Preprints.org

Article

Not peer-reviewed version

IgG Glycosylation Analysis in Patients with Ring14 Syndrome Unveils Novel Pathomechanisms and New Therapy Perspectives

Angela Messina [†], Angelo Palmigiano [†], Donata Agata Romeo, Luisa Sturiale, Enrico Parano, Marco Crimi, Annunziata Carrese Cirillo, Enrico Parano, Rita Barone, Domenico Garozzo [*,‡]

Posted Date: 13 April 2026

doi: 10.20944/preprints202604.0844.v1

Keywords: Ring 14; N-glycans; IgG; CDG; IVIG

Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2026 doi:10.20944/preprints202604.0844.v1

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

IgG Glycosylation Analysis in Patients with Ring14 Syndrome Unveils Novel Pathomechanisms and New Therapy Perspectives

Angela Messina [1,†], Angelo Palmigiano [1,†], Donata Agata Romeo [1], Luisa Sturiale [1], Enrico Parano [2], Marco Crimi [3,4], Annunziata Carrese Cirillo [5], Enrico Parano [2], Rita Barone [7] and Domenico Garozzo [1,*,‡]

[1] CNR – Institute for Polymers, Composites and Biomaterials (IPCB), Catania, Italy
[2] CNR - Institute for Biomedical Research and Innovation (IRIB), Catania, Italy
[3] Ring14 Italy, Via Flavio Gioia 5, 42124, Reggio Emilia, Italy
[4] Kaleidos SCS, Scientific Office, Via Andrea Moretti, 24121 Bergamo, Italy
[5] Azienda Unità Sanitaria Locale di Reggio Emilia, Reggio Emilia, Italy
[6] Child Neurology and Psychiatry Section, Department of Clinical and Experimental Medicine, University of Catania, Catania
* Correspondence: domenico.garozzo@cnr.it
† These authors contributed equally.
‡ Current address: CNR - Institute for Biomedical Research and Innovation (IRIB), Catania, Italy.

Abstract

Ring chromosome 14 (RC14) syndrome is an ultra-rare disorder characterized by drug-resistant epilepsy, intellectual disabilities, autism, and recurrent infections, suggesting a possible underlying immune dysregulation. We analyzed immunoglobulin G (IgG) N-glycosylation profile in six RC14 patients and compared it with age-matched healthy controls using ultra-high performance liquid chromatography (UHPLC) coupled with fluorescence detection (FLR) and high-resolution electrospray ionization mass spectrometry (ESI-MS). Patients showed decreased galactosylation and sialylation, resembling pro-inflammatory patterns observed in autoimmune diseases. These alterations were not observed in total serum glycoproteins, indicating a selective effect on IgG. One patient treated with intravenous immunoglobulin (IVIG) showed clinical improvement, which led us to investigate around causality.

Keywords: Ring 14; N-glycans; IgG; CDG; IVIG

1. Introduction

Ring chromosome 14 (RC14) syndrome is an ultra-rare genetic disorder caused by a structural rearrangement of chromosome 14, leading to the formation of a ring-shaped chromosome. This abnormality results in genomic instability and variable loss of genetic material, producing heterogeneous clinical manifestations.

The syndrome was first described in 1971 by Gilgenkrantz and colleagues [Gilgenkrantz et al. 1971]. Since its initial identification, RC14 syndrome has remained a subject of ongoing research due to its rarity and the complexity of its genetic basis. The precise prevalence and incidence of RC14 syndrome remain unknown, reflecting the challenges in diagnosing and reporting such rare chromosomal disorders. To date, fewer than 100 patients have been reported worldwide (Rinaldi et al. 2017). The size of the terminally deleted region varies, ranging from less than one to several megabases.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2026 doi:10.20944/preprints202604.0844.v1

RC14 syndrome is characterized by distinctive facial features, developmental delay evolving in severe intellectual disability, postnatal microcephaly, retinal abnormalities, and drug-resistant epilepsy [Incecik et al. 2013; Zelante et al. 1991].

Children and adults with RC14 have a marked susceptibility to infections, including severe and recurrent respiratory infections requiring hospitalization, representing a major cause of death in patients with RC14. In addition to possible increased vulnerability due to hypoxia and feeding difficulties with aspiration risk, immune dysregulation has been reported in clinical cohorts [Rinaldi et al. 2017; Vaisfeld et al. 2024].

Furthermore, fever episodes may trigger rapid clinical deterioration, especially in the presence of comorbidities such as epilepsy or malnutrition, supporting an improper immunological response in RC14 patients. As immunoglobulin G (IgG) levels remain consistent, functional, rather than quantitative IgG abnormalities may be implicated such as aberrant glycosylation since it can profoundly influence IgG function [Krawczun et al.1984; Krištić et al. 2024]. N-glycosylation is the most common glycosylation process in humans, with about 80–90% of glycoproteins being N-glycosylated. In the N-glycosylation pathway a glycan is linked to the nitrogen of an asparagine (Asn) residue, usually within an "Asn-X-Ser/Thr" sequence (where X is any amino acid except proline). The N-glycosylation process consists of two main steps: (i) synthesis and attachment of the oligosaccharide in the ER (cytosol and endoplasmic reticulum, (ER)), and (ii) maturation by trimming and elongation in both the ER and Golgi [Stanley et al. 2022]. This biosynthesis involves about a hundred enzymes resulting in a heterogeneous N-glycosylation producing a diversity in sugar structures (glycans) attached to proteins creating different glycoforms that affect protein function, folding, stability, and immune response.

N-Glycosylation is essential for IgG function, influencing its interaction with immune cells and activation of immune responses. Patterns in IgG glycosylation affect effector functions such as cytotoxicity and immune cell activation [Arnold et al. 2007; Cobb 2020; Krištić et al. 2024, Subedi & Barb 2015].

N-glycosylation can be affected by genetic [Ondruskova et al. 2021; Sturiale et al. 2011], physiological, metabolic, and external factors such as inflammation and other disease conditions [Krištić et al.2024].

Here, we present the IgG N-glycomic profile of six patients with proven RC14 and the comparison with healthy individuals. We found remarkable differences that might be related to the observed susceptibility to recurrent infections thus unveiling possible mechanisms and therapeutic venues.

2. Materials and Methods

2.1. Patients

This observational cross-sectional study included six Italian patients with RC14.

Inclusion criteria: (1) a confirmed diagnosis of RC14 syndrome established through standard cytogenetic analysis and molecular characterization; (2) documented clinical features consistent with the syndrome, including epilepsy, developmental delay/intellectual disability, and ophthalmological abnormalities; (3) availability of adequate serum volume to permit IgG purification and subsequent N-glycan profiling; and (4) informed consent obtained from patients, allowing the use of biological samples for research purposes.

