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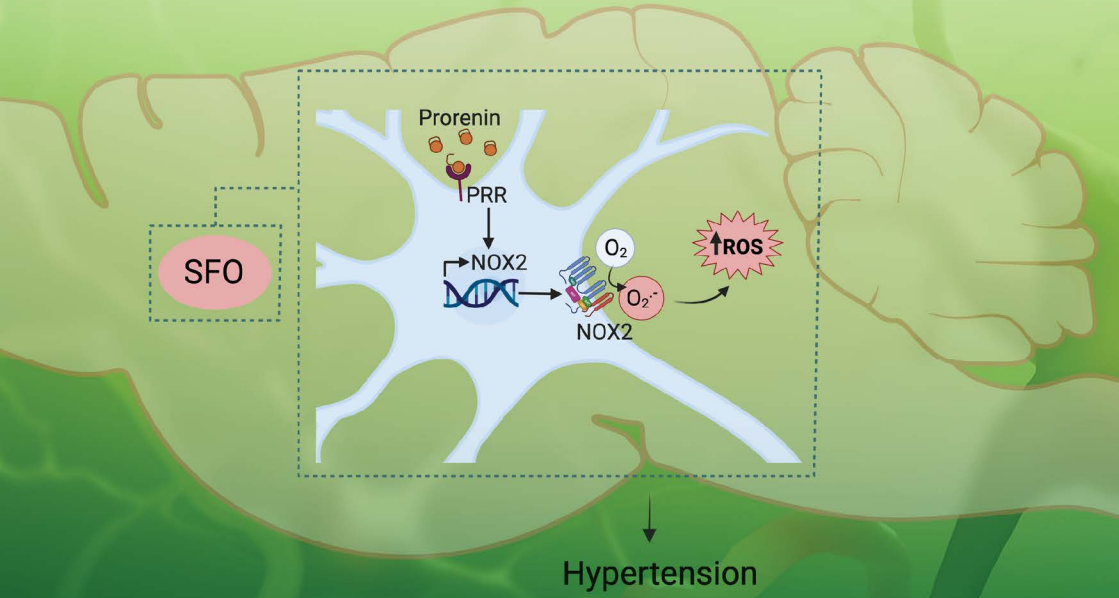

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Editorial Board Member

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Institute of Molecular Chemistry of Reims, UMR CNRS 7312, Université Reims Champagne, Ardenne, 51687 Reims, France
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Editorial Board Member
Department of Biotechnology and Biomedicine, Technical University of Denmark, DK-2800 Kgs Lyngby, Denmark
Interests: biophysics; amyloid fibrils; self-assembly; kinetics; protein folding; biosensing; microfluidics; calorimetry; biomaterials; high throughput methods

Dr. María Ángela Burrell Bustos (https://sciprofiles.com/profile/1625828)
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Editorial Board Member
Department of Pathology, Anatomy and Physiology, University of Navarra, Pamplona, Spain
Interests: cell biology; histology; adipose tissue; gut endocrinology; obesity
Special Issues, Collections and Topics in MDPI journals

Dr. Vito Calderone (https://sciprofiles.com/profile/592849)
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Editorial Board Member
Magnetic Resonance Center and Department of Chemistry, University of Florence, 50019 Sesto Fiorentino, Italy
Interests: X-ray protein crystallography; mitochondrial proteins; metalloproteins; structure-based drug design; protein–protein complexes; structural biology
Special Issues, Collections and Topics in MDPI journals

Dr. Matteo Cameli (https://sciprofiles.com/profile/1292732)
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Editorial Board Member
Department of Medical Biotechnologies, Division of Cardiology, University of Siena, 53100 Siena, Italy
Interests: heart failure; atrial fibrillation; echocardiography; hypertension; heart; cardiology; transesophageal echocardiography; cardiovascular system; cardiac function; electrocardiographyh
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Editorial Board Member
Baranello lab, Block 7B, CMB, Karolinska Institutet, 171 77 Stockholm, Sweden
Interests: topoisomerases; RNA Polymerase I and II transcription; Myc; ribosomal DNA; transcription regulation

Dr. Jordi Camps (https://sciprofiles.com/profile/91836)
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Editorial Board Member
Unitat de Recerca Biomèdica (Biomedical Research Unit), Universitat Rovira i Virgili, Hospital Sant Joan de Reus, Institut d'Investigació Sanitària Pere Virgili, Reus, Spain
Interests: oxidative stress; inflammation; metabolism; non-communicable diseases; infectious diseases
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Prof. Dr. Mario Capecchi
Website (http://capecchi.genetics.utah.edu/)
Editorial Board Member
Department of Human Genetics and Biology, Howard Hughes Medical Institute, University of Utah, Salt Lake City, UT 84112, USA
Interests: molecular genetic analysis of mammalian development; neurogenesis; organogenesis; patterning of the vertebral column; limb development; modeling of human disease in the mouse, from cancer to neuropsychiatric disorders

Prof. Dr. Gordon G. Carmichael (https://sciprofiles.com/profile/206602)
Website (http://facultydirectory.uchc.edu/profile?profileId=3078)
Editorial Board Member
Genetics & Developmental Biology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-3301, USA
Interests: long noncoding RNAs; antisense RNA; RNA editing; RNA processing

Dr. Bridget Carragher
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Editorial Board Member
1. New York Structural Biology Center, New York, NY, USA
2. Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY, USA
Interests: cryo electron microscopy (cryoEM); protein structure

Dr. Gianfranca Carta (https://sciprofiles.com/profile/371846)
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Editorial Board Member
Department of Biomedical Sciences, University of Cagliari, Cagliari 09124, Italy
Interests: lipid nutrition; saturated and poliunsaturated fatty acids; palmitic acid; omega-3 fatty acids; conjugated linoleic acid; endocannabinoid

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Editorial Board Member
Research School of Chemistry, College of Physical and Mathematical Sciences, The Australian National University, Canberra, ACT 0200, Australia
Interests: peptide and protein structure; function and interactions; molecular chaperone proteins; protein aggregation

Prof. Dr. Omar Cauli (https://sciprofiles.com/profile/89020)
Website (https://www.uv.es/uvweb/departamento-enfermeria/es/investigacion/grupos-investigacion/-frailty-research-organized-group-/miembros-del-grupo-1285857900444.html)
Editorial Board Member
Department of Nursing, University of Valencia, 46010 Valencia, Spain
Interests: cognitive impairment; frailty syndrome; neurodevelopemntal disorders; depression; neuropathy; sleep; envirnomental factors; comorbidty; immune alterations; metabolic alterations; biomarkers
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Dr. Nicolas Cenac (https://sciprofiles.com/profile/1133043)
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Editorial Board Member
Institut de Recherche en Santé Digestive - (IRSD), 31024 Toulouse, France
Interests: polyunsaturated lipid metabolites; short chain fatty acid; bile acids; bacterial metabolites; microbiota; bacterial lipids; visceral pain; lipid signaling; lipid identification by mass spectrometry; lipid quantification by mass spectrometry

Prof. Dr. Piotr Ceranowicz (https://sciprofiles.com/profile/182492)
Website (https://www.usosweb.uj.edu.pl/kontroler.php?_action=katalog2/osoby/pokazOsobe&os_id=73166)
Editorial Board Member
Department of Physiology, Faculty of Medicine, Jagiellonian University Medical College, 31-531 Cracow, Poland
Interests: experimental studies of the gastrointestinal tract; acute pancreatitis; colitis; gastric et duodenal ulcer; physiology; pathophysiology; ghrelin; obestatin; inflammation; digestive system; gut microbiota; renal diseases; diet; nutrition

Prof. Dr. Jijie Chai
★ (https://clarivate.com/highly-cited-researchers/2022) **Website (http://life.tsinghua.edu.cn/lifeen/info/1149/1252.htm)**
Editorial Board Member
Beijing Advanced Innovation Center for Structural Biology, Tsinghua-Peking Joint Center for Life Sciences, Center for Plant Biology, School of Life Sciences, Tsinghua University, 100084 Beijing, China
Interests: immunity; nucleotide binding, leucine repeat receptors (NLRs); receptor-like receptors (RLKs)

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1. Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH, 44106-4970, USA
2. Department of Neuroscience, School of Medicine, Case Western Reserve University, Cleveland, OH, 44106-4970, USA
Interests: Ion Channels; protein dynamics; EPR; Cryo-EM; Electrophysiology

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Editorial Board Member
Centre de Recherche en Transplantation et Immunologie (CRTI) INSERM UMR1064, Université de Nantes, 44093 Nantes, France
Interests: endothelial cell biology; transplantation immunology; innate immunity; CD8 T cells; infection; HCMV; inflammation; cell signaling; biomarkers; MHC; antibodies
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Prof. Dr. Chryssostomos Chatgilialoglu (https://sciprofiles.com/profile/18772)
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1. Research Director, ISOF, Consiglio Nazionale delle Ricerche, Via P. Gobetti 101, 40129 Bologna, Italy
2. Center for Advanced Technology, Adam Mickiewicz University, Uniwersytetu Poznanskiego 10, 61-614 Poznan, Poland
Interests: free radical chemistry; biomimetic chemistry; organic synthesis; reaction mechanism; analytical protocols for biomarkers of radical stress; oxidative DNA damage; lipid modification; fatty acid-based lipidomics
Special Issues, Collections and Topics in MDPI journals

Prof. Dr. Jen-Tsung Chen (https://sciprofiles.com/profile/314991)
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Editorial Board Member
Department of Life Sciences, National University of Kaohsiung, Kaohsiung 811, Taiwan
Interests: bioactive compounds; chromatography techniques; medicinal plants; phytochemicals; plant biotechnology; plant growth regulators; plant secondary metabolites
Special Issues, Collections and Topics in MDPI journals

Dr. Tai Cheng Chen (https://sciprofiles.com/profile/13082)
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Core Assay Laboratory, Clinical Translational Science Institute, Boston University, School of Medicine, Rm M-1022, 715 Albany St., Boston, MA 02118, USA
Interests: vitamin D; enzymology; biomarker analyses; cancers; metabolism; adipogenesis

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Department of Oral Biology, Oral Cancer Research Institute, and BK21 FOUR Project, Yonsei University College of Dentistry, Seoul 03722, Korea
Interests: cancer bone invasion; tumor bone microenvironment; osteolytic factors; chemokines; periodontitis and carcinogenesis; phytochemicals; cancer chemoprevention

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Instituto de Investigación e Innovación en Ciencias Biomédicas de Cádiz, INiBICA, 11009 Cádiz, Spain
Interests: inflammasomes; aging; autophagy; rare diseases
Special Issues, Collections and Topics in MDPI journals

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Editorial Board Member
Pathophysiology of Parkinson's disease, Paris Brain Institute (ICM), Pitié-Salpêtrière Hospital, Paris, France
Interests: molecular and cellular mechanisms underlying Parkinson’s disease; biology of Parkinson’s disease-linked proteins (PINK1, Parkin, alpha-synuclein); mitochondrial biology; mitochondrial quality control; protein aggregation

Dr. Benoit Coulombe (https://sciprofiles.com/profile/1611929)
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Editorial Board Member
Department of Biochemistry and Molecular Medicine, Université de Montréal, Montréal, QC H3T 1J4, Canada
Interests: RNA polymerase; PAQosome; protein-protein interactions; protein networks; leukodystrophy; single-cell proteomics; cell-based interceptive medicine; translational proteomics; biomarkers

Prof. Dr. Olivier Coux
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Editorial Board Member
Centre de Recherches de Biochimie Macromoléculaire (CRBM), CNRS-UMII UMR5237, Universités Montpellier 1 and 2, 1919 Route de Mende, 34293 Montpellier CEDEX 05, France
Interests: proteasome and its regulators; p53 and Cdc25B ubiquitylation and degradation
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2. Institute for Research and Inovation in Health (i3S), University of Porto, 4099-002 Porto, Portugal
Interests: evidence-based medicine; phytochemistry; phytopharmacology; drug discovery; natural products biochemistry; bioactive molecules; functional foods; nutraceuticals; fungal and bacterial infections; resistance to antimicrobials
Special Issues, Collections and Topics in MDPI journals

