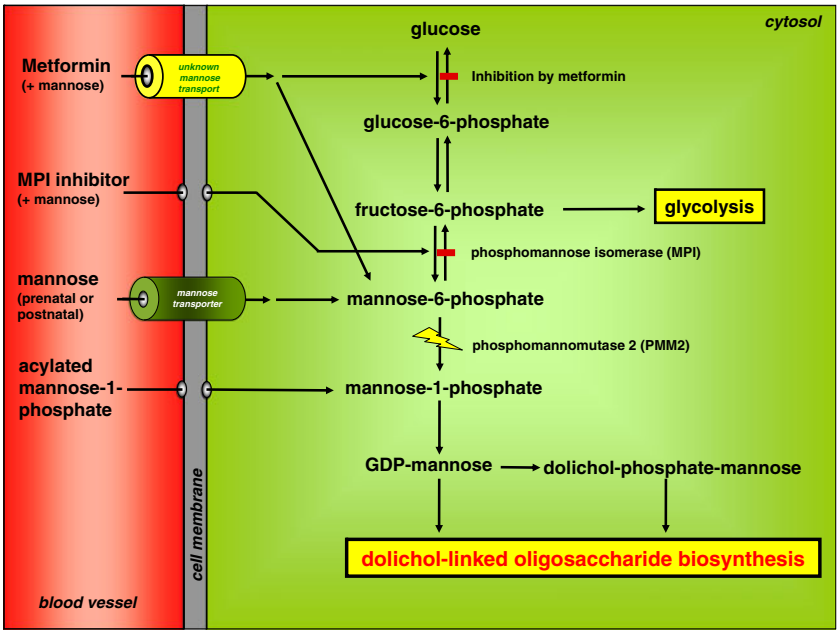

Fig. 3 *Therapeutic approaches for PMM2-CDG.* Phosphomannomutase 2 (PMM2) catalyzes the conversion of mannose-6-phosphate to mannose-1-phosphate in the cytosol. Due to reduced enzymatic activity (indicated by a yellow flash) reduced levels of GDP-mannose and dolichol-phosphate-mannose is the result, leading to an impaired dolichol-linked oligosaccharide biosynthesis in the cell. To enhance the allocation of full-length oligosaccharides several therapeutic approaches are envisaged. Since all PMM2-CDG patients exhibit a distinct residual PMM2 activity the addition of Metformin or of an inhibitor for phosphomannose isomerase together with mannose potentially promotes the flux of mannose towards the synthesis of lipid-linked oligosaccharides. Besides, application of mannose already during pregnancy might have a positive effect on organ development and thereby on the overall clinical outcome. In contrast to the abovementioned approaches, a therapy with acylated mannose-1-phosphate would bypass the PMM2 blockage. Red boxes indicate biochemical inhibition

patient-derived fibroblasts the biochemical defect could be corrected by either addition of mannose or reduction of the glucose concentration [38, 39] to cell culture medium, neither oral supplementation of PMM2 patients with mannose over a period of six months nor intravenous mannose supplementation for three weeks led to any measurable positive effect on coagulation abnormalities or IEF serum transferrin patterns [40–42], assumably due to transferring the surplus of mannose to glycolysis.

Since an increase of the PMM2's substrate pool failed as therapy, one newer approach for healing this deficiency is to bypass the defective enzyme by application of the product of PMM2, mannose-1-phosphate. Unfortunately, mannose-1-phosphate is not capable to pass cellular membranes due to its hydrophilic nature, which necessitates synthesis of membrane-permeable acylated derivatives of mannose-1-phosphate [43–45; Fig. 3]. Although these derivatives cured the biochemical defect in cell culture, neither its toxicity nor its effectiveness are yet clear and still have to be determined. Besides the difficulties in synthesis, the amount of material is small, making a lifelong therapy for PMM2-CDG patients questionable.

As opposed to this, Metformin is used since many years as oral drug in the biguanide class to treat *e.g.* diabetes type 2. By addition of Metformin to the cell culture medium of PMM2-CDG fibroblasts positive stimulation of D-mannose transport in these cells was shown that helped to correct the dolichol-linked oligosaccharide biosynthesis and subsequently the hypoglycosylation of nascent glycoproteins, potentially due to activation of an alternative mannose-selective transport system ([46]; Fig. 3). If Metformin can serve as therapeutic enhancer for mannose transportation in PMM2 patients needs to be determined.

Another approach is to reduce the enzymatic activity of phosphomannose isomerase (MPI) on the one hand and to carefully increase the mannose-6-phosphate level in the cells on the other hand (Fig. 3), which could lead to a higher flux of mannose into the affected dolichol-linked oligosaccharide biosynthesis. In this regard it was recently shown that a MPI inhibitor from the benzoisothiazolone series indeed enhanced the metabolic flow of substrate into the depleted glycosylation pathway in the cell culture system [47, 48]. Anyway, how this substance is tolerated by humans and how the medication together with mannose works, has to be resolved. Outcome of both therapeutic approaches with Metformin but as well as with MPI inhibitors together with mannose will be foremost depending on the residual PMM2 activity of the respective patient, meaning that especially the severely affected patients will have the least profit. But anyhow, a little bit is better than nothing.

Establishing a therapy is difficult and the cell culture system has clear limits when estimating its beneficial outcome, also demonstrating the necessity to analyse a therapeutic approach in an entire organism. For that reason since many years a lot of animal models have been generated in the field of N- and O-glycosylation. Beside knockout, knockin and hypomorphic mouse lines [49, 50] also drosophila [51, 52], zebrafish [53–55] and caenorhabditis models [56, 57] were established and even more are planned for different types of the CDG hereafter. These animal models will help to understand the pathophysiology of the diseases and will be an essential tool to study therapeutic approaches.

Very recent results in a hypomorphic mouse model for PMM2-CDG might give hope for a future therapy for women at risk for a PMM2-CDG child. After feeding pregnant dams with mannose, the lethality of compound-heterozygous embryos was overcome and normal life was possible thereafter, indicating that mannose treatment in the patients might have been started too late [58].

Besides, an auspicious therapeutic concept for the dystroglycanopathies evaluated in different mouse lines showed that by adenoviral-transmitted gene transfer of *Large,* expression of the protein in Large-deficient mice or an upregulation of Large expression in fukutin- and PomGnT1-deficient mouse lines was achieved, respectively. This led to enhancement of the glycosylation status of alpha-dystroglycan and thus to a decrease in muscle disorder [59, 60]. Although the biochemical path treaded by this kind of therapy is not that clear, the approach looked safe and might also be useful for other types of dystroglycanopathies [50, 61].

Conclusion

The field of glycosylation deficiencies has grown rapidly especially in the last decade and is assumed to further expand. Nevertheless, the low number of CDG patients worldwide limits commercial interests in the development of therapeutic approaches so far: Therefore, most research attempts are currently carried out by research groups from universities and hospitals. The successful treatment of some CDG types that has been achieved in recent years yet indicates that research on new therapeutics for so far untreatable CDG is essential and merits further intensified investigations in the future.

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