This study was based solely on information and investigations that were carried out as part of the routine clinical care of RC14 patients. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee at Policlinico "G. Rodolico-San Marco" Catania and with the 1964 Helsinki declaration and its later amendments.

Table 1. Summarized clinical data of the patients in this study. (Further clinical data can be requested to the corresponding author).

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2026 doi:10.20944/preprints202604.0844.v1

Patient N°	1	2	3	4	5	6
Code	**RING11510M**	**RING13910M**	**RING38010F**	**RING13310F**	**RING23711M**	**RING12110M**
Age (years) at study time	**20**	**30**	**10**	**22**	**14**	**16**
14q deletion size	2 Mb	1.5 Mb	4.95 Mb	0.5 Mb	0.35 Mb	2.5 Mb
Monosomic cells on Peripheral blood	20%	20%	n.a.	18%	12%	19%
Age at seizure onset	15 months	2 months	8 months	9 months	3 months	4 months
Epileptic phenotype*	Generalized Clonic	Generalized tonic-clonic	Generalized Clonic	Focal to bilateral tonic-clonic seizures	Generalized tonic-clonic	Clonic
Main infectious events recorded	Pneumonia, sepsis, otitis, sinusitis	Bronchiolitis, otitis, recurrent upper respiratory infections	Bronchiolitis, pneumonia	Lyme disease, otitis, pneumonia, gastroenteritis	Bronchiolitis, urinary tract infections	Otitis, bronchiolitis, pneumonia
Autoimmune disorders	Celiac disease	None	Type 1 diabetes	None	None	None

2.2. IgG Purification from Whole Serum

IgG was isolated from serum by affinity chromatography using a 96-well Protein G Spin Plate for IgG Screening (Thermo Fisher Scientific, Waltham, MA USA). Prior to use, the spin plate and reagents were equilibrated to room temperature for approximately 30 minutes. The plate was then mounted on a Positive Pressure-96 Processor (Waters corporation, Milford, MA, USA), and the resin beads were conditioned with three washes of 200 µL Binding Buffer (Phosphate-buffered saline PBS, pH 7.2) under 5–10 mbar pressure. Serum samples (50 µL) were diluted with 150 µL Binding Buffer to maintain a proper ionic strength and pH for optimal binding, then transferred to the wells assembled on a wash plate. IgG binding was promoted by shaking at 1000 RPM for 60 minutes at room temperature. Then, the plate was mounted on the Positive Pressure-96 Processor and the solution in the wells drained away. To avoid non-specific binding proteins, the resin was washed four times with 300 µL PBS, discarding the flow-through after each step. The plate was once again removed from the Positive Pressure-96 Processor, and the bottom was gently tapped dry on a lint-free paper.

For elution, the purification plate was assembled onto a 96-well collection plate preloaded with 60 µL Neutralization Buffer (1 M Tris-HCl, pH 9) per well to preserve IgG integrity. Bound IgG was eluted twice with 200 µL of 0.1 M formic acid (pH 2.7). The purity of the recovered fractions was verified by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). Eluates were subsequently transferred to Eppendorf tubes, dried under vacuum, and reconstituted in 200 µL PBS 20 mM. A 9 µL aliquot of the reconstituted IgG solution was then used for glycan release.

2.3. Enzymatic Release of N-Glycans, Labelling and ESI-MS Analysis

The technique used in this study to analyze the N-glycan profile of serum extracted IgG has been reported as suitable for studying the glycosylation of IgG and monoclonal antibodies. [Messina et al. 2021, Lauber et al. 2015].

Enzymatic release and fluorescent labelling of N-glycans were performed by the GlycoWorks *Rapi*Fluor™ -MS N-Glycan kit (Waters Corporation, Milford, MA, USA). All reactions and purification steps were performed in the kit's 96-well plate format.

Labelled N-glycans, derivatized at the glycosylamine residue of the terminal chitobiose epitope [20, 33], were subjected to chromatographic separation using a Vanquish™ UHPLC system (Thermo Scientific™, model 83L6BL3). The UHPLC was coupled with a Vanquish™ Fluorescence Detector

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2026 doi:10.20944/preprints202604.0844.v1

(Thermo Scientific™, model VC-D50-A) which was further connected in series to an Orbitrap Exploris™ 120 high-resolution mass spectrometer (Thermo Scientific™ Inc., Bremen, Germany). Chromatographic separation was achieved using a hydrophilic interaction liquid chromatography (HILIC) column (ACQUITY UPLC Glycan BEH Amide, 130 Å, 1.0 mm × 150 mm, 1.7 µm; Waters Corporation). The column operated at 60 °C with a flow rate of 0.1 mL/min. Mobile phase A consisted of 200 mM ammonium formate (pH 4.4), while mobile phase B was acetonitrile. A gradient from 25% to 46% mobile phase A over 35 minutes was applied.

ESI-MS analyses were performed under the following conditions: ion transfer tube temperature 275 °C, vaporizer temperature 250 °C, spray voltage 3.30 kV. Spectra were acquired in positive ion mode at a resolution of 120,000 FWHM at 200 m/z. Each sample was analyzed in triplicate, producing nine chromatograms per patient. No substantial intra-sample variation was observed.

2.4. Protein N-Glycosylation Analyses by MALDI-MS

N-glycans preparation from whole serum (10 µl) consisted of glycoprotein denaturation by RapiGest™ SF Surfactant (Waters Corporation, Milford, MA, USA) in 50 mM ammonium bicarbonate buffer, followed by reduction in 5 mM Dithiothreitol (DTT, Sigma) at 56 °C for 30 min, alkylation in 15 mM iodoacetamide (IAA, Sigma) in the dark at room temperature for 45 min, and peptide-N-glycosydase F (PNGase F) digestion (2 U, Roche, Molecular Biochemicals, Mannheim, Germany) overnight at 37 °C. The released glycans were therefore purified by two chromatographic steps on C18 Sep-Pak cartridges (Waters Corporation, Milford, MA, USA) and by solid-phase-extraction (SPE) on HyperSep™ Hypercarb™ cartridges (Thermo Scientific™, Bellefonte, PA, USA) [Messina et al., 2019; Palmigiano et al., 2018]. The obtained N-glycans were therefore permethylated in a DMSO/NaOH slurry followed by ICH3 addition, according to the method originally developed by Ciucanu and Kerek (1984) to enhance glycan detection sensitivity upon MALDI-MS. Mass spectra of permethylated N-glycans, dissolved in MeOH at an estimated concentration of about 10 pmol/µL, were performed using 5-chloro-2-mercaptobenzothiazole (CMBT, 10 mg/ml in 80:20 MeOH/H2O, v/v) as matrix [Sturiale et al., 2021]. MALDI TOF and MALDI TOF/TOF analyses were conducted on a 4800 Proteomic Analyzer (AB Sciex), equipped with a Nd:YAG laser operating at a wavelength of 255 nm with <500-ps pulse and 200 Hz firing rate. Mass spectra of permethylated N-glycans were acquired in positive polarity and in reflector mode, allowing detection of monoisotopic masses with mass accuracy below 75 ppm. Data were processed using DataExplorer™ 4.9 software. Structural assignments were based on molecular weight identification, knowledge of the N-glycan biosynthetic pathway and MS/MS analyses. N-glycan species were identified by bioinformatic tools, such as GlycoMod (http://web.expasy.org/glycomod/) and glycoworkbench v2.1 [Ceroni et al., 2008] and by tools provided by the consortium for functional glycomics (http://functionalglycomics.org).