Prof. Dr. Richard D. Cummings (https://sciprofiles.com/profile/2225484)
Website (https://urldefense.proofpoint.com/v2/url?u=https-3A__ncfg.hms.harvard.edu_people_richard-2Dd-2Dcummings&d=DwIFaQ&c=WknmpdNpvrlj2B5K1aWVqL1SOiF30547pqSuOmtwXTQ&r=NExnm-ud1KdRNDEAVID3SW4PMQHEpsNs21J4THtjM50&m=Elhp-j85OweQWA50sCF9GYbHPtROL6EiEsX3xm3RCQQ&s=oT)
Editorial Board Member
Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, 02115, USA
Interests: glycobiology; glycan binding proteins; glycoconjugates; lectins and galectins; inflammation

Prof. Dr. Daniel M. Czajkowsky (https://sciprofiles.com/profile/397679)
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Editorial Board Member
School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China
Interests: molecular biophysics; chromatin structure and function; bacterial pore-forming toxins; nanopores
Special Issues, Collections and Topics in MDPI journals

Dr. Gabriella D'Orazi (https://sciprofiles.com/profile/698167)
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Editorial Board Member
Department of Research, IRCCS Regina Elena National Cancer Institute, 00144 Rome, Italy
Interests: tumor biology; p53; HIPK2; molecular oncology; apoptosis; autophagy; resistance to therapies; solid tumors
Special Issues, Collections and Topics in MDPI journals

Prof. Dr. Arik Dahan (https://sciprofiles.com/profile/83515)
Website (https://www.longdom.org/editor/arik-dahan-10086)
Editorial Board Member
Department of Clinical Pharmacology, School of Pharmacy, Ben-Gurion University of the Negev, Beer-Sheva 8410501, Israel
Interests: oral drug absorption; intestinal permeability; drug solubility; drug dissolution; biopharmaceutics classification system (BCS); drug delivery and targeting

Prof. Dr. Massimo Dal Monte (https://sciprofiles.com/profile/190070)
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Editorial Board Member
Department of Biology, University of Pisa, via San Zeno, 31, 56127 Pisa, Italy
Interests: retinopathy of prematurity (ROP); retinopathies; retinal physiology; somatostatin; beta adrenoceptors; melanoma; food supplement; neurodegeneration
Special Issues, Collections and Topics in MDPI journals

Dr. Dirk Dannenberger (https://sciprofiles.com/profile/28666)
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Editorial Board Member
Institute for Muscle Biology and Growth, Leibniz Institute for Farm Animal Biology (FBN), Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany
Interests: lipids in farm animals; lipid metabolism; lipidomics; membrane microdomains; n-3/n-6 PUFA

Dr. Gary W. Daughdrill
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Editorial Board Member
Department of Cell Biology, Microbiology and Molecular Biology, University of South Florida, 4202 East Fowler Ave, ISA2015, Tampa, FL 33620, USA
Interests: IDP

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Editorial Board Member
Human and Animal Physiology, Department of Animal Sciences, Wageningen University and Research, 6708 WD Wageningen, The Netherlands
Interests: metabolism; mitochondria, gut health; immunometabolism; polyamines; post-translational modifications; epigenetics; protein acylation; extracellular flux analysis

Prof. Dr. Philippe De Deurwaerdère (https://sciprofiles.com/profile/384091)
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Editorial Board Member
Centre National de la Recherche Scientifique (Unité Mixte de Recherche 5287), CEDEX, 33076 Bordeaux, France
Interests: monoamines; neurochemistry; addiction; Parkinson's disease; schizophrenia; neuropharmacology; mood disorders
Special Issues, Collections and Topics in MDPI journals

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Editorial Board Member
NemaLab-ICAAM, Departamento de Biologia, Universidade de Évora, 7002-554 Évora, Portugal
Interests: plant nematology; plant pathology (phytopathology); forest pathology; biological control; phytochemistry

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Editorial Board Member
School of Life Sciences, Tsinghua University, Beijing, China
Interests: method development in proteomics/metabolomics/chemical biology; biomarker discovery; understanding mechanisms underlying aging and associated diseases
Special Issues, Collections and Topics in MDPI journals

Prof. Dr. Umesh Desai (https://sciprofiles.com/profile/944119)
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1. Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298, USA
2. Drug Discovery and Development, Institute for Structural Biology, Virginia Commonwealth University, Richmond, VA 23219, USA
Interests: drug discovery; chemical biology; biological macromolecules; glycosaminoglycans; coagulation factors; cancer; viral infection; bio-mimetic design; enzyme mechanisms; computational biology; high throughput screening
Special Issues, Collections and Topics in MDPI journals

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Editorial Board Member
Department of Pharmacy, University “G. d’Annunzio” Chieti-Pescara, Chieti, Italy
Interests: neurodegenerative diseases; prodrugs; nanomedicine
Special Issues, Collections and Topics in MDPI journals

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Editorial Board Member
Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA
Interests: translational control; translational recoding; frameshifting; virology; RNA; RNA viruses

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Editorial Board Member
Department of Experimental Medicine, University of Perugia, Perugia, Italy
Interests: cell biology; cancer biology; skeletal muscle regeneration; neurodegeneration; aging; tissue engineering
Special Issues, Collections and Topics in MDPI journals

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Prof. Dr. Wen-ji Dong
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Editorial Board Member
Department of Integrated Physiology and Neuroscience Washington State University, Pullman, WA 99164, USA
Interests: protein bioassay; paper-based disease diagnosis; protein engineering; fluorescence spectroscopy; myofilament proteins; exosomes detection; biosensors

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Editorial Board Member
Center of Life Sciences, Skolkovo Institute of Science and Technology, Skolkovo, Russia Faculty of Chemistry, Moscow State University, Moscow, Russia Belozersky Research Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia
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Interests: epigenomics; cancer genomics; chromosome topology; oncogene regulation; computional biology; systems biology

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Special Issues, Collections and Topics in MDPI journals

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Editorial Board Member
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Special Issues, Collections and Topics in MDPI journals

Prof. Dr. Vincent Ellis (https://sciprofiles.com/profile/905838)

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School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK
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School of Pharmacy, Chapman University, Orange, CA, USA
Interests: glycoproteins; inflammation; macrophages; extracellular matrix proteins
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Special Issues, Collections and Topics in MDPI journals

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Interests: cell signaling; phosphorylation; cytoskeleton; cell motility; protein phosphatase; smooth muscle

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Department of Biomedical and Biotechnological Sciences, University of Catania, Via Santa Sofia, 97, 95123 Catania, Italy
Interests: systemic and organ specific autoimmune diseases; cellular and molecular processes; immune activation and suppression; functional role of molecules; new target-specific interventions; regulation; biological functions; potential therapies
Special Issues, Collections and Topics in MDPI journals

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Editorial Board Member
Department of Diagnostic and Biomedical Sciences, School of Dentistry, The University of Texas Health Science Center at Houston, Houston, TX 77054, USA
Interests: extracellular matrix; heparan sulfate; prostate cancer; salivary gland; tissue engineering; hyaluronic acid; cell adhesion; bone metastasis

Dr. Ramin M. Farahani (https://sciprofiles.com/profile/2872208)
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Interests: neurogenesis; mitochondria; notch signalling pathway

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Editorial Board Member
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Special Issues, Collections and Topics in MDPI journals

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Department of Medicine, Washington University in St. Louis, St. Louis, MO 63110, USA
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Special Issues, Collections and Topics in MDPI journals

Prof. Dr. Michele Fornaro (https://sciprofiles.com/profile/1401759)
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Editorial Board Member
Department of Anatomy, College of Graduate Studies and Chicago College of Osteopathic Medicine, Midwestern University, Downers Grove, IL 60515, USA
Interests: plasticity of the peripheral nervous system; nerve regeneration; adulthood and development
Special Issues, Collections and Topics in MDPI journals

Prof. Dr. Carola Yvette Förster (https://sciprofiles.com/profile/1231227)
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Editorial Board Member
Julius-Maximilians-Universitat Wurzburg, Department of Anesthesia and Critical Care, Wurzburg, Germany
Interests: cerebrovascular biology; cardiovascular biology; brain-heart; brain cancer; neuroinflammation; ischemic brain injury; systems biology and mathematical modeling
Special Issues, Collections and Topics in MDPI journals

Dr. José María Frade
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Editorial Board Member
Department of Molecular, Cellular and Developmental Neurobiology, Cajal Institute, CSIC, Avda. Doctor Arce, 37, E-28002 Madrid, Spain
Interests: molecular and cellular neurobiology

Prof. Dr. Hanne Frøkiær (https://sciprofiles.com/profile/1439712)
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Editorial Board Member
Department of Veterinary and Animal Sciences, Faculty of Health and Medical Science, University of Copenhagen, 2100 Copenhagen, Denmark
Interests: dietary components; food related microorganisms; environmental microbiota; immune system

Dr. Pio Maria Furneri (https://sciprofiles.com/profile/1248018)
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Interests: bacteriocins; prebiotics; probiotics; antibiotic; natural products; drug delivery systems; bacterial pathogenesis; antiviral natural compounds; antiproliferative natural compounds; disinfectants; antimycotics, synbiotics
Special Issues, Collections and Topics in MDPI journals

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Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan
Interests: peptide and protein engineering; in-cell chemistry; drug delivery

Dr. Maria E. Gaczyńska (https://sciprofiles.com/profile/175552)
Website (https://www.uthscsa.edu/academics/medicine/profile/gaczynska)
Editorial Board Member
Department of Molecular Medicine, Institute of Biotechnology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78245, USA
Interests: proteasome; allosteric proteasome regulators; ubiquitin-proteasome pathway in cancer, aging and immune response; protein allostery; protein biophysics; scanning probe microscopy; atomic force microscopy; single-cell biophysics; circulating tumor cells
Special Issues, Collections and Topics in MDPI journals

Dr. Raul Gainetdinov (https://sciprofiles.com/profile/1163598)
Website (http://biomedinstitute.spbu.ru/en/rgainetdinov_en)

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Editorial Board Member
Institute of Translational Biomedicine, St. Petersburg State University, St. Petersburg 199034, Russia
Interests: dopamine; serotonin; trace amines; schizophrenia; depression; Parkinson's disease; knockout mice; psychostimulants; antipsychotics; antidepressants; mood stabilizers; cognitive enhancers
Special Issues, Collections and Topics in MDPI journals

Dr. Zhangguo Gao (https://sciprofiles.com/profile/654528)
Website (https://www.niddk.nih.gov/about-niddk/staff-directory/biography/gao-zhanguo)
Editorial Board Member
Laboratory of Bioorganic Chemistry, Molecular Recognition Section, NIDDK, National Institutes of Health, Bldg. 8A, Room B1A-23, 9000 Rockville Pike, Bethesda, MD 20892-0810, USA
Interests: molecular pharmacology; chemical biology; GPCRs; adenosine receptors; P2Y receptors; allosteric modulation; GPCR signaling

Prof. Dr. María S. García Gutiérrez (https://sciprofiles.com/profile/1394579)
Website (https://www.umh.es/contenido/Estudiantes/:persona_109981/datos_es.html)
Editorial Board Member
Instituto de Neurociencias, Miguel Hernández University, Av. Ramón y Cajal s/n, 03550 San Juan de Alicante, Alicante, Spain
Interests: cannabinoid receptors; psychiatry; neuroglia; animal models; neuropharmacology
Special Issues, Collections and Topics in MDPI journals

Dr. Maria Gasset (https://sciprofiles.com/profile/376734)
Website (https://www.csic.es/en/investigation/research-groups/bioconformatics-and-protein-assemblies)
Editorial Board Member
Institute of Physical-Chemistry Rocasolano (IQFR), Spanish National Research Council (CSIC), 28006 Madrid, Spain
Interests: protein folding; self-assembly; amyloids; food allergens; protein-membrane interactions

