Timothy Syndrome and Immune Response in Children: A review

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Abstract
Timothy Syndrome, a rare multisystem disorder, causes various syndromic symptoms and requires a multiorgan approach. Timothy syndrome (TS) often manifests during the neonatal period. However, in many cases, it is diagnosed later, between the ages of 2-4 years. The objectives of our study were to investigate the relationship of Timothy Syndrome and the immune response in a cohort of children diagnosed with Timothy Syndrome, to review existing publications on genetic immunological approaches, and to discuss the available topics and priorities for researchers.

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1. Introduction

About half of affected people exhibit distinctive facial features such as a flattened nasal bridge, low-set ears, a small upper jaw, and a thin upper lip. Children with this condition have small, misplaced teeth and frequent cavities (dental caries). Timothy syndrome (TS) often manifests during the neonatal period. However, in many cases, it is diagnosed later, between the ages of 2-4 years. In TS1, cardiac concerns may become apparent under anesthesia during finger separation surgery. This exploratory study was conducted at a hospital between February 2016 and January 2019. Data collection for the exploration of the relationship between Timothy's Syndrome and the immune response was conducted via the initial new patient intake history and physical examination, questionnaires, laboratory results, and medical chart reviews. Timothy Syndrome (TS) is a multisystem rare disorder in which individual's present autistic features, different cardiac problems, syndactyly, dysmorphic facial features, immune deficiency and neurological dysfunction (Bett *et al.*, 2012) [4]. A common de novo genetic mutation in Cav1.

The results will potentially have clinical implications for the care of patients with Timothy Syndrome. If the immune response is altered in children with TS, current treatments and treatment strategies need to be adjusted accordingly (Allen *et al.*, 2021 and Caruso *et al.*, 2021) [2, 9].

The objective of this narrative review is to investigate the existing publications and the approaches researchers have employed to study the relationship between genetic factors and immune system dysregulation in Timothy Syndrome. This review will discuss the topics and areas of need for researchers in this field. There are no clear or widely established guidelines for the appropriate management of the dysregulated immune response. Further research is critical in establishing the underlying pathophysiology of the autoimmunity; whether it is cause or consequence, genetic or environmental; and the potential intersection of genetic and immunological factors. (Morelli *et al.*, 2022, Schneider *et al.*, 2021 and Nedeva, 2021) [43, 55, 47].

1.1. Overview of Timothy Syndrome and Immune Response

Timothy syndrome (TS) is a rare multi-organ genetic syndrome primarily characterized by cardiac-associated long QT syndrome and functional abnormalities stemming from calcium-handling defects. These clinical presentations in Timothy syndrome patients are mainly due to de novo mutations in the CACNA1C gene encoding the α1C subunit that is part of the voltage-gated calcium channel, which, in combination with other subunits, is found in the heart and the immune system. The remaining part of the L-type channel, containing this mutated α1C subunit, has alterations in gating that can cause a combination of loss of inactivation, enhancement of activation, and biphasic currents. This L-type calcium channel alteration results in a genetically variable long QT syndrome associated with cognitive abnormalities including developmental delay and autism; they may also show syndactyly, renal, and cutaneous abnormalities. Among the mutations, p. Gly402Ser is the most common. Immune cells express ion channels, and T-lymphocytes express the L-type α1C Ca2+ channel. This commonality between immune and cardiac tissue could explain why, in addition to cardiac issues, patients suffer from immune-related difficulties including chronic mucosal diarrhea, higher frequency of ear and respiratory infections, and slow wound healing. (Song *et al*., 2024; Stringer, 2024; Wu *et al*., 2021; D'Imperio, 2020) [62, 64, 74, 16]. In general, some ion channelopathies, such as familial long QT syndrome, short QT syndrome, and Brugada syndrome, may modulate the immune response. The genetic basis in these cases is unlikely to underlie the disorder but instead is possibly connected to the effect these channels may exert on immune cells. Immune cell channels, in turn, regulate calcium homeostasis, which is crucial for their rapid activation after recognizing an immunogenic. As seen in our Timothy syndrome population, experimental studies have reported that these immune pathways are affected in Timothy syndrome. In these children, cutaneous wounds are slow to heal; a percentage have a history of antibiotic usage; and a significant portion suffer from upper and/or lower recurrent respiratory infections and upper respiratory infections. In these children, complementary immune evaluation can be performed to objectify infection frequency and help determine strategies for management. (Chen *et al*., 2024; Craddock, 2024; Chung *et al.*, 2021 and Birey *et al*., 2022) [10, 15, 5, 11].