2.5. Statistical Analysis

Specificity and relevance of quantitative differentiations were established by ANOVA p-values, and results were expressed as mean values ±SD. Statistical significance was set at $p \leq 0.05$.

3. Results and Discussion

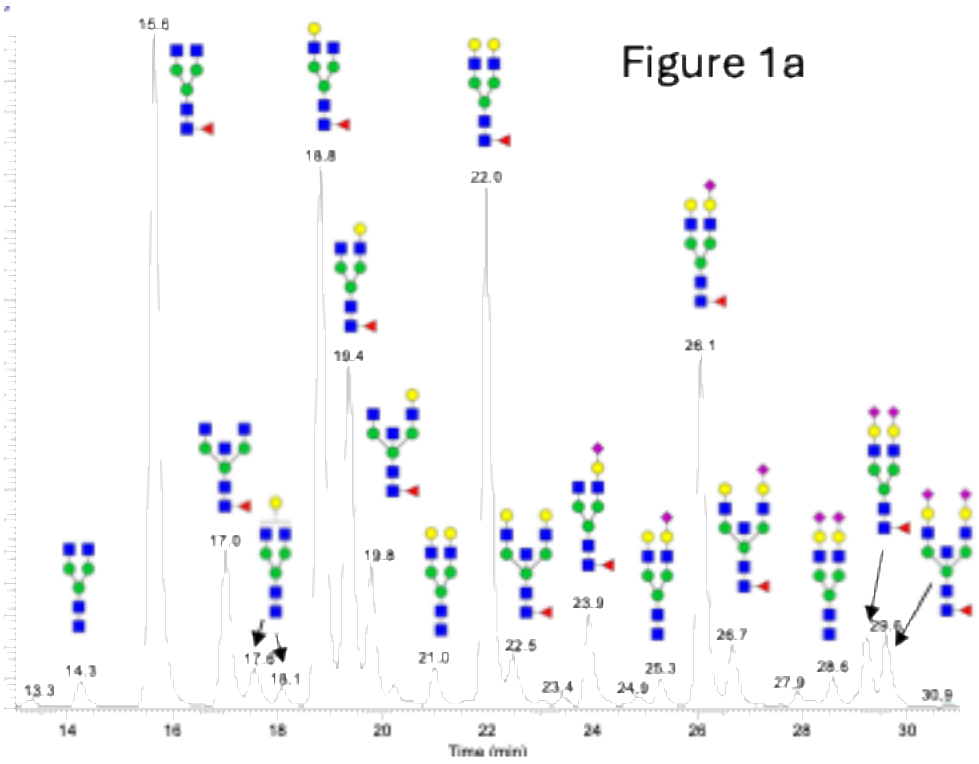
3.1. IgG N-Glycosylation Profiles in RC14 Patients

We used chromatographic separation of the IgG N-glycans allowing isomeric structures characterization [Messina et al 2021] and high sensitivity thanks to the fluorescence labelling, with the possibility of unambiguously identifying all the chromatographic peaks by ESI MS and ESI MS/MS. All IgG N-glycans were identified with the most abundant ones detailed in Figure 1 and in Table 2.

The HILIC-UHPLC-FLR profiles of the IgG N-glycans from RC14 patients show clear differences when compared to age-matched healthy controls, mainly consisting of decreased galactosylation and sialylation (Figures 1a-1b and Supplementary Figures S1-S5).

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2026 doi:10.20944/preprints202604.0844.v1



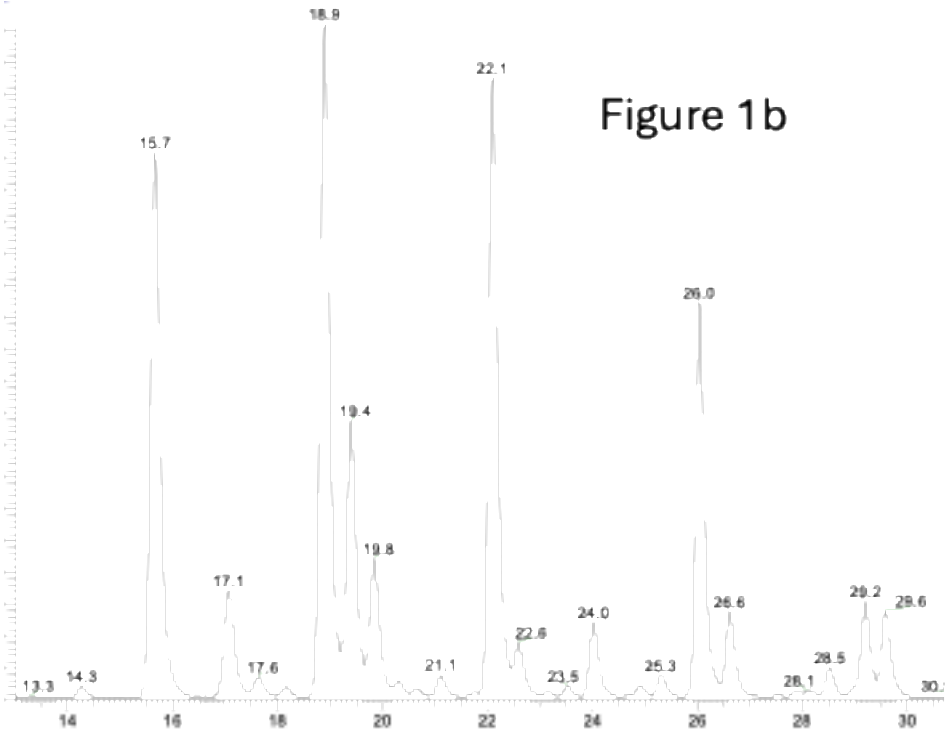
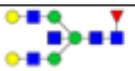
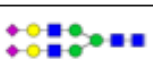


Figure 1. HILIC-UPLC-FLR chromatograms of the IgG N-glycans extracted from patient 1 (**1a**) and from a healthy control (**1b**). Most abundant N-glycans are detailed and illustrated in Figure 1a. Glycan symbols: GlcNAc, blue square; Man, green circle; Gal, yellow circle; NeuAc, purple lozenge; Fuc, red triangle.