Prof. Dr. Juan Carmelo Gómez-Fernández (https://sciprofiles.com/profile/734777)
Website (https://www.researchgate.net/profile/Juan_Gomez-Fernandez)
Editorial Board Member
Department of Biochemistry and Molecular Biology A, Universidad de Murcia, Murcia, Spain
Interests: biomembranes; model membranes; molecular interactions in membranes; membrane biophysics; NMR of membranes; calorimetry of membranes; FTIR; X-ray diffraction of membranes; protein kinases C; phosphoinositides; liposomes; lipid nanostructures

Prof. Dr. Daniela Grimm (https://sciprofiles.com/profile/1291908)
Website (http://mtrm.med.ovgu.de/Team/Daniela+Grimm.html)
Editorial Board Member
1. Department of Microgravity and Translational Regenerative Medicine, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany
2. Department of Biomedicine, Aarhus University, Aarhus, Denmark
Interests: space medicine; translational regenerative medicine; tissue engineering; cancer research; biomarker
Special Issues, Collections and Topics in MDPI journals

Prof. Dr. Dirk Grimm (https://sciprofiles.com/profile/457496)
Website (https://grimm-labs.com/)
Editorial Board Member
Dept. of Infectious Diseases/Virology, Medical Faculty, University of Heidelberg, 69120 Heidelberg, Germany
Interests: AV vectors; RNA interference; Infectious diseases; induced pluripotent stem cells; Gene/genome engineering; non-coding RNAs; CRISPR

Prof. Dr. Frank G. Grosveld (https://sciprofiles.com/profile/12858)
Website (https://www.nbic.nl/about-nbic/nbic-faculty/details/prof-dr-frank-g-grosveld/index.html)
Editorial Board Member
Erasmus Medical Center (Faculty of Medicine), Department of Cell Biology, Erasmus University Rotterdam, Dr Molewaterplein 50, 3015GE Rotterdam, The Netherlands
Interests: transcription factors/gene regulation; genomic interactions; genomic structure development and differentaition of the mammalian hematopoietic system

Prof. Dr. Jörg Gsponer (https://sciprofiles.com/profile/1127174)
Website (https://biochem.ubc.ca/person/joerg-gsponer/)
Editorial Board Member
Michael Smith Laboratories, Department of Biochemistry & Molecular Biology, University of British Columbia, Vancouver, BC V6T 1Z4, Canada
Interests: structural bioinformatics; molecular modeling; intrinsically disordered proteins; protein evolution; allostery; protein interactions; phase separation

Prof. Dr. Jianguo Gu (https://sciprofiles.com/profile/2258152)
Website (https://www.tohoku-mpu.ac.jp/laboratory/drg/index.html)
Editorial Board Member

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Institute of Molecular Biomembrane and Glycobiology, Tohoku Medical and Pharmaceutical University, Sendai, Japan
Interests: glycobiology; cell adhesion; cancer

Dr. Zheng Guo
Website (https://pure.au.dk/portal/en/persons/zheng-guo(67372258-2bc9-4585-98c9-1b93c60d0ca9).html)
Editorial Board Member
Department of Engineering, Faculty of Technical Science, Aarhus University, 8000 Aarhus, Denmark
Interests: bio-refining; green synthesis; biocatalysts; enzyme engineering; lipid chemistry; ionic liquids; enzyme discovery; new chemistry

Dr. Michael Hackenberg (https://sciprofiles.com/profile/846090)
Website (https://bioinfo2.ugr.es/ceUGR/)
Editorial Board Member
Computational Epigenomics Lab, Department of Genetics, Faculty of Science, University of Granada, 18071 Granada, Spain
Interests: computational genomics; bioinformatics; small RNA detection and prediction; miRNA targets; small RNAs at the interface between parasites and hosts; NGS data analysis; epigenomics; epitranscriptomics
Special Issues, Collections and Topics in MDPI journals

Dr. Marc W. Halterman (https://sciprofiles.com/profile/1896167)
Website (https://renaissance.stonybrookmedicine.edu/neurovascular_protection_lab)
Editorial Board Member
Department of Neurology, Renaissance School of Medicine, Stony Brook University, Stony Brook, NY 11794, USA
Interests: ischemia/stroke; neuroprotection; vascular biology; tetracyclines; neutrophil biology

Dr. Mohamed Hammadeh (https://sciprofiles.com/profile/2354985)
Website (https://www.researchgate.net/profile/Mohamad-Eid-Hammadeh)
Editorial Board Member
Department of Obstetrics and Gynecology, University of Saarland, 66421 Homburg, Germany
Interests: molecular biology and biochemistry of reproductive medicine; epigenetic; cytokines; assisted reproduction

Dr. Aleš Hampl
Website (https://www.muni.cz/en/people/47170-ales-hampl)
Editorial Board Member
Faculty of Medicine, Department of Histology andEmbryology, Masaryk University, Kamenice 3, 625 00 Brno, Czech Republic
Interests: genetic stability of stem cells; modelling diseases using stem cells; biomaterials; tissue engineering; tissue regeneration; gamete development; early embryogenesis

Prof. Dr. Byung Woo Han (https://sciprofiles.com/profile/374283)
Website (https://snupharm.snu.ac.kr/ko/node/164)
Editorial Board Member
College of Pharmacy, Seoul National University, Seoul, Korea
Interests: structural biology; tumor microenvironment; protein degradation pathway; aminoacyl-tRNA synthetase; immunology
Special Issues, Collections and Topics in MDPI journals

Prof. Dr. Kyungsook Han (https://sciprofiles.com/profile/677294)
Website (http://bclab.inha.ac.kr)
Editorial Board Member
Department of Computer Engineering, Inha University, Incheon, Korea
Interests: machine learning; protein-binding motif; protein-RNA interaction; cancer; gene correlation network; protein-protein interaction

Prof. Dr. Fumio Hanaoka (https://sciprofiles.com/profile/13234)
Website (https://www.nig.ac.jp/nig/about-nig/directors-welcome)
Editorial Board Member
National Institute of Genetics, 1111 Yata, Mishima, Shizuoka 411-8540, Japan
Interests: molecular mechanisms of translesion synthesis and nucleotide excision repair; understanding the cellular responses to DNA damages; interactions between cell cycle control and DNA repair
Special Issues, Collections and Topics in MDPI journals

Prof. Dr. Yusuf A. Hannun (https://sciprofiles.com/profile/1259966)
Website (https://www.stonybrook.edu/commcms/biochem/research/_faculty/hannun/#ResearchDescription)
Editorial Board Member

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1. Department of Pharmacology, Stony Brook University, Stony Brook, NY 11794, USA
2. Department of Medicine, Stony Brook University, Stony Brook, NY 11794, USA
3. Cancer Center, Stony Brook University, Stony Brook, NY 11794, USA

Interests: bioactive lipids; ceramide; sphingolipids; sphingomyelinases; protein kinase C; protein phosphatases

Special Issues, Collections and Topics in MDPI journals

Dr. Christophe Hano (https://sciprofiles.com/profile/296514)

Website (https://www.univ-orleans.fr/fr/lblgc/christophe-hano)

Editorial Board Member

Department of Biological Chemistry, Faculty of Sciences and Technologies, University of Orleans, Chartres, France

Interests: chemistry of natural products; analytical methods; HPLC; LC-MS; polyphenols; ethnopharmacology; history of pharmacy

Special Issues, Collections and Topics in MDPI journals

Dr. Mark Harrison (https://sciprofiles.com/profile/250459)

Website (https://staff.qut.edu.au/staff/md.harrison)

Editorial Board Member

Science and Engineering Faculty, Queensland University of Technology, Brisbane 4001, Australia

Interests: lignocellulose; cellulose; hemicellulose; oligosaccharides; xylooligosaccharides; fructooligosaccharides; lignin; phenolics; anthocyanins; anthocyanadins; flavonoids; plant secondary metabolites; fung

Prof. Dr. Terry Hébert (https://sciprofiles.com/profile/561358)

Website (http://www.medicine.mcgill.ca/pharma/hebertlab/profiles/hebert_terry.htm)

Editorial Board Member

Department of Pharmacology and Therapeutics, McGill University, Room 1303 McIntyre Medical Sciences Building, 3655 Promenade Sir-William-Osler, Montreal, QC H3G 1Y6, Canada

Interests: cellular signalling; G protein-coupled receptors; stem cells; heart disease; drug discovery

Prof. Dr. Thomas Helleday

Website (http://ki.se/en/people/thohel)

Editorial Board Member

Torsten and Ragnar Söderberg Professor of Translational Medicine Science for Life Laboratory, Division of Translational Medicine and Chemical Biology Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Box 1031, S-171 21 Stockholm, Sweden

Interests: DNA damage signalling; homologous recombination at replication forks in mammalian cells

Special Issues, Collections and Topics in MDPI journals

Dr. Jaap J. Van Hellemond (https://sciprofiles.com/profile/2224807)

Website1 (https://www.erasmusmc.nl/en/research/researchers/hellemond-van-jaap) **Website2 (https://scholar.google.com/citations?user=4XAFposAAAAJ&hl=nl&oi=ao)**

Editorial Board Member

Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, ‘s Gravendijkwal 230, Rotterdam, The Netherlands

Interests: host-parasite interactions; parasites; parasitic infections; external quality assessment schemes; helminths; schistosomiasis; free living amoeba; energy metabolism

Dr. Gregory Henderson (https://sciprofiles.com/profile/1681152)

Website (https://www.purdue.edu/hhs/nutr/directory/faculty/henderson-greg.html)

Editorial Board Member

College of Health and Human Sciences, West Lafayette, IN, USA

Interests: lipid metabolism; lifestyle factors; energy balance; exercise; diet selection

Prof. Dr. Wolf-Dietrich Heyer (https://sciprofiles.com/profile/894939)

Website (http://micro.ucdavis.edu/heyer)

Editorial Board Member

Professor and Chair, Department of Microbiology & Molecular , University of California, Davis, One Shields Avenue, Davis, CA 95616, USA

Interests: regulation and mechanisms of homologous recombination; genome stability; DNA damage response

Special Issues, Collections and Topics in MDPI journals

Dr. Stanley M Hileman (https://sciprofiles.com/profile/1763452)

Website (https://directory.hsc.wvu.edu/Profile/29892)

Editorial Board Member

Department of Physiology and Pharmacology, West Virginia University, Morgantown, WV, USA

Interests: neurobiology; nutrition; reproduction; obesity

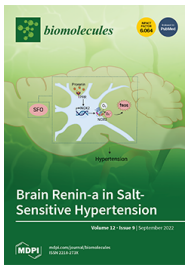
Special Issues, Collections and Topics in MDPI journals

Dr. Aleš Horák

Website (https://www.paru.cas.cz/en/staff/profile/40/)

Editorial Board Member

Biomolecules, Volume 12, Issue 9 (September 2022) – 163 articles



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Biomolecules **2022**, *12*(9), 1323; **https://doi.org/10.3390/biom12091323 (https://doi.org/10.3390/biom12091323)** - 19 Sep 2022

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The Paradoxical Role of Circulating Ketone Bodies in Glycemic Control of Individuals with Type 2 Diabetes: High Risk, High Reward? (/2218-273X/12/9/1318)

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Injectable Peptide Hydrogel Encapsulation of Mesenchymal Stem Cells Improved Viability, Stemness, Anti-Inflammatory Effects, and Early Stage Wound Healing (/2218-273X/12/9/1317)

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The Role of Disordered Regions in Orchestrating the Properties of Multidomain Proteins: The SARS-CoV-2 Nucleocapsid Protein and Its Interaction with Enoxaparin (/2218-273X/12/9/1302)

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The Association of Metformin, Other Antidiabetic Medications and Statins on the Prognosis of Rectal Cancer in Patients with Type 2 Diabetes: A Retrospective Cohort Study (/2218-273X/12/9/1301)