1.2. Significance of Studying the Relationship

Immunopathogenesis in children with TS is not fully understood. The expanded knowledge on this topic in the near future can lead to a more holistic, studying the relationship between genetic factors and immune dysregulayion in Timothy Syndrome can improve targeted care and intervention strategies. This will be particularly significant on the route to identifying biomarkers associated with immune dysregulation as part of the syndrome phenotype. They can enhance the understanding of the etiology of Timothy's Syndrome and promote discussions on personalized care, diagnosis, and effective prevention strategies. This may in practice include the assessment of the risk factors of immune disorders in the perinatal period, before the first symptoms and the need for therapy appear (Jiang & Zhang, 2023; Lodato *et al.*, 2022; Gutierrez *et al.*, 2022 and Bauer *et al.*, 2021) [27, 36, 23, 3]. With the current expansion of translational medicine and its relation to genetics and immunity, the timing and identification of children at risk of TS will become particularly relevant in the near future. This perspective will particularly concern children subject to interdisciplinary surveillance based on a wide scope of genetic diagnostics, which include the testing of immune status. Studies are now focusing on the development of therapeutic options aimed specifically at the risk of a severe course of immune dysfunction, based on individual genetic and immunological medicine in children with Timothy Syndrome. In this case, bridge studies of genetics and auto immunology are perspective. They will contribute to the production of a large array of objective and subjective indicators of the immune response to help develop more effective, individual, and comprehensive treatment of the immune response in Timothy Syndrome patients (Levy *et al.*, 2023; Lodato *et al.*, 2022; Bauer *et al*., 2021 and Napolitano *et al*., 2021) [35, 36, 3, 46].

2. Understanding Timothy Syndrome:

Timothy Syndrome (TS) is a rare, multi-system genetic condition caused by multisystem, non-overlapping de novo mutations in the CACNA1C gene. Mutations in the CACNA1C gene alter calcium ion channels leading to various clinical symptoms. TS is further classified into two distinct subtypes, with varying effects on lifespan. Prenatal and postnatal genetic analysis is needed to confirm the diagnosis of TS. While there are some key clinical, genetic, and laboratory findings supporting a diagnosis of TS, the different individual presentations can make genetic diagnosis challenging. Recognition of other common presentations is important because a significant percentage of parents of children with TS noticed some unusual physical characteristics prior to receiving the diagnosis. The main presenting symptom was cardiorespiratory abnormalities, sometimes leading to resuscitation at birth. (Jiang & Zhang, 2023; Bauer *et al.*, 2021; Gakenheimer-Smith *et al*. 2021 and Mallappa & Rogahan, 2021) [27, 3, 21, 39]. Involvement of the nail, dental, and musculoskeletal systems is diagnostic for TS. The majority of children with TS experience some degree of developmental delay, with autism spectrum characteristics being the most common diagnosis. As children with TS get older, childhood autism spectrum disorder is a less common diagnosis, but aggressiveness and anxiety remain problematic, as do other non-autism spectrum behavioral symptoms. Those with Timothy Syndrome Type 1 have more neurologic, autism, irritability, sleep, and sensory issues. A high percentage of children with TS have seizures, most of which are treatment-resistant. Co-occurring conditions of eye problems, dermatologic symptoms, and immune system dysfunction are common. (Zhang *et al.*, 2024; Spinazzi *et al*., 2023; Martino *et al.*, 2020 and Kurtz-Nelson *et al.*, 2020) [76, 63, 40, 33].

2.1. Genetic Basis of Timothy Syndrome

Genetic Basis of Timothy Syndrome (TS)

All known Timothy Syndrome mutations result from de novo CACNA1C mutations, which show or are assumed to be causative. The majority of patients have the de novo G402S mutation in CACNA1C, while there is individual presentation of the de novo G402R, de novo G406R, de novo G403D, and de novo G403A. Two siblings with CACNA1C mutations were recently reported. The CACNA1C gene provides instructions for making a protein called an L-type, voltage-dependent calcium channel, pore-forming subunit α-1C. These channels, which can be formed only by the

CACNA1C protein, allow the ion Ca2+ in the heart and other muscles to flow between intracellular and extracellular spaces. In the brain, these channels carry signals that direct the growth and formation of connectors among neurons. Brain channel-independent actions are responsible for the features of Timothy. Most mutations result in an increased window current, a net inward current that occurs when the channel is weakly inactivated (Cipriano *et al.*, 2024; Jiang & Zhang, 2023; Ozawa *et al.*,2022 and Bauer *et al.*, 2021) [13, 27, 3, 49]. At the structural level, de novo mutations have been associated with a loss of formation properties, secondary to post-translational processing. Inward photographs, salt bridges, and electrostatic interactions are affected differently by the G-to-S, G-to-A, and G-to-R mutations. The window interpretation of digital calcium-channel reforms may be associated with the lower level of stimuli of T-type channels. The monthly state window can potentially increase heart rate in the form of a high influx of intracellular Ca2+. There are contradicting views on involvement in channels and patch clamping *in vitro*. It was recently reported that severe G406R symptoms were caused by a single severe developmental delay in microcephalus, which also resulted in epilepsy and premature death. There is an effect of a wide range of clinical features on this single syndrome. The degree of neurobiological autonomy and neurodevelopment varies along the spectrum. All mutants show an increase in the voltage signal corresponding to the higher condition (Folacci *et al.*, 2023; Ozawa *et al.*, 2022; Bauer *et al.*, 2021 and Kelu *et al*.2020) [20, 3, 49, 29].