Table 2. Structural and mass information of the IgG N-glycans investigated.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2026 doi:10.20944/preprints202604.0844.v1

Glycan (Oxford notation)	Structure	$[M+2H]^{+2}$ Calculated	$[M+2H]^{+2}$ Measured	Error (ppm)
A2		814.8378	814.8380	0,25
FA2		887.8668	887.8679	1,24
FA2B		989.4065	989.4071	0,61
A2G1a		895.8643	895.8649	0,67
A2G1b		895.8643	895.8649	0,67
FA2G1a		968.8932	968.8932	0,00
FA2G1b		968.8932	968.8945	1,34
FA2BG1a		1070.4329	1070.4334	0,47
A2G2		976.8907	976.8918	1,13
FA2G2		1049.9196	1049.9207	1,05
FA2BG2		1151.4593	1151.4619	2,26
FA2G1S1		1114.4409	1114.4427	1,62
A2G2S1		1122.4384	1122.4396	1,06
FA2G2S1		1195.4673	1195.4698	2,09
FA2BG2S1		1297.0070	1297.0097	2,08
A2G2S2		1267.9861	1267.9880	1.49
FA2G2S2		1341.0150	1341.0183	2,46

The IgG N-glycan profiles among patients are similar but markedly different from controls showing FA2 (agalactosylated glycan) as the most intense peak, whereas the two most intense species in healthy controls always correspond to FA2G1a (monogalactosylated glycan) and FA2G2 (digalactosylated glycan). Additional differences include increases in bisected and afucosylated N-glycans. ANOVA analysis (Figure 2) reveals seven N-glycans with significant intensity differences: patients exhibit an increase of the afucosylated species A2G1 and the agalactosylated structures FA2 and FA2B, while healthy controls have higher levels of digalactosylated and sialylated glycans.

The straightforward explanatory hypothesis may implicate a direct correlation between the aberrant IgG N-glycosylation observed in patients with RC14 syndrome and the underlying chromosomal rearrangement. However, this has yet to be proven, as no genes encoding galactosyltransferases or sialyltransferases are located on chromosome 14. By contrast, the *FUT8* gene, encoding the enzyme α-(1,6)-fucosyltransferase (FUT8) essential for core fucosylation of N-

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2026 doi:10.20944/preprints202604.0844.v1

glycans, maps on chromosome 14q23.3, usually not involved into rearrangement/deletions leading to typical chromosome 14 ring structures [Miyoshi, E., 2002]. It is worth noting that haploinsufficiency of the genes included in the deletion is only one of the possible pathogenic mechanisms, which involves several other factors such as ring chromosome instability during cell division and the effects of the chromosome's new conformation on gene expression. Therefore, the lack of candidate genes within the region that is typically deleted does not in itself exclude an underlying genetic basis. This is also an element to take into consideration when investigating phenotypic differences among patients.

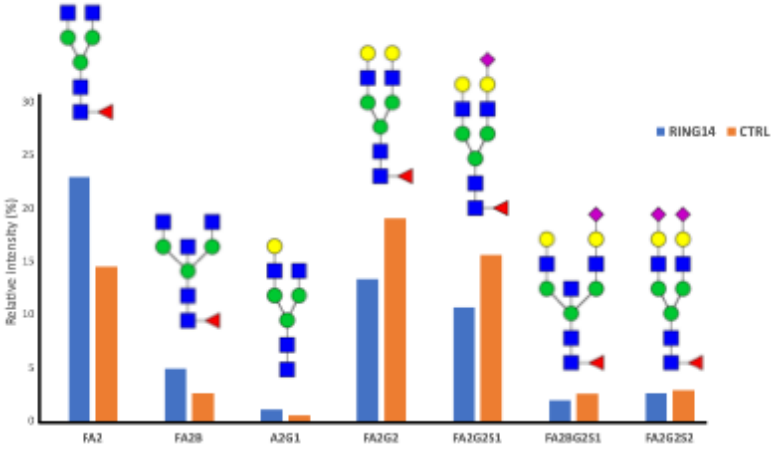


Figure 2. Comparison of statistically significant ($p < 0.01$) relative intensities between control subjects (CTRL) and RC14 syndrome patients. N-Glycans are indicated using the Oxford Notation (see Table 2). Glycan symbols: GlcNAc, blue square; Man, green circle; Gal, yellow circle; NeuAc, purple lozenge; Fuc, red triangle.

To investigate a possible generalized N-glycosylation defect owing to chromosomal defect underlying RC14 we performed the N-glycosylation analysis on total serum proteins and on serum transferrin, one of the most abundant serum liver-derived glycoprotein, used as a valuable biomarker in the diagnosis of Congenital Disorders of Glycosylation (CDG) [Sturiale et al 2011].

The MALDI mass spectra of permethylated N-glycans released from the total serum of patient 3 and of a control subject are shown in Figure 3a and 3b respectively. No galactosylation or sialylation defects are evident, although some peaks in the 2800–5000 mass range, corresponding to antennary fucosylated N-glycans, are slightly more intense in the RC14 patient when compared to the control. A similar profile was observed for serum and serum transferrin of all the patients included in this study (data not shown). Since no decreases in galactosylation and sialylation are observed in total serum and serum transferrin N-glycans of patients affected by RC14 syndrome, it appears that the observed increase in agalactosylated and asialylated N-glycans and the concurrent decrease of galactosylated and sialylated glycoforms of serum IgG do not relate to the structural chromosomal RC14 rearrangement.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2026 doi:10.20944/preprints202604.0844.v1