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Generation and Functional Characterization of PLAP CAR-T Cells against Cervical Cancer Cells (/2218-273X/12/9/1296)

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Functional and Structural Impact of Deleterious Missense Single Nucleotide Polymorphisms in the NR3C1, CYP3A5, and TNF-α Genes: An In Silico Analysis

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Abstract: Human diseases are generally influenced by SNPs (single nucleotide polymorphisms). The mutations in amino acid residues generated by deleterious SNPs contribute to the structural and functional diversity of the encoded protein. Tumor necrosis factor-α (TNF-α), Glucocorticoid receptor gene (NR3C1), and Cytochrome P450 3A5 (CYP3A5) play a key role in glucocorticoid resistance susceptibility in humans. Possible causative mutations could be used as therapeutic targets and diagnostic markers for glucocorticoid resistance. This study evaluated the missense SNPs of TNF-α, NR3C1, and CYP3A5 to predict their impact on amino acid changes, protein interaction, and functional stability. The protein sequence of dbSNP was obtained and used online in silico method to screen deleterious mutants for the in silico analysis. In the coding regions of TNF-α, NR3C1, and CYP3A5, 14 deleterious mutations were discovered. The protein functional and stability changes in the amino acid between native and mutant energy were identified by analyzing the changes in the hydrogen bonding of these mutants from native, which were all measured using Swiss PDB and PyMOL. F446S and R439K had the highest root-mean-square deviation (RMSD) values among the 14 deleterious mutants. Additionally, the conserved region of amino acid protein interaction was analyzed. This study could aid in the discovery of new detrimental mutations in TNF-α, NR3C1, and CYP3A5, as well as the development of long-term therapy for corticosteroid resistance in several inflammatory diseases. However, more research into the deleterious mutations of the *TNF-α*, *NR3C1*, and *CYP3A5* genes is needed to determine their role in corticosteroid resistance.

Keywords: glucocorticoid resistance; computational study; pharmacogenomic; precision medicine; missense mutation; SNP

1. Introduction

Glucocorticoids are pharmacological agents used to treat a variety of illnesses. Glucocorticoids can be endogenous or therapeutic, can affect nearly every tissue in the body, and play an important role in human physiology [1]. However, when patients are treated chronically with glucocorticoids, they develop glucocorticoid resistance or sensitivity, making

them more vulnerable to chronic diseases such as asthma, heart disease, and depression [2]. One of the limitations of steroid therapy is the development of resistance to the beneficial effects of glucocorticoids on targeting the tissues [3].

During acute inflammation, macrophages produce many inflammatory cytokines, one of which is TNF-α. It is a proinflammatory cytokine that includes cytotoxic and also immunoregulatory activity. It plays a significant role in B cells, dendritic, and T cells as an immunoregulator and is involved in the execution of the cell death process [4,5]. TNF-α, a potent proinflammatory cytokine, causes an acute form of glucocorticoid resistance. TNF-α has a significant and widespread effect on glucocorticoid transcriptional performance but no effect on nuclear translocation, dimerization, or DNA binding capacity [6]. The human glucocorticoid receptor gene *NR3C1* has nine exons and is located on chromosome 5q31–32. The glucocorticoid receptor polymorphism has an effect on glucocorticoid receptor function, which could be a key factor in glucocorticoid therapy resistance [7]. The *NR3C1* gene, specifically its variants, may influence glucocorticoid treatment towards the target disease. Sensitivity, and treatment outcomes. The exogenous and endogenous molecular mechanisms of glucocorticoids are mainly influenced by intracellular steroid receptors, and NR3C1 encodes them. Glucocorticoid sensitivity is mostly found in the *NR4C1* genes with several SNPs [8]. CYP3A5 belongs to the CYP3A subfamily, and it has a primary role in the metabolism of half of the drugs prescribed globally. Polymorphism in the *CYP3A5* gene severely affects the metabolism and increases the disease condition [9]. The inflammatory transcription gene is downregulated when multiple anti-inflammatory genes are activated or through an independent process synthesis process when the glucocorticoid binds to NR3C1 and CYP3A5 [10,11]. In many cases, mutations or polymorphisms in the *NR3C1* and *CYP3A5* genes may be responsible for glucocorticoid resistance, and treatments are impaired.

Recently, many studies have focused on factors affecting the glucocorticoid function at the molecular level and the genetic variants that played a major role in influencing the mechanism of glucocorticoids. The primary function of glucocorticoids is to suppress the expression of inflammatory genes in a variety of ways via the cytoplasmic receptor interaction in which glucocorticoids interact and inhibit nuclear factor-kappa. (NF-KB) [12]. The resistance of glucocorticoids is caused by the mutation in the amino acids of a gene such as TNF-α, NR3C1, and CYP3A5, while several polymorphisms of this gene are involved in glucocorticoids' toxicity and response. Thus, due to this condition of glucocorticoid resistance, the anti-inflammatory drugs of glucocorticoids cannot act as a powerful medicine. To understand the pattern of corticosteroid resistance in patients, computational studies are performed on the polymorphisms of the genes such as *TNF-α*, *NR3C1*, and *CYP3A5*.

2. Material and Methods

2.1. Prediction and Screening of Deleterious SNPs

Using the national center for biotechnology information (NCB1, 1998, United states) and UnitProtKB (Developed by Georgetown university, United states, 2002), the protein sequence and gene of the human TNF-α (Uniport ID: P01375), NR3C1 (Uniport ID: P04150), and CYP3A5 (Uniport ID: P20815) were collected. SNPs are obtained from the dbSNP database (https://www.ncbi.nlm.nih.gov/snp/) (accessed on 22 November 2021) for the *TNF-α*, *NR3C1*, and *CYP3A5* genes. Among them, 403 missense mutations of TNF-α, NR3C1, and CYP3A5 were used for computational analysis.

2.1.1. SIFT and I-Mutant Tools

The normal function of the protein depends on the correct folding of the protein for exhibiting its stability. If not, it may lead to many pathological diseases. Misfolded protein, which leads to loss of their stability, can be studied using the SIFT (https://sift.bii.a-star.edu.sg/) (Introduced in 2001 and supported by bioinformatics institute in Singapore) (accessed on 28 November 2021) and I-Mutant tools (https://folding.biofold.org/cgi-bin/i-mutant2.0.cgi) (accessed on 3 December 2021) [13,14]. Sorting intolerance from tolerance

was performed on the human nsSNPs available in dbSNP [15]. The phenotypic effect on changes in an amino acid is calculated using this software, and the physiochemical and homology sequences are used for prediction. SIFT predictions are primarily based on amino acid physiochemical properties and sequence homology [16]. The neural network-based web server I-Mutant predicts protein mutations that stabilize or destabilize the protein structure [17].

2.1.2. SNP and GO and PolyPhen-2 Tools

The missense mutation causes a change in amino acids by affecting the structure and function of the protein, which were predicted by the SNP and GO (https://snps-and-go.biocomp.unibo.it/snps-and-go/) (accessed on 10 December 2021) and polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) (accessed on 15 December 2021) tools [18,19]. The SNP and GO server gathered data from a variety of sources, including the protein sequence, SNP local sequence environment, and protein sequence profile. The information from the gene ontology database was analyzed using this server [19]. The values are mentioned after the prediction from zero to a positive number. The value of zero indicates that the SNP has no impact on protein structure, while the positive numbers indicate severe consequences.

2.2. Prediction of Site-Directed Mutagenesis

TNF-α (3ALQ) and NR3C1 (1P93) crystal structures are collected from the PDB. The CYP3A5 structure is not available in PDB, and so it was modeled and obtained through SWISS model software (https://swissmodel.expasy.org/) (accessed on 21 December 2021). The amino acid substitution from native to mutant was performed using Swiss PBD viewer 4.1.0 (Developed in 1994 by Nicolas Guex, Swiss institute of bioinformatics, Switzerland. These structures are energy minimized using Swiss PDB viewer 4.1.0 to generate lower energy conformation of a protein structure. During the protein structure modeling, some of the faulty bonded and non-bonded interactions cause structural geometry errors. The energy minimization process is critical for optimizing errors. For the geometry optimization process, the steepest descent algorithm is used [20]. The RMSD value was calculated using PyMOL software 2.5.2 (Created by Warren Lyford Delano and commercialized by schrodinger). The hydrogen bonding variation between the native and mutant was visualized using Swiss PBD viewer 4.1.0 [21,22].

2.3. Prediction of Relative Surface Accessibility

Environmental factors affect protein folding through chemicals or temperature when they are exposed, so it is necessary to evaluate the surface accessibility of protein. NetsurfP 2.0 helps to predict the relative surface accessibility of amino acids, and values are calculated as a Z-score from the network reliability score [23].

2.4. Analysis of Conserved Amino Acid Residues

The ConSurf server (https://consurf.tau.ac.il) (accessed on 23 December 2021) was used for identifying the position of the amino acid based on evolutionary conservation [24]. The FASTA sequence of the TNF-α, NR3C1, and CYP3A5 proteins is provided to the ConSurf server. The color scheme gives a conservation score from 1–9 (Score 9 means the most conserved amino acid whereas 1 means variable amino acid). ConSurf's conservation scores represent a relative measure of evolutionary conservation at each target chain sequence site. The lowest score in a protein represents the most conserved position.

2.5. String

The protein–protein interaction studies are important to analyze since the mutated protein continuously affects the other protein during the diseased condition. This helps to study the mechanism of the diseased condition for targeting the source protein and other

corresponding proteins. The predicted version of protein–protein interaction information was analyzed using String server 11.5 [25].

3. Results and Discussion

TNF-α, *NR3C1*, and *CYP3A5* are three genes that play a key role in glucocorticoid resistance and were chosen for computational SNP analysis. Previous computational analysis studies have aided in predicting functional non-synonymous SNPs associated with the *BCL11A* gene [26]. In our current study, we used in silico tools to screen and analyze the SNPs with the deleterious condition and their impact on the TNF-α, NR3C1, and *CYP3A5* genes. Missense variants can also affect the structure of the protein by affecting the interaction, stability, and solubility of the protein. To evaluate the effect of a missense mutation on protein structure and function, the SNPs are mapped into the protein structure and validated through in silico [27–29]. The human gene of *TNF-α*, *NR3C1*, and *CYP3A5* contains a total of 1119 missense mutations in the NCBI dbSNP database. In this study, randomly, 403 missense mutations of TNF-α (118), NR3C1 (141), and CYP3A5 (144) were retrieved from the dbSNP database. Finding SNPs responsible for specific characteristics using molecular techniques looks to be costly. As a result, in silico techniques can help in genetic association studies and acquire a better understanding of the parent protein's functional and structural characteristics [30]. The selected nsSNPs were tested by SIFT, I-Mutant, polyphen-2, and SNP and GO tools to see if they changed protein stability due to mutation and deleterious. Previously, it was reported that many deleterious SNPs from *BCL11B*, *VDR*, and *CYP24A1* gene are identified using these tools [1,26]

SIFT prediction helps to analyze the function of the protein in case of a change in amino acid and allows for the prioritization of substitutions for further investigation [31]. It speculates on whether or not the substitution is deleterious or tolerated. SIFT values of less than 0.05 are harmful, while values higher than 0.05 are harmless. The selected missense mutations of SNPs were tested by I-Mutant to investigate the change in protein stability due to mutation [32]. PolyPhen-2 is used for studying protein function and structure through information obtained from phylogenetic, structural, and sequence analysis. Deleterious (1.0) and tolerated (0.0) SNPs are identified based on the score value [18]. SNPs and GO can predict mutation-induced disease using protein sequence and functional protein annotation. A probability score greater than 0.5 indicates that the mutation has a disease-related effect on the parent protein function [19]. The findings from SNPs of TNF-α, NR3C1, and CYP3A5 indicate that among the 403 missense mutation, 14 SNPs were predicted from various tools such as SNP, I-Mutant, SNP and GO, polyphen-2 and are presented in Table 1. From the outcomes of these four servers, it was concluded that in TNF-α, there were five deleterious SNPs with rsIDs of rs11574936 (I194N), rs140654183 (T181N), rs190788828 (K87T), rs369510319 (R158H), and rs566451995 (A172V). Then, from NR3C1, there were five deleterious nsSNPs with rsIDs of rs104893913 (R477H), rs104893909 (I559N), rs104893914 (G679S), rs121909726 (L753F), rs6190 (R23T), rs6189 (E22D), and rs104893911 (V571A). Further, CYP3A5 had four deleterious nsSNPs with rsIDS of rs41279854 (F446S), rs13220949 (R439K), rs72552791 (Y53C), and rs140521496 (P416S). It was previously reported that mutations and small deletions in the *NR3C1* gene were the cause of generalized glucocorticoid resistance syndrome [33].

Table 1. Prediction of the effect of SNPs by using SIFT, I-Mutant, SNP&GO, and PolyPhen-2 server.

Gene	Uniprot ID	SNP ID	Amino Acid Changes	SIFT	I-Mutant	SNP&GO	Polyphen-2
TNF-α	P01375	rs11574936	I194N	Damaging	Decrease	Disease	Disease
		rs140654183	T181N	Damaging	Decrease	Disease	Disease
		rs190788828	K87T	Damaging	Decrease	Disease	Disease
		rs369510319	R158H	Damaging	Decrease	Disease	Disease
		rs566451995	A172V	Damaging	Decrease	Disease	Disease
		rs104893913	R477H	Damaging	Decrease	Disease	Disease
NR3C1	P04150	rs104893909	I559N	Damaging	Decrease	Disease	Disease
		rs104893914	G679S	Damaging	Decrease	Disease	Disease
		rs121909726	L753F	Damaging	Decrease	Disease	Disease
		rs6190	R23T	Damaging	Decrease	Disease	Disease
		rs6189	E22D	Damaging	Decrease	Disease	Disease
		rs104893911	V571A	Damaging	Decrease	Disease	Disease
CYP3A5	P20815	rs41279854	F446S	Damaging	Decrease	Disease	Disease
		rs13220949	R439K	Damaging	Decrease	Disease	Disease
		rs72552791	Y53C	Damaging	Decrease	Disease	Disease
		rs140521496	P416S	Damaging	Decrease	Disease	Disease

The native amino acid of the TNF-α, NR3C1, and CYP3A5 proteins was changed to the mutant amino acid using the Swiss PDB for comparative modeling. The modeling of the 3D structure of the protein with mutant and native residues helps to visualize the changes in the amino acids and their structural modification of a protein. The superimposed structure with native to mutant amino acid (Figures 1–3) was created with PyMOL. The structure analysis of TNF-α (PDB ID: 3ALQ) was performed using Swiss PDB. The software maps the SNPs by replacing the amino acid with its mutant and testing for various properties. The RMSD is a commonly used metric for comparing values predicted by a model or estimator to values observed. The RMSD value can be used to measure the backbone distance between the proteins in superimposed structures. The values of RMSD are calculated based on the square root of the averaged square error. The translation and rotation of one structure with respect to the other is a common way to compare the structures of biomolecules or solid bodies to minimize RMSD [13,34]. The greater the RMSD will be when it is a loop, and these scores are measured by comparing the RMSD between the native and mutant [35]. The RMSD calculated by PyMOL revealed that the amino acid changes I194N, T181N, K87T, R158H, and A172V in the TNF-α protein have scores of 0.04, 0.02, 0.03, 0.03, and 0.03 (Table 2). Similarly, RMSD values for the NR3C1 and CYP3A5 proteins are mentioned in Tables 3 and 4. According to the previous report, RMSD analysis revealed a difference in values between mutant and native on the *NR3C1* gene, which causes glucocorticoid resistance [36]. The structure and function of protein rely heavily on hydrogen bonds and other nonbonding interactions [37]. Hence, the Swiss PDB viewer was used to examine hydrogen bonding patterns in both native and mutant structures of both proteins. A change in the position of the hydrogen bond was observed in the proteins TNF-α, NR3C1, and CYP3A5. These findings suggest that these mutations may significantly impact the protein's structure, function, and stability compared to the native form. Figures 4–6 represent the changes in the hydrogen bond. Previous research has shown that a missense mutation in the human glucocorticoid receptor resulted in glucocorticoid resistance by disrupting the hydrogen bond [38]. The structure and functions of proteins are influenced by solvent accessibility and hydrophobicity [39]. Polar side chains in proteins are more likely to be exposed to the solvent, whereas hydrophobic residues are more likely to be buried deep within the protein, away from the solvent. Protein stability improves as the area of water-accessible hydrophobic surface decreases [40,41]. The above-mentioned variants of TNF-α, NR3C1, and CYP3A5 were evaluated for solvent accessibility and stability using the NetsurfP server 2.0. The obtained results are mentioned in Tables 5–7. Mutations in buried sites are more likely to disrupt the protein structure. After further investigation, it was discovered that the mutant type relative solvent accessibility (RSA), and accessible surface area (ASA) values of TNF, NR3C1, and CYP3A5 have changed compared to the native type. The same difference was seen in the Z fit score, indicating that SNP has an

impact on protein structure changes. It was previously shown that glucocorticoid resistance is caused by protein structural alteration in the glucocorticoid receptor [42].

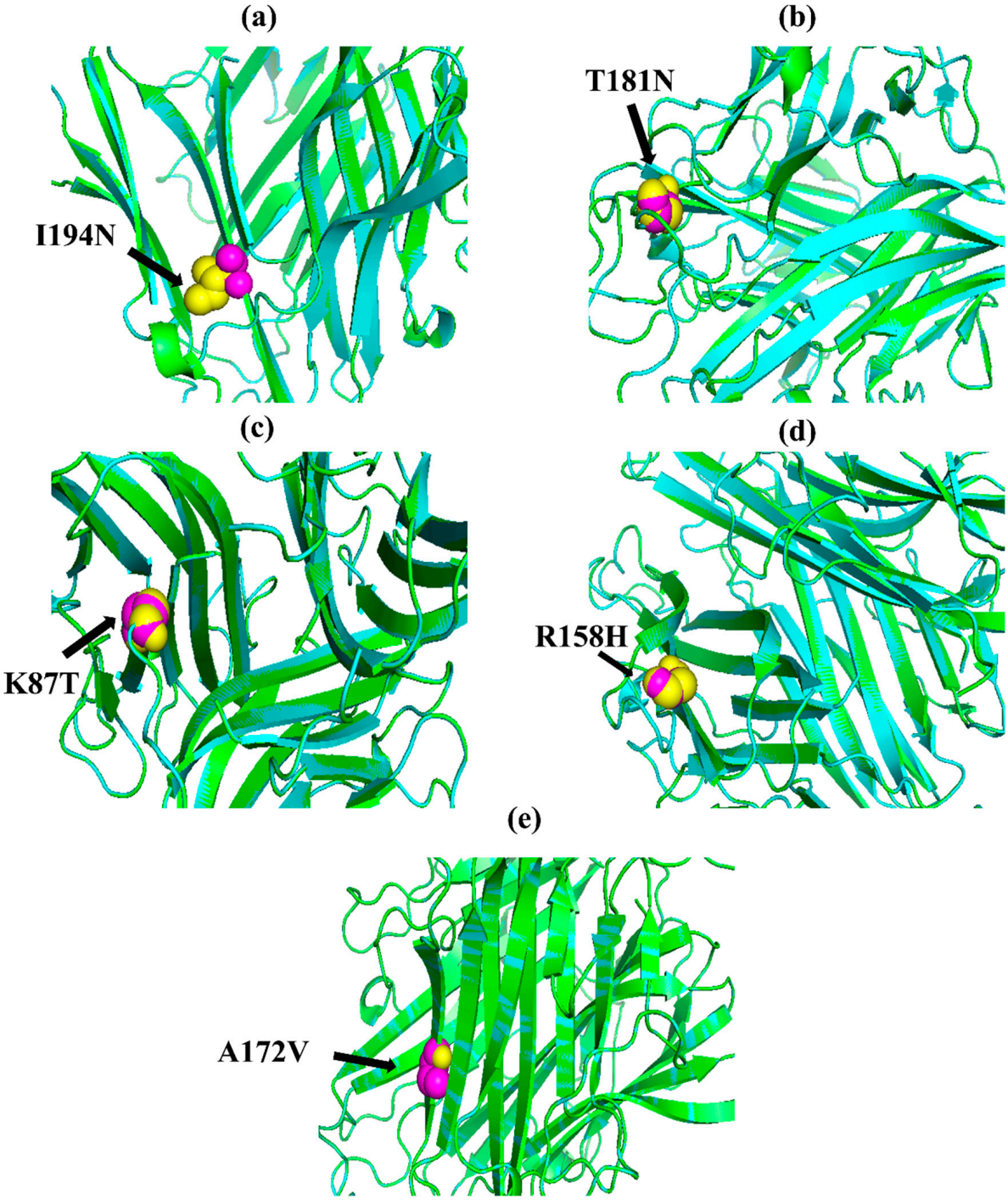


Figure 1. PyMOL was used to visualize 3D structure comparison modeling of the TNF-α protein. (**a**) At position 194, the native amino acid isoleucine (yellow) with the mutant amino acid asparagine (purple). (**b**) At position 181, the native amino acid threonine (yellow) with the mutant amino acid asparagine (purple). (**c**) The native amino acid lysine (yellow) with the mutant amino acid threonine (purple) at position 87. (**d**) The native amino acid arginine (yellow) is with the mutant amino acid histidine (purple) at position 158. (**e**) At position 172, the native amino acid alanine (yellow) with the mutant amino acid valine (purple).