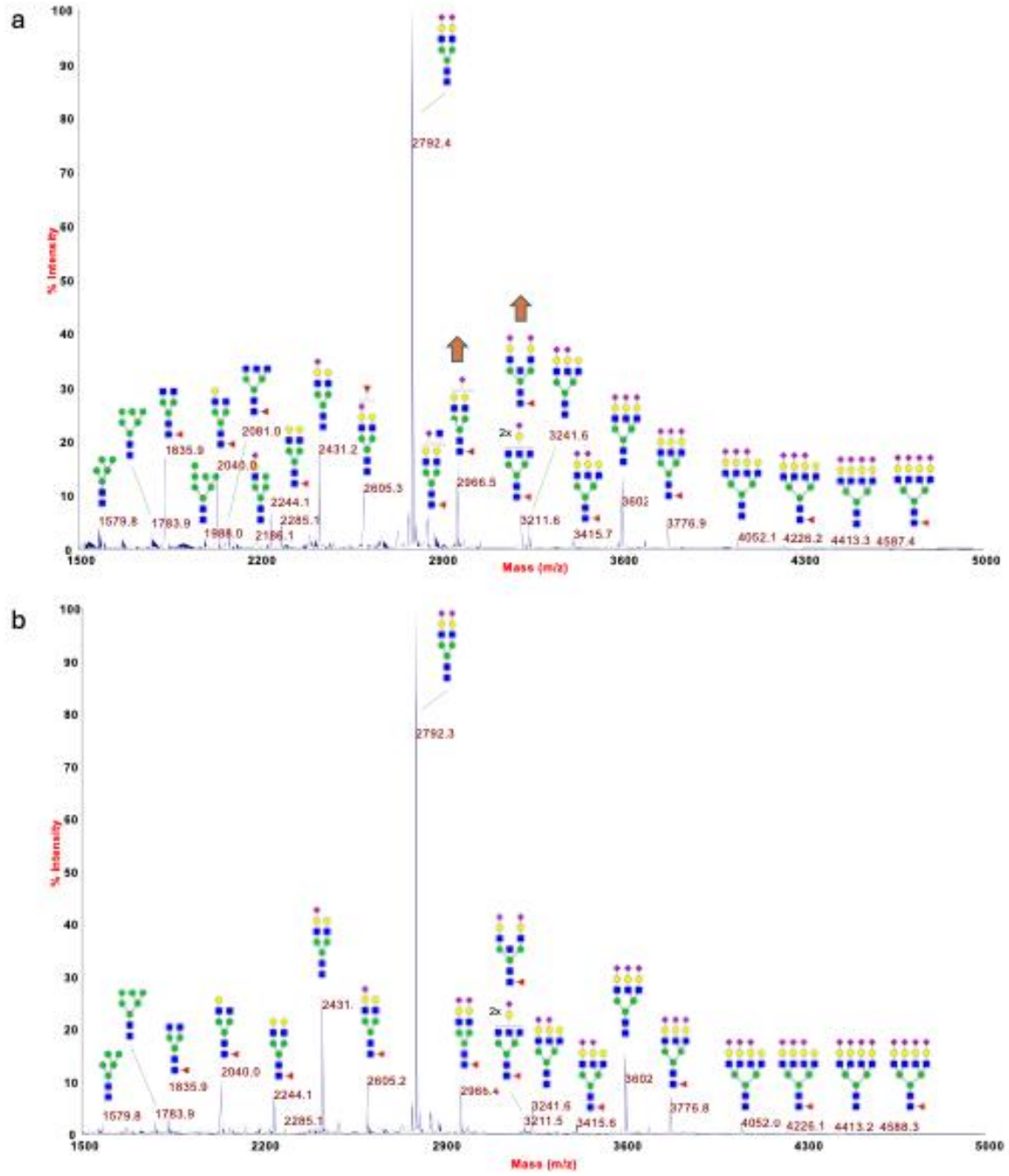


Figure 3. MALDI TOF mass spectrum of serum N-glycans from patient 3 (a) and from a healthy subject (b). Glycan symbols: GlcNAc, blue square; Man, green circle; Gal, yellow circle; NeuAc, purple lozenge; Fuc, red triangle.

3.2. Effects of N-Glycosylation on IgG Functions

Glycosylation is critical for IgG functionality, modulating how IgGs interact with immune cells and activate immune responses. The specific patterns of IgG glycosylation, especially on the Fc region, determine its role in effector functions like antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and regulating immune cell activation [Arnold et al.2007; Cobb 2020; Krištić 2024; Subedi & Barb 2015].

Over 95 % of IgGs are core fucosylated in healthy individuals. IgG antibodies lacking core fucose have significantly increased affinity for both FcγRIIIa and FcγRIIIb receptors compared with IgG

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2026 doi:10.20944/preprints202604.0844.v1

with core-fucosylated glycans [Okazaki 2004; Shields 2002] and this results in a strong increase of ADCC and phagocytosis [Bruggeman 2017; Pereira et al. 2018; Shinkawa 2003].

Galactosylation is critical for IgG effector functions as well. IgG antibodies containing galactose show higher binding affinities for the C1q component of the classical complement pathway, which has also been associated with enhanced complement cascade activation [Gudelj 2018; Malhotra et al. 1995, Rademacher 1997]. Furthermore, Galectin 3 binds specifically to galactosylated IgG [Heyl et al. 2016]. Notably, IgG antibodies missing galactose are found at higher abundance in inflammatory and autoimmune diseases [Albrecht et al. 2014].

Sialylation also increases the anti-inflammatory IgG activity [Kaneko 2016; Samuelsson et al. 2001] by promoting their binding with the C-type lectin DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin) which is expressed on some monocytes, regulatory macrophages and dendritic cells [Anthony et al. 2008a, Anthony et al. 2013]. Furthermore, sialylation acts as a crucial switch between pro-inflammatory and anti-inflammatory IgG functions, particularly in pathogenic IgG autoantibodies [Ohmi et al. 2016]. Sialylation reduces antibody pathogenicity and can even improve active disease in animal models when applied both in vitro and in vivo [Ohmi et al. 2016; Pagan et al. 2018; Schwab & Nimmerjah 2013].

In conclusion, according to present data, the simultaneous increase in agalactosylated and asialylated N-glycans with the concurrent decrease of galactosylated and sialylated glycoforms might enhance the proinflammatory IgG structures while decreasing the anti-inflammatory ones. Based on the abnormal IgG glycosylation, patients might be prone to increased inflammatory response which could explain their recurrent infections as common feature of RC14 syndrome [Rinaldi et al. 2017; Vaisfeld et al. 2024], despite serum IgG levels do not fall below the normal levels [Krawczun et al.1984].

3.3. Comparison with Other Diseases

The observed IgG glycosylation pattern resembles those occurring in autoimmune diseases characterized by a marked increase of undergalactosylated and undersialylated IgG glycoforms [Gińdzieńska-Sieśkiewicz et al. 2016; Gudelj et al. 2018; Malhotra et al.1995; Mayboroda et al 2023; Parekh et al. 1985; Sun et al 2019]

Rheumatoid arthritis (RA) is one of the most common immune mediated inflammatory diseases. Over the past 25 years, the management of this condition has been revolutionized, resulting in substantially higher levels of disease remission and better long-term outcomes. RA therapies focus on controlling inflammation and immune response with drugs like conventional DMARDs (methotrexate), IVIG administration [Anthony et al 2008b; Anthony et al. 2013; Katz-Agranov 2015; Schwab & Nimmerjah 2013; Schwab & Nimmerjah 2014], monoclonal antibodies [Bossaller, L., & Rothe, A. (2013)]. (adalimumab, etanercept, tocilizumab), and JAK inhibitors (tofacitinib) [Bossaller, L., & Rothe, A. (2013)].