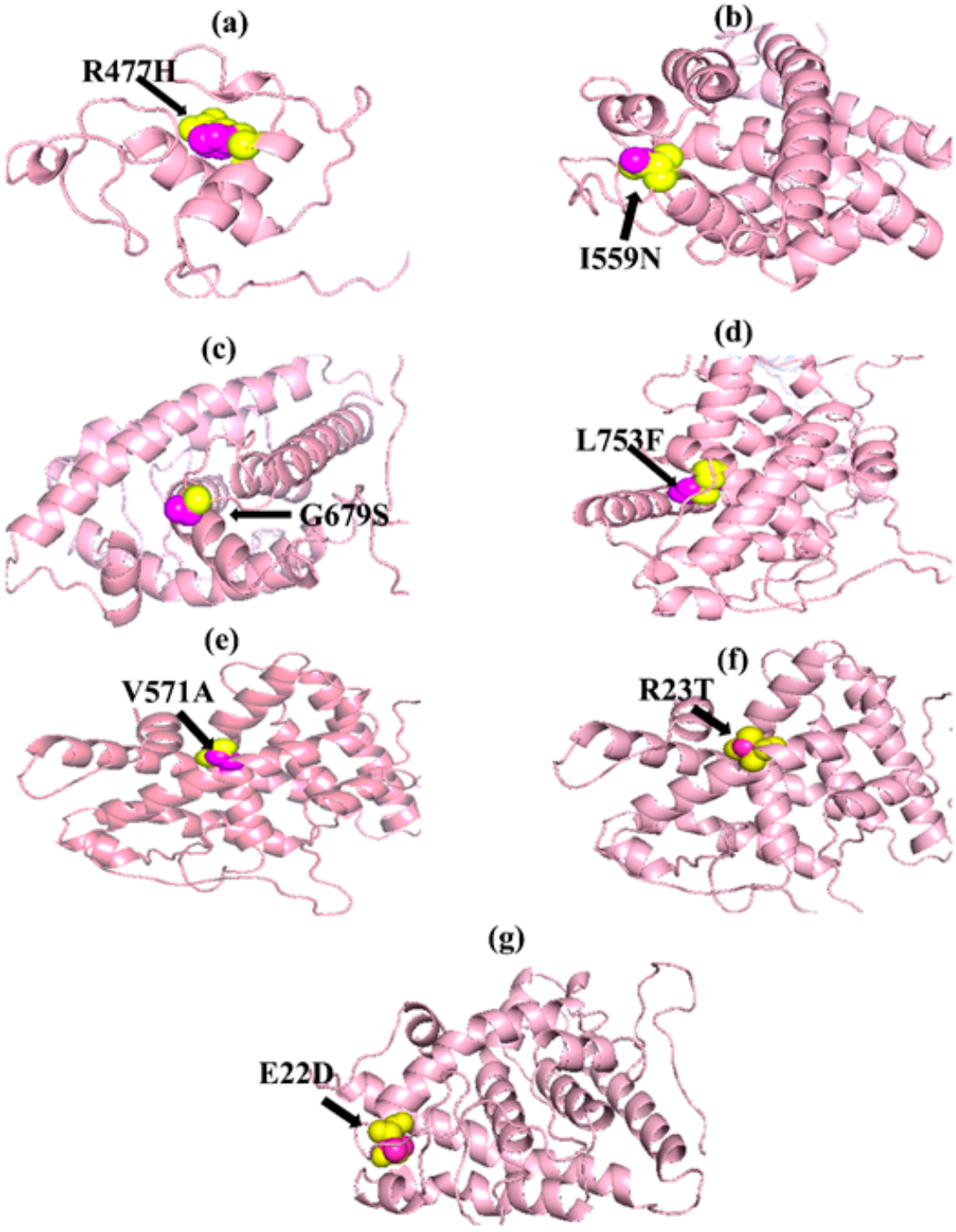


Figure 2. PyMOL was used to visualize 3D structure comparison modeling of the NR3C1 protein. (**a**) The native amino acid arginine (yellow) with the mutant amino acid histidine (purple) at position 477. (**b**) At position 559, the native amino acid isoleucine (yellow) with the mutant amino acid asparagine (purple). (**c**) A sphere-shaped native amino acid glycine (yellow) with a mutant amino acid serine (purple) at position 679. (**d**) At position 753, the native amino acid leucine (yellow) with the mutant amino acid phenylalanine (purple). (**e**) At position 571, the native amino acid valine (yellow) with the mutant amino acid alanine (purple). (**f**) At position 23, the native amino acid arginine (yellow) with the mutant amino acid threonine (purple). (**g**) At position 22, the native amino acid glutamic acid (yellow) with the mutant amino acid aspartic acid (purple).

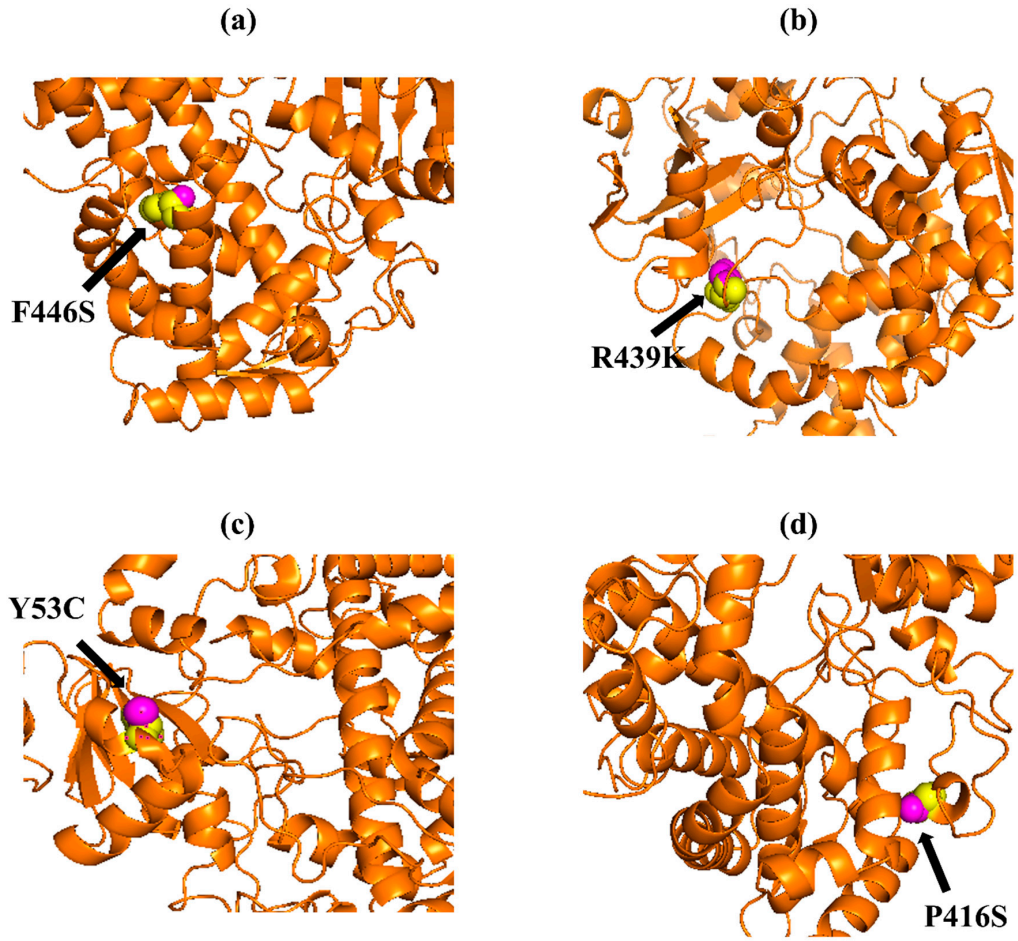


Figure 3. PyMOL was used to visualize 3D structure comparison modeling of the CYP3A5 protein. (**a**) A sphere-shaped native amino acid phenylalanine (yellow) with a mutant amino acid serine (purple) at position 446. (**b**) The native amino acid arginine (yellow) with the mutant amino acid lysine (purple) at position 439. (**c**) A sphere-shaped native amino acid tyrosine (yellow) with a mutant amino acid cysteine (purple) at position 53. (**d**) The native amino acid proline (yellow) with the mutant amino acid serine (purple) at position 416.

Table 2. RMSD value of TNF-α protein.

Gene	Uniprot ID	SNP ID	Amino Acid Change	RMSD(Å)
TNF-α	P01375	rs11574936	I194N	0.04
		rs140654183	T181N	0.02
		rs190788828	K87T	0.03
		rs369510319	R158H	0.03
		rs566451995	A172V	0.03

Table 3. RMSD value of NR3C1 protein.

Gene	Uniprot ID	SNP ID	Amino Acid Change	RMSD(Å)
NR3C1	P04150	rs104893913	R477H	1.7
		rs104893909	I559N	1.9
		rs104893914	G679S	1.8
		rs121909726	L753F	2.3
		rs6190	R23T	1.6
		rs6189	E22D	1.7
		rs104893911	V571A	2.9

Table 4. RMSD value of CYP3A5 protein.

Gene	Uniprot ID	SNP ID	Amino Acid Change	RMSD(Å)
CYP3A5	P20815	rs41279854	F446S	5.3
		rs13220949	R439K	5.3
		rs72552791	Y53C	4.9
		rs140521496	P416S	4.6
		rs104893911	V571A	2.9

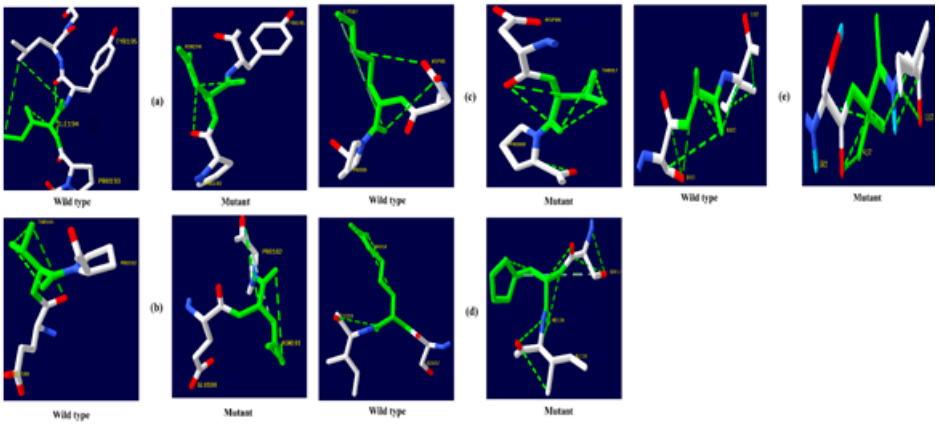


Figure 4. TNF-α hydrogen bonding results (**a**) I194N, (**b**) T181N, (**c**) K87T, (**d**) R158H, and (**e**) A172V.

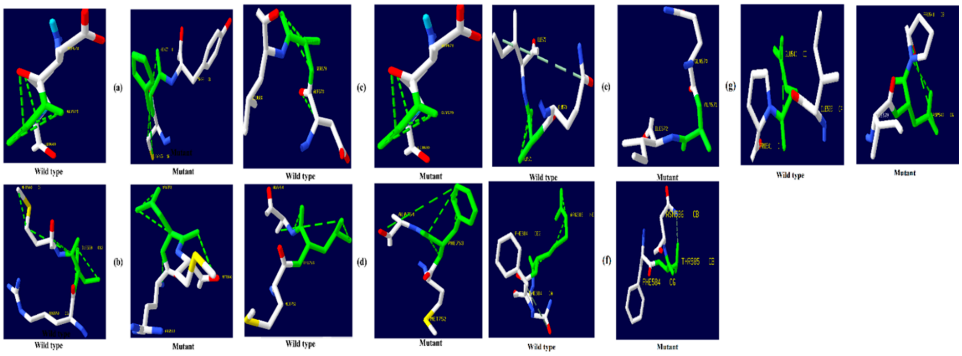


Figure 5. NR3C1 protein hydrogen bonding results (**a**) R477H, (**b**) I559N, (**c**) G679S, (**d**) L753F, (**e**) V571A, (**f**) R23T, and (**g**) E22D.

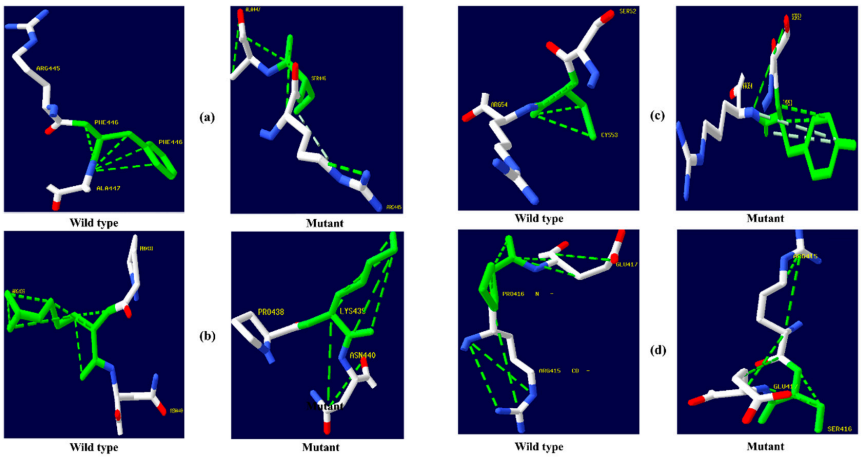


Figure 6. CYP3A5 protein hydrogen bonding results (**a**) F446S, (**b**) R439K, (**c**) Y53C, and (**d**) P416S.

Table 5. Prediction of relative surface accessibility of TNF-α through NetsurfP.

Gene	Mutation	NetsurfP			
		Class	RSA	ASA	Z Fit Score
TNF-α	I194N	Buried	0.067	12.45	−0.365
		Exposed	0.462	67.578	−1.221
	T181N	Exposed	0.501	69.489	−1.623
		Exposed	0.565	82.643	−0.709
	K87T	Exposed	0.349	71.789	0.357
		Exposed	0.368	51.055	0.588
	R158H	Buried	0.189	43.166	0.513
		Buried	0.161	29.213	−0.081
	A172V	Exposed	0.469	51.717	−1.707
		Exposed	0.432	66.383	−1.871

Table 6. Prediction of relative surface accessibility of NR3C1 through NetsurfP.