All the above therapies also result in a counterbalancing of IgG glycosylation restoring the anti-inflammatory glycan pattern [Gińdzieńska-Sieśkiewicz, E. et al. (2016); Lundstrom et al. (2017) Pasek et al. (2008); Zhou et al (2021)]

3.4. A Preliminary Therapeutic Insight for RC14 Patients?

These evidences would suggest the possibility of therapies with IVIG or monoclonal antibody to manage untreatable infections in RC14 patients. Among the patients involved in this study, patient 1 received five doses of IVIG (0.3 g/Kg) during a hospital stay for pneumonia. The therapy, along with others, not only proved useful in treating pneumonia but patient 1 subsequently showed a reduction in seizures from 5–6/week to one/week. Furthermore, the Understanding Rare Chromosome and Gene Disorders (UNIQUE) website, about the RC14, reports that “The research group that recently identified the loss of IGH (Immunoglobulin Heavy Locus) genes as a possible cause of repeated infection also suspects that giving intravenous immunoglobulin may help with seizure control. In this context, the family of a 12-year-old reported to UNIQUE that better seizure

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2026 doi:10.20944/preprints202604.0844.v1

control and fewer respiratory infections went hand in hand" [https://www.rarechromo.org/media/information/Chromosome%2014/Ring%2014%20FTNW.pdf]

These facts are not so surprising since IVIG are sometimes used to treat drug-resistant epilepsy [Elsayed et al. 2022; Emmi et al. 2002; González-Castillo et al. 2020], being also adopted as typical first-line agent against autoimmune epilepsy [Castagnola et al. 2022; Husari & Dubey 2019; Silverman et al. 2025; Yang et al. 2020]. The overall findings suggest that in RC14 patients treated with IVIG, seizure reduction might reflect a decrease of neuroinflammation associated with the altered IgG N-glycan profiles.

In sum we foresee that patients with RC14 syndrome, or some of them, might have a proinflammatory condition predisposing to severe and long-lasting infections as well as to inflammatory related neurological concerns such as epilepsy. If this is the case, IVIG administration and/or monoclonal antibodies administration might be effective in balancing the inflammatory response and ultimately decreasing seizures frequency and infection duration.

4. Conclusions

The IgG N-glycan profile of RC14 patients differ markedly from controls, showing decreased fucosylation as well as galactosylation and sialylation. One patient benefited clinically from IVIG treatment, supporting the hypothesis that targeting IgG glycosylation may hold therapeutic potential. However, the observed clinical improvement cannot be exclusively attributed to IVIG and should be interpreted with caution.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.Org, Figures S1–S5 "HILIC-UPLC-FLR chromatograms of the IgG N-glycans extracted from patients 2-6".

Funding: This research was partially funded by the European Union – Next Generation EU, Mission 4 Component 1, Project "NGLYNEU" P.I Rita Barone, CUP E53D23009730006. Ministry of University and Research—PRIN (Research Projects of National Relevant Interest) 2022 (ID: 202255RLB4).

Institutional Review Board Statement: This study was based solely on information and investigations that were carried out as part of the routine clinical care of RC14 patients. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee at Policlinico "G. Rodolico-San Marco" Catania and with the 1964 Helsinki declaration and its later amendments.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Acknowledgments: Ring 14 Italia, Ring 14 International Onlus and Stefania Azzali are acknowledged for support and helpful discussion.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Albrecht, S., Unwin, L., Muniyappa, M., & Rudd, P. M. (2014). Glycosylation as a marker for inflammatory arthritis. *Cancer biomarkers: section A of Disease markers*, *14*(1), 17–28. https://doi.org/10.3233/CBM-130373
2. Anthony, R. M., Wermeling, F., Karlsson, M. C., & Ravetch, J. V. (2008a). Identification of a receptor required for the anti-inflammatory activity of IVIG. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(50), 19571–19578. https://doi.org/10.1073/pnas.0810163105
3. Anthony, R. M., Nimmerjahn, F., Ashline, D. J., Reinhold, V. N., Paulson, J. C., & Ravetch, J. V. (2008b). Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. Science (New York, N.Y.), 320(5874), 373–376. https://doi.org/10.1126/science.1154315
4. Anthony, R. M., Kobayashi, T., Wermeling, F., & Ravetch, J. V. (2011). Intravenous gammaglobulin suppresses inflammation through a novel T(H)2 pathway. *Nature*, *475*(7354), 110–113. https://doi.org/10.1038/nature10134

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2026 doi:10.20944/preprints202604.0844.v1

5. Arnold, J. N., Wormald, M. R., Sim, R. B., Rudd, P. M., & Dwek, R. A. (2007). The impact of glycosylation on the biological function and structure of human immunoglobulins. *Annu. Rev. Immunol.*, *25*(1), 21-50. https://doi.org/10.1146/annurev.immunol.25.022106.141702
6. Bossaller, L., & Rothe, A. (2013). Monoclonal antibody treatments for rheumatoid arthritis. *Expert Opinion on Biological Therapy*, *13*(9), 1257–1272. https://doi.org/10.1517/14712598.2013.811230
7. Bruggeman, C. W., Dekkers, G., Bentlage, A. E. H., Treffers, L. W., Nagelkerke, S. Q., Lissenberg-Thunnissen, S., Koeleman, C. A. M., Wuhrer, M., van den Berg, T. K., Rispens, T., Vidarsson, G., & Kuijpers, T. W. (2017). Enhanced Effector Functions Due to Antibody Defucosylation Depend on the Effector Cell Fcγ Receptor Profile. *Journal of immunology (Baltimore, Md.: 1950)*, *199*(1), 204–211. https://doi.org/10.4049/jimmunol.1700116
8. Costagliola, G., Depietri, G., Michev, A., Riva, A., Foiadelli, T., Savasta, S., Bonuccelli, A., Peroni, D., Consolini, R., Marseglia, G. L., Orsini, A., & Striano, P. (2022). Targeting Inflammatory Mediators in Epilepsy: A Systematic Review of Its Molecular Basis and Clinical Applications. *Frontiers in neurology*, *13*, 741244. https://doi.org/10.3389/fneur.2022.741244
9. Ceroni, A., Maass, K., Geyer, H., Geyer, R., Dell, A., & Haslam, S. M. (2008). GlycoWorkbench: a tool for the computer-assisted annotation of mass spectra of glycans. *Journal of proteome research*, *7*(4), 1650–1659. https://doi.org/10.1021/pr7008252
10. Cobb B. A. (2020). The history of IgG glycosylation and where we are now. *Glycobiology*, *30*(4), 202–213. https://doi.org/10.1093/glycob/cwz065
11. Elsayed, W., Khattab, Y. A., & Khattab, R. A. (2022). Immunoglobulin use in childhood epilepsy: Literature review. International Journal of Health Sciences, 6(S1), 9787-9794. https://doi.org/10.53730/ijhs.v6nS1.7289
12. Emmi, L., & Chiarini, F. (2002). The role of intravenous immunoglobulin therapy in autoimmune and inflammatory disorders. *Neurological sciences: official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology*, *23 Suppl 1*, S1–S8. https://doi.org/10.1007/s100720200010
13. Gińdzieńska-Sieśkiewicz, E., Radziejewska, I., Domysławska, I., Klimiuk, P. A., Sulik, A., Rojewska, J., Gabryel-Porowska, H., & Sierakowski, S. (2016). Changes of glycosylation of IgG in rheumatoid arthritis patients treated with methotrexate. *Advances in medical sciences*, *61*(2), 193–197. https://doi.org/10.1016/j.advms.2015.12.009
14. Gilgenkrantz, S., Cabrol, C., Lausecker, C., Hartleyb, M. E., & Bohe, B. (1971, March). The Dr syndrome. Study of a further case (46, XX, 14r). In *Annales de Genetique* (Vol. 14, No. 1, pp. 23-31).
15. González-Castillo, Z., Solórzano Gómez, E., Torres-Gómez, A., Venta Sobero, J. A., & Gutiérrez Moctezuma, J. (2020). Intravenous immunoglobulin G as adjuvant treatment in drug-resistant childhood epilepsy. Inmunoglobulina G intravenosa como tratamiento adyuvante en epilepsia infantil farmacorresistente. *Neurologia*, *35*(6), 395–399. https://doi.org/10.1016/j.nrl.2017.09.005
16. Gudelj, I., Lauc, G., & Pezer, M. (2018). Immunoglobulin G glycosylation in aging and diseases. *Cellular immunology*, *333*, 65–79. https://doi.org/10.1016/j.cellimm.2018.07.009
17. Husari, K.S., Dubey, D. Autoimmune Epilepsy. *Neurotherapeutics* 16, 685–702 (2019). https://doi.org/10.1007/s13311-019-00750-3
18. Heyl, K. A., Karsten, C. M., & Slevogt, H. (2016). Galectin-3 binds highly galactosylated IgG1 and is crucial for the IgG1 complex mediated inhibition of C5aReceptor induced immune responses. *Biochemical and biophysical research communications*, *479*(1), 86–90. https://doi.org/10.1016/j.bbrc.2016.09.038
19. Incecik, F., Hergüner, M. O., Mert, G., Erdem, S., & Altunbaşak, S. (2013). Ring chromosome 14 syndrome presenting with intractable epilepsy: a case report. *The Turkish Journal of Pediatrics*, *55*(5), 549-551.
20. Jang, Y., Kim, D. W., Yang, K. I., Byun, J. I., Seo, J. G., No, Y. J., Kang, K. W., Kim, D., Kim, K. T., Cho, Y. W., Lee, S. T., & Drug Committee of Korean Epilepsy Society (2020). Clinical Approach to Autoimmune Epilepsy. *Journal of clinical neurology* (Seoul, Korea), 16(4), 519–529. https://doi.org/10.3988/jcn.2020.16.4.519
21. Katz-Agranov, N., Khattri, S., & Zandman-Goddard, G. (2015). The role of intravenous immunoglobulins in the treatment of rheumatoid arthritis. *Autoimmunity reviews*, *14*(8), 651-658. https://doi.org/10.1016/j.autrev.2015.04.003

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2026 doi:10.20944/preprints202604.0844.v1

22. Kaneko, Y., Nimmerjahn, F., & Ravetch, J. V. (2006). Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science (New York, N.Y.)*, *313*(5787), 670–673. https://doi.org/10.1126/science.1129594
23. Krawczun, M., Melink, G., Cervenka, J., & Opitz, J. M. (1984). Ring chromosome 14 and immunoglobulin locus. *American journal of medical genetics*, *17*(2), 465-469.
24. Krištić, J., & Lauc, G. (2024). The importance of IgG glycosylation—What did we learn after analyzing over 100,000 individuals. *Immunological Reviews*, *328*(1), 143-170. https://doi.org/10.1111/imr.13407
25. Lauber, M. A., Yu, Y. Q., Brousmiche, D. W., Hua, Z., Koza, S. M., Magnelli, P., Guthrie, E., Taron, C. H., & Fountain, K. J. (2015). Rapid Preparation of Released N-Glycans for HILIC Analysis Using a Labeling Reagent that Facilitates Sensitive Fluorescence and ESI-MS Detection. *Analytical chemistry*, *87*(10), 5401–5409. https://doi.org/10.1021/acs.analchem.5b00758
26. Lundstrom SL, Hensvold AH, Rutishauser D, Klareskog L, Ytterberg AJ, Zubarev RA and Catrina AI, (2017). IgG Fc galactosylation predicts response to methotrexate in early rheumatoid arthritis, Arthritis Res Ther 19 182. https://link.springer.com/article/10.1186/s13075-017-1389-7
27. Malhotra, R., Wormald, M. R., Rudd, P. M., Fischer, P. B., Dwek, R. A., & Sim, R. B. (1995). Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. *Nature medicine*, *1*(3), 237–243. https://doi.org/10.1038/nm0395-237
28. Mayboroda, O. A., Lageveen-Kammeijer, G. S. M., Wuhrer, M., & Dolhain, R. J. E. M. (2023). An Integrated Glycosylation Signature of Rheumatoid Arthritis. *Biomolecules*, *13*(7), 1106. https://doi.org/10.3390/biom13071106
29. Messina, A., Palmigiano, A., Bua, R. O., Romeo, D. A., Barone, R., Sturiale, L., ... & Garozzo, D. (2019). CSF N-glycoproteomics using MALDI MS techniques in neurodegenerative diseases. In *Cerebrospinal Fluid (CSF) Proteomics: Methods and Protocols* (pp. 255-272). New York, NY: Springer New York.
30. Messina, A., Palmigiano, A., Esposito, F., Fiumara, A., Bordugo, A., Barone, R., Sturiale, L., Jaeken, J., & Garozzo, D. (2021). HILIC-UPLC-MS for high throughput and isomeric N-glycan separation and characterization in Congenital Disorders Glycosylation and human diseases. *Glycoconjugate journal*, *38*(2), 201–211. https://doi.org/10.1007/s10719-020-09947-7
31. Miyoshi, E., Taniguchi, N. (2002). α6-Fucosyltransferase (FUT8). In: Taniguchi, N., et al. Handbook of Glycosyltransferases and Related Genes. Springer, Tokyo. https://doi.org/10.1007/978-4-431-67877-9_34
32. Ohmi, Y., Ise, W., Harazono, A., Takakura, D., Fukuyama, H., Baba, Y., Narazaki, M., Shoda, H., Takahashi, N., Ohkawa, Y., Ji, S., Sugiyama, F., Fujio, K., Kumanogoh, A., Yamamoto, K., Kawasaki, N., Kurosaki, T., Takahashi, Y., & Furukawa, K. (2016). Sialylation converts arthritogenic IgG into inhibitors of collagen-induced arthritis. *Nature communications*, *7*, 11205. https://doi.org/10.1038/ncomms11205
33. Ondruskova, N., Cechova, A., Hansikova, H., Honzik, T., & Jaeken, J. (2021). Congenital disorders of glycosylation: Still "hot" in 2020. *Biochimica et biophysica acta. General subjects*, *1865*(1), 129751. https://doi.org/10.1016/j.bbagen.2020.129751
34. Pagan, J. D., Kitaoka, M., & Anthony, R. M. (2018). Engineered Sialylation of Pathogenic Antibodies In Vivo Attenuates Autoimmune Disease. *Cell*, *172*(3), 564–577.e13. https://doi.org/10.1016/j.cell.2017.11.041
35. Palmigiano, A., Messina, A., Sturiale, L., & Garozzo, D. (2018). Advanced LC-MS methods for N-glycan characterization. In *Comprehensive Analytical Chemistry* (Vol. 79, pp. 147-172). Elsevier. https://doi.org/10.1016/bs.coac.2017.06.009
36. Parekh, R. B., Dwek, R. A., Sutton, B. J., Fernandes, D. L., Leung, A., Stanworth, D., Rademacher, T. W., Mizuochi, T., Taniguchi, T., & Matsuta, K. (1985). Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. *Nature*, *316*(6027), 452–457. https://doi.org/10.1038/316452a0
37. Pasek M, Duk M, Podbielska M, Sokolik R, Szechinski J, Lisowska E and Krotkiewski H, Galactosylation of IgG from rheumatoid arthritis (RA) patients--changes during therapy, Glycoconj J 23 (2006) 463–71. https://doi.org/10.1186/s13075-018-1540-0
38. Pereira, N. A., Chan, K. F., Lin, P. C., & Song, Z. (2018). The "less-is-more" in therapeutic antibodies: Afucosylated anti-cancer antibodies with enhanced antibody-dependent cellular cytotoxicity. *mAbs*, *10*(5), 693–711. https://doi.org/10.1080/19420862.2018.1466767