Gene	Mutation	NetsurfP			
		Class	RSA	ASA	Z Fit Score
NR3C1	R477H	Buried	0.155	35.449	−0.288
		Buried	0.178	32.324	−0.183
	I559N	Exposed	0.378	70.004	−1.158
		Exposed	0.341	49.878	−0.937
	G679S	Exposed	0.389	30.591	−1.456
		Exposed	0.389	45.626	−1.597
	L753F	Buried	0.028	5.145	0.956
		Buried	0.029	5.84	0.777
	R23T	Exposed	0.396	90.661	−0.661
		Exposed	0.427	59.225	−0.744
	E22D	Exposed	0.446	77.829	−1.100
		Exposed	0.580	83.549	−1.691
	V571A	Exposed	0.100	15.324	−0.021
		Exposed	0.078	8.629	−0.142

Table 7. Prediction of relative surface accessibility of CYP3A5 through NetsurfP.

Gene	Mutation	NetsurfP			
		Class	RSA	ASA	Z Fit Score
CYP3A5	F446S	Buried	0.076	15.293	−0.242
		Buried	0.078	9.165	−0.177
	R439K	Buried	0.162	37.19	−0.931
		Buried	0.174	35.854	−1.07
	Y53C	Buried	0.107	22.887	−1.153
		Buried	0.095	13.38	−0.499
	P416S	Buried	0.122	17.34	−0.13
		Buried	0.127	14.873	−0.116

The evolutionary rate is calculated in ConSurf based on the evolutionary relationship between the protein and its homologs and the amino acid similarity as reflected in the substitutions matrix. The residues R158 and A172 are conserved and exposed with a score of 8 in the TNF-α protein (Figure 7). The R477, G679, and L753 residues in the NR3C1 protein are highly conserved and exposed, with a score value of 9 (Figure 8). The residues R439 and P416 in the CYP3A5 protein, on the other hand, are highly conserved, whereas the other residues F446 and Y53 are variable (Figure 9) [24]. Further, the interaction of the TNF-α, NR3C1, and CYP3A5 proteins with other corresponding proteins which may affect the signaling pathway was studied using the STRING database. Both confidence and evidence

views have been shown in Figures 10–12. It has been observed that there is a strong functional association of TNF-α protein with IL10, RIPK1, TNFRSF1B, TRADD, BIRC2, IKBKG, FADD, TNFAIP3, TRAF2, and TNFRSF1A. For the interaction of NR3C1 protein, it was found by NCOA2, NCOA1, FKBP5, FKBP4, HSPA4, HSP90AA1, JUN, CREBBP, and SMARCA4. The CYP3A5 protein was found to interact with EPHX1, CYP2C19, CYP2B6, CYP4A22, CYP4A11, CYP2A6, CYP1A1, CYP2A13, CYP1A2, and CYP2B6. The results showed that mutation in the residues of these proteins showed that changes in amino acids could interfere with other associated proteins. We sought to anticipate SNPs that can change protein expression and function in three interconnected genes in this work (*TNF-α*, *NR3C1*, and *CYP3A5*). Mutations in these genes have been linked to a variety of disorders. Interestingly, our in silico studies reveal the detrimental nature of these SNPs. As a result, our data obscure the possibility that these mutations alter gene expression and protein structure. As a result, alterations in amino acids in a specific location may be linked to glucocorticoid resistance. As a result, our research could help refine SNP prediction by identifying SNPs with a high potential for complexity.

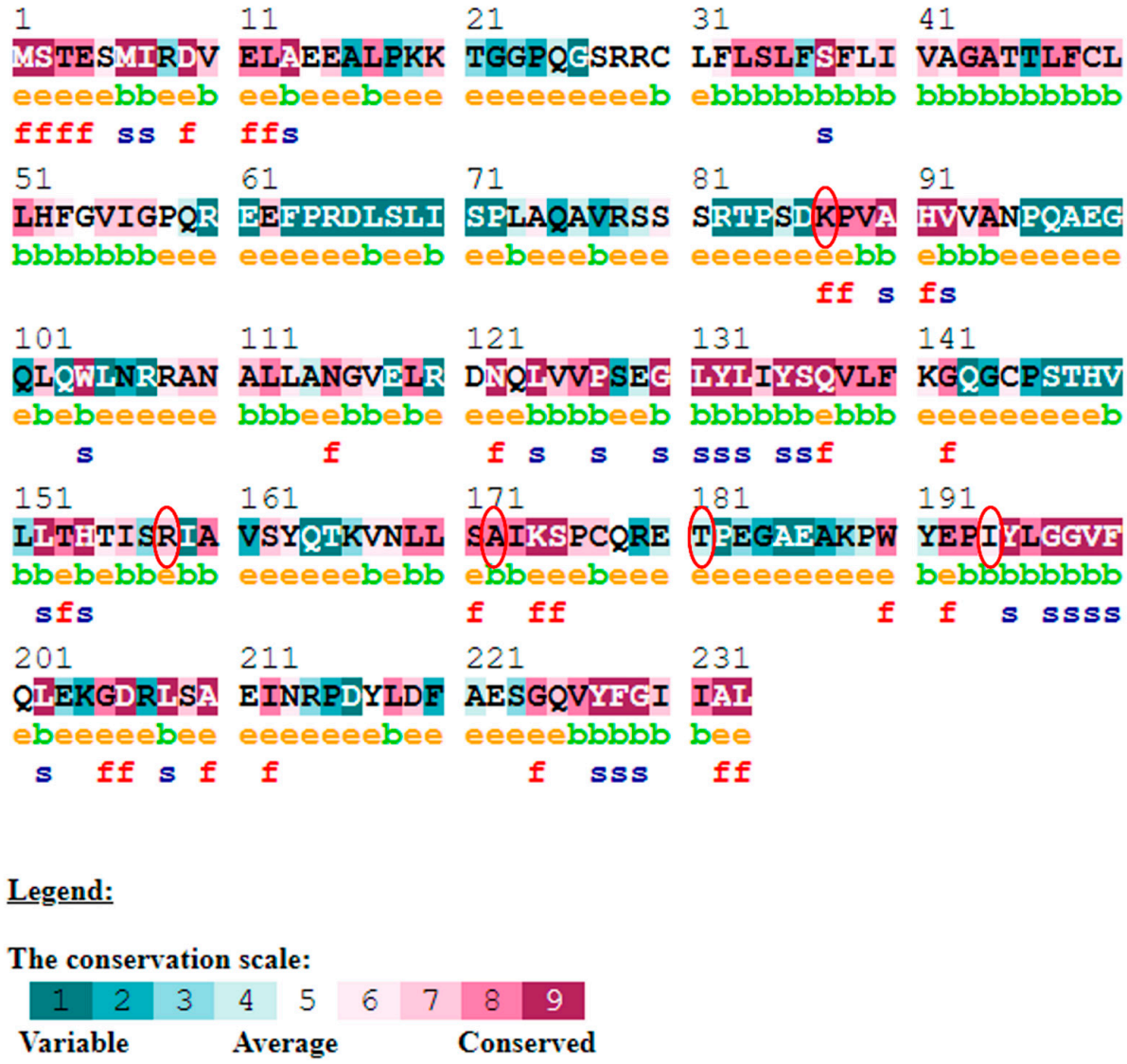


Figure 7. ConSurf server predicted conserved amino acids in TNF-protein. Based on the conservation scale, the amnio acids are ranked as variable, average, and conserved.

Figure 8. The ConSurf server predicted conserved amino acids in the NR3C1 protein. Based on the conservation scale, the amnio acids are ranked as variable, average, and conserved.

Figure 9. The ConSurf server predicted conserved amino acids in the CYP3A5 protein. Based on the conservation scale, the amnio acids are ranked as variable, average, and conserved.

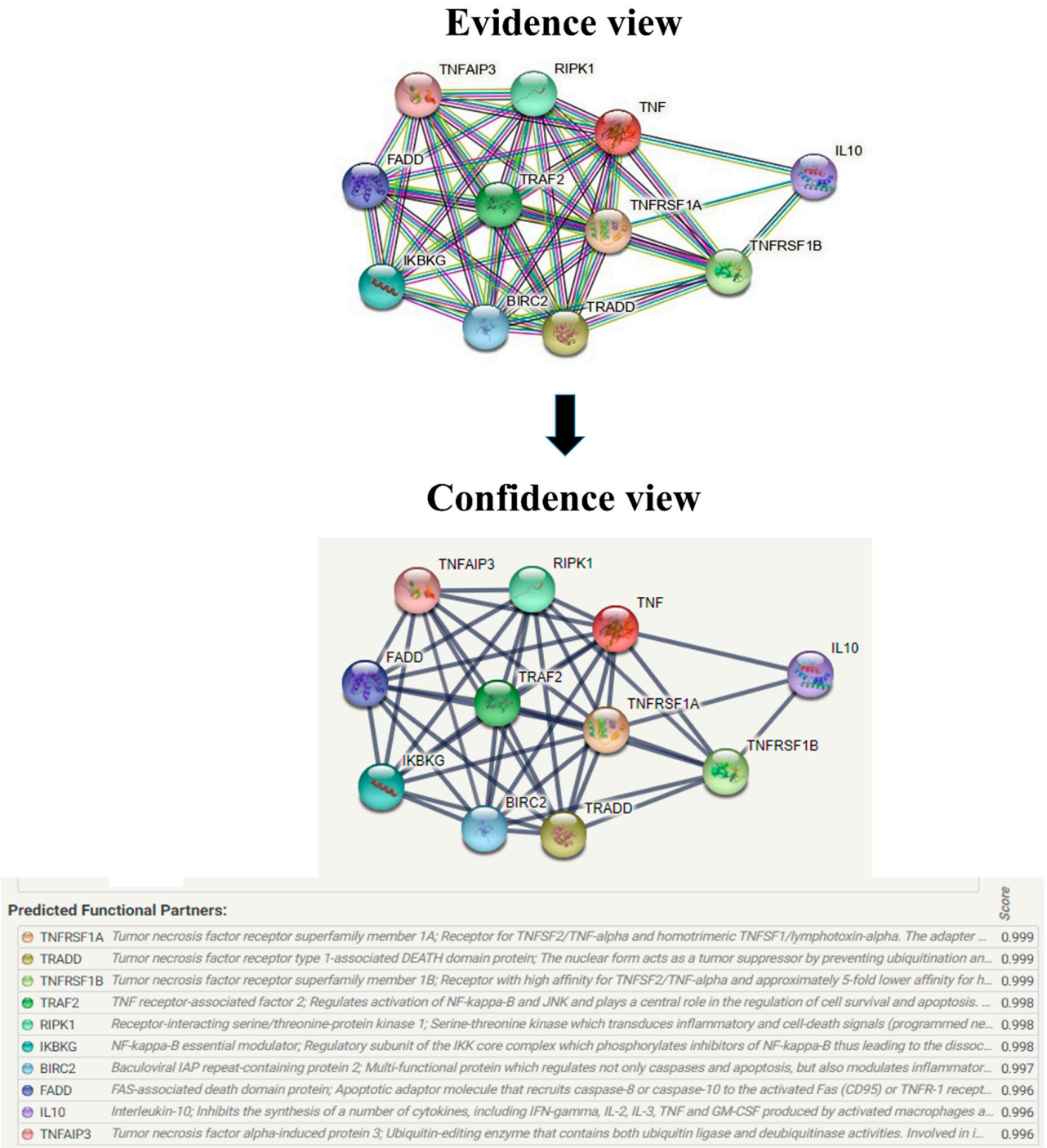
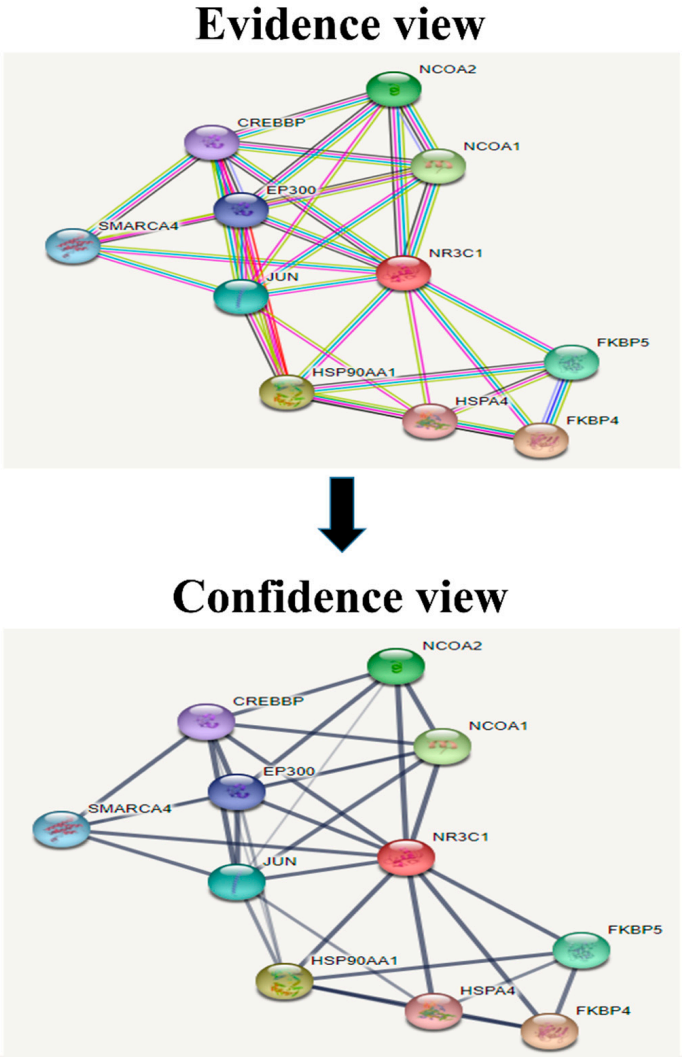