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2026 doi:10.20944/preprints202604.0844.v1

39. Rademacher, T. W., Williams, P., & Dwek, R. A. (1994). Agalactosyl glycoforms of IgG autoantibodies are pathogenic. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(13), 6123–6127. https://doi.org/10.1073/pnas.91.13.6123
40. Rinaldi, B., Vaisfeld, A., Amarri, S., Baldo, C., Gobbi, G., Magini, P., Melli, E., Neri, G., Novara, F., Pippucci, T., Rizzi, R., Soresina, A., Zampini, L., Zuffardi, O., & Crimi, M. (2017). Guideline recommendations for diagnosis and clinical management of Ring14 syndrome-first report of an ad hoc task force. *Orphanet journal of rare diseases*, *12*(1), 69. https://doi.org/10.1186/s13023-017-0606-4
41. Samuelsson, A., Towers, T. L., & Ravetch, J. V. (2001). Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science (New York, N.Y.)*, *291*(5503), 484–486. https://doi.org/10.1126/science.291.5503.484
42. Schwab, I., & Nimmerjahn, F. (2013). Intravenous immunoglobulin therapy: how does IgG modulate the immune system? *Nature reviews. Immunology*, *13*(3), 176–189. https://doi.org/10.1038/nri3401
43. Shinkawa, T., Nakamura, K., Yamane, N., Shoji-Hosaka, E., Kanda, Y., Sakurada, M., Uchida, K., Anazawa, H., Satoh, M., Yamasaki, M., Hanai, N., & Shitara, K. (2003). The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. *The Journal of biological chemistry*, *278*(5), 3466–3473. https://doi.org/10.1074/jbc.M210665200
44. Silverman M, Nagesh D, Ho E, Santoro JD. Immunotherapy for management of refractory and super-refractory status epilepticus. *Practical Neurology* (US). 2025;24(8):16-22.
45. Stanley, P., Moremen, K. W., Lewis, N. E., Taniguchi, N., & Aebi, M. (2022). N-Glycans. In A. Varki (Eds.) et. al., *Essentials of Glycobiology*. (4th ed., pp. 103–116). Cold Spring Harbor Laboratory Press. https://doi.org/10.1101/glycobiology.4e.9
46. Subedi, G. P., & Barb, A. W. (2015). The Structural Role of Antibody N-Glycosylation in Receptor Interactions. *Structure (London, England: 1993)*, *23*(9), 1573–1583. https://doi.org/10.1016/j.str.2015.06.015
47. Sun, D., Hu, F., Gao, H., Song, Z., Xie, W., Wang, P., Shi, L., Wang, K., Li, Y., Huang, C., & Li, Z., (2019). Distribution of abnormal IgG glycosylation patterns from rheumatoid arthritis and osteoarthritis patients by MALDI-TOF-MS^{n}. *The Analyst*, *144*(6), 2042–2051. https://doi.org/10.1039/c8an02014k
48. Sturiale, L., Barone, R., & Garozzo, D. (2011). The impact of mass spectrometry in the diagnosis of congenital disorders of glycosylation. *Journal of inherited metabolic disease*, *34*(4), 891–899. https://doi.org/10.1007/s10545-011-9306-8
49. Sturiale, L., Nassogne, M. C., Palmigiano, A., Messina, A., Speciale, I., Artuso, R., Bertino, G., Revencu, N., Stephénne, X., De Castro, C., Matthijs, G., Barone, R., Jaeken, J., & Garozzo, D. (2021). Aberrant sialylation in a patient with a *HNF1α* variant and liver adenomatosis. *iScience*, *24*(4), 102323. https://doi.org/10.1016/j.isci.2021.102323
50. Vaisfeld, A., Crimi, M., & Rinaldi, B. (2024). Ring Chromosome 14. In *Human Ring Chromosomes: A Practical Guide for Clinicians and Families* (pp. 215-220). Cham: Springer International Publishing.
51. Zelante, L., Torricelli, F., Calvano, S., Mingarelli, R., & Dallapiccola, B. (1991, January). Ring chromosome 14 syndrome. Report of two cases, including extended evaluation of a previously reported patient and review. In *Annales de genetique* (Vol. 34, No. 2, pp. 93-97).
52. Zhou, X., Motta, F., Selmi, C., Ridgway, W. M., Gershwin, M. E., & Zhang, W. (2021). Antibody glycosylation in autoimmune diseases. *Autoimmunity reviews*, *20*(5), 102804. https://doi.org/10.1016/j.autrev.2021.102804

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.