Figure 10. Overview of TNF-α network construction using String server 11.5. The evidence view and confidence view are given.



Predicted Functional Partners:

		Score
FKBP4	*Peptidyl-prolyl cis-trans isomerase FKBP4; Immunophilin protein with PPIase and co-chaperone activities. Component of steroid receptors heterocom...*	0.999
HSP90AA1	*Heat shock protein HSP 90-alpha; Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target ...*	0.999
NCOA1	*Nuclear receptor coactivator 1; Nuclear receptor coactivator that directly binds nuclear receptors and stimulates the transcriptional activities in a horm...*	0.999
NCOA2	*Nuclear receptor coactivator 2; Transcriptional coactivator for steroid receptors and nuclear receptors. Coactivator of the steroid binding domain (AF- ...*	0.999
FKBP5	*Peptidyl-prolyl cis-trans isomerase FKBP5; Immunophilin protein with PPIase and co-chaperone activities. Component of unligated steroid receptors h...*	0.999
JUN	*Transcription factor AP-1; Transcription factor that recognizes and binds to the enhancer heptamer motif 5'-TGA[CG]TCA-3'. Promotes activity of NR5A...*	0.998
SMARCA4	*Swi/snf related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4; Transcription activator BRG1; Involved in transcripti...*	0.998
EP300	*Histone acetyltransferase p300; Functions as histone acetyltransferase and regulates transcription via chromatin remodeling. Acetylates all four core ...*	0.997
CREBBP	*CREB-binding protein; Acetylates histones, giving a specific tag for transcriptional activation. Also acetylates non-histone proteins, like NCOA3 and FO...*	0.993
HSPA4	*Heat shock protein family A member 4; Belongs to the heat shock protein 70 family*	0.993

Figure 11. The protein NR3C1 interacts with a total of 10 other proteins. The evidence view and confidence view are given, while the dark blue lines denoting a strong correlation.

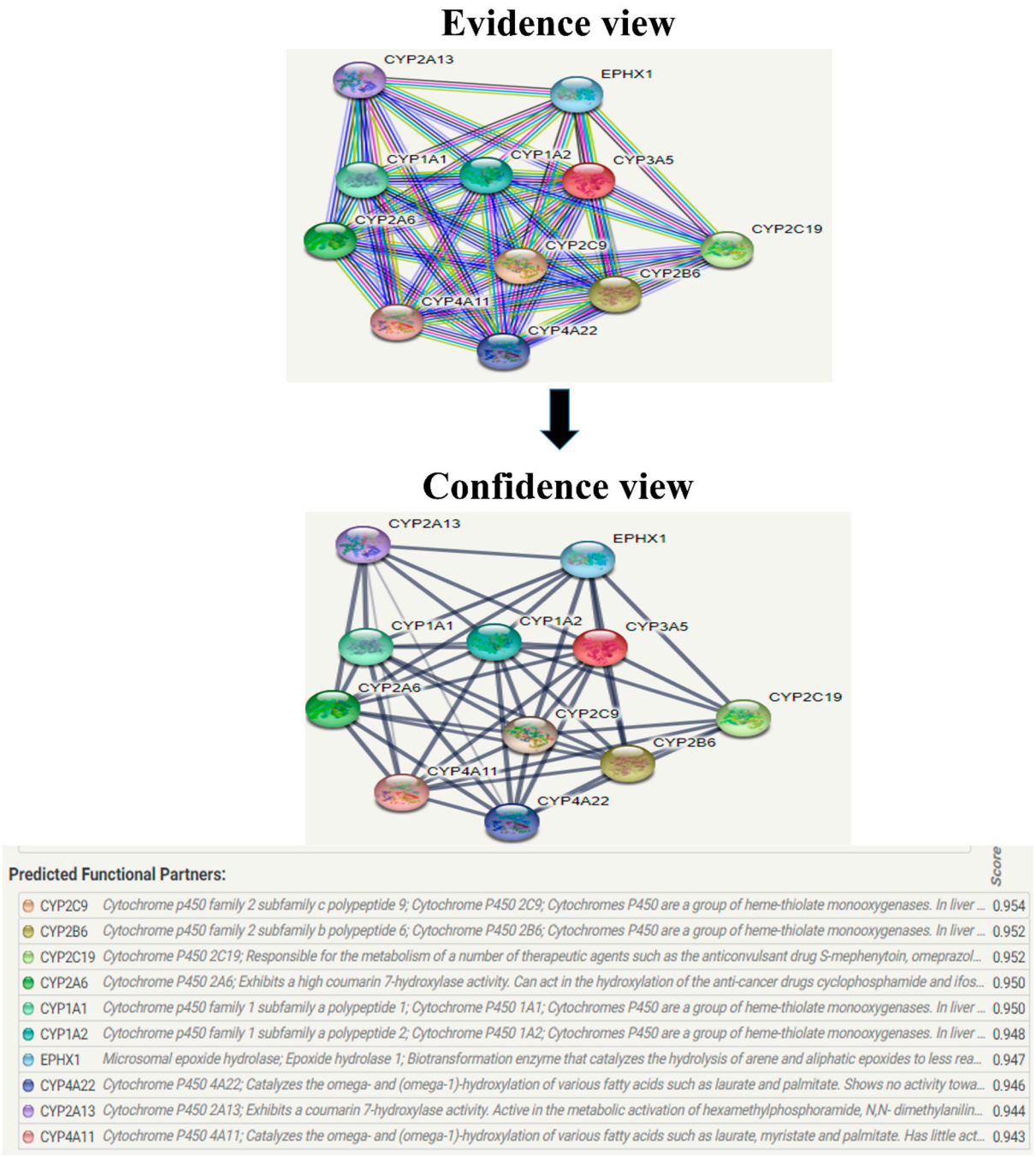


Figure 12. CYP3A5 interacts with a total of 10 different proteins. The evidence view and confidence view are given, while dark blue lines indicating a strong link.

4. Conclusions

According to the findings, mutants of TNF-α, NR3C1, and CYP3A5 are highly deleterious, and their presence can result in protein under-expression. Similarly, mutants of TNF-α, NR3C1, and CYP3A5 have been discovered to affect protein structure and stability, potentially leading to protein dysfunction. As a result of these mutations, glucocorticoid resistance may develop. The comparative in silico analysis of these gene variants showed a potential application for large-scale research. The current study will also aid experimental geneticists in their large-scale SNP analysis and assist in finding functional variation from the perspectives of structure, expression, evolution, physiochemical property, and phenotypes. This bioinformatics research will need to be looked at further in our future human clinical trials to see if the in silico study can be linked to the clinical trial. However, since

TNF-α, NR3C1, and CYP3A5 are involved in a key mechanism of glucocorticoid resistance, their nsSNPs can aid in diagnosing and treating the condition.

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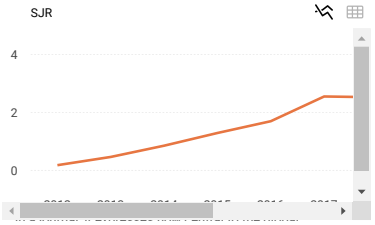

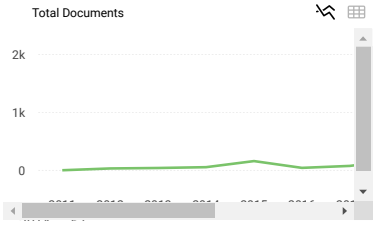

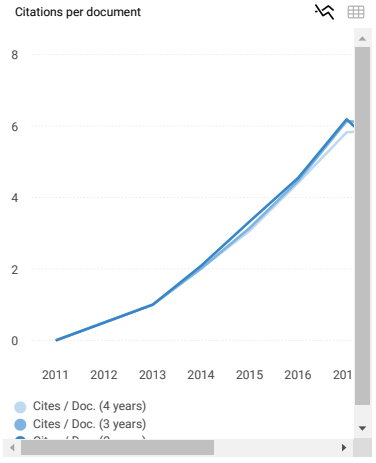

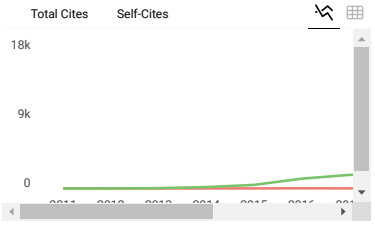

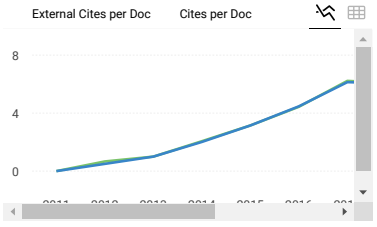

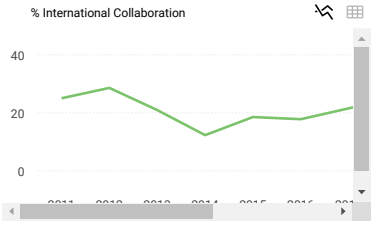

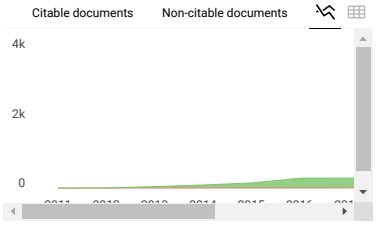

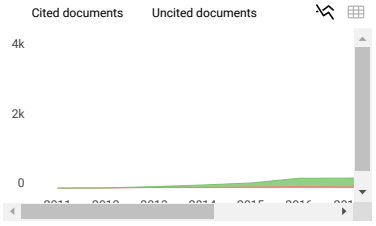

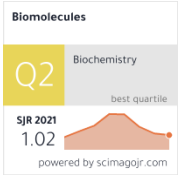

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