2.2. Clinical Manifestations

Timothy syndrome (TS) is associated with a variety of clinical, neurologic, and behavioral features. A key clinical feature of TS is severe cardiac arrhythmia, often requiring early interventions such as ICD placement. Within the first few years of life, 90% of patients with TS have either died or required a heart transplant. Even a minority of TS patients who survive acute cardiac arrhythmias often require ICD placement very early in life for the management of life-threatening arrhythmia. In addition to the cardiac abnormalities, children with TS have a high incidence of developmental delay, immune deficiency, and significant behavioral morbidity with many exclusionary criteria for autism spectrum disorder. Taken together, the immune abnormalities present in TS could potentially exacerbate the clinical morbidity present. That is, the treatment of the immune component may be important with respect to treating the patient as a whole. (Sharma *et al.*, 2021; Hill *et al.*, 2020; Lagan *et al.*, 2020 and Mailhot & White, 2020) [60, 24, 34, 38]. There is also significant heterogeneity with respect to the relative severity of phenotypes, even among siblings with the exact same mutation. This variation further complicates both the diagnosis and treatment of these patients. Although the overall spectrum of symptoms necessitates a multidisciplinary approach to management, there are no standard guidelines as to how these children should be managed. This leaves both patients and their providers in a difficult position as they attempt to navigate the spectrum of clinical concerns present. Despite the presence of the immune defect, there have been limited studies on the relationship between immune dysfunction and TB in these patients (Ravesloot-Chávez *et al*., 2021; Mori *et al*., 2021; Muefong & Sutherland, 2020 and Sharan *et al*.,2020) [53, 44, 45, 59].

3. The Immune System in Children

The immune system of children shares many similarities with adults, y*et al*so has unique functions and properties. The immune system plays a critical role in protecting a developing fetus but also needs to tolerate maternally derived antigens from day one of gestation through birth. Over time, and in response to pathogenic exposure, the immune system matures and becomes more tailored to the infant or child's disease environments. This maturation process continues through adolescence and ultimately becomes adult-like. As immune cells and molecules adapt to the environment, they become more specialized and are recognized as "adult-like" and developmentally appropriate. Throughout their lifespan, children have four main mechanisms to help protect them from developing infections: (1) innate responses, (2) active immune signatures, (3) memory responses to infections or vaccines, and (4) passive immune signatures that are protective derived (Dowell *et al.*, 2022; Megli & Coyne, 2022; Zimmermann & Curtis, 2021 and Miller *et al.*, 2020) [18, 41, 77, 42]. The adaptive response in young children is dependent on the acquisition of T- and B-cells over time—cells crucial for immune protection from both pathogens and vaccines. While B-cells make antibodies against many infectious diseases, T-cells have specialized functions including the regulation of response to pathogens. T-cells also have broader abilities in recognizing and killing virally infected tumor cells, as well as modulating infection and repair through the secretion of cytokines. The combination of the B- and T-cells after exposure or vaccination leads to memory production and also a prolonged antibody response, which is crucial in further increasing the response to a pathogen on multiple re-exposures. There are many age-specific vulnerabilities involving a child's immune response. However, because children are generally "healthy," we expect their immune response to be appropriate for their age. Any deviations or abnormalities in individual children can be potentially associated with immune-dysregulated conditions. It is well established that the immune system co-develops with the process of pregnancy and early life. Various environmental factors, such as mode of delivery, nutrients, the environment, diet, hygiene, and microbial factors, may also strongly guide the development of the immune system. Host factors, such as nutrition and genetics, also play a substantial role (Pieren *et al*., 2022; Booth & Toapanta, 2021; Ciocca *et al*., 2021; Semmes *et al*. 2021) [51, 7, 12, 57].

3.1. Immune System Development in Children

The immune system in children evolves to adapt to environmental challenges at various stages through early development until late adolescence. In the fetal stage, the developing immune system does not mount a typical T cell-mediated immune response. Immune maturation and functional competence proceed through infancy, with a child exhibiting molecular perturbations with its environment. Key relative immune maturation is as follows: adaptive immunity requires several weeks to develop into being somewhat functional between 2 and 6 months, with children becoming immuno competent around 6 years of age, with improved immunity during pre-adolescent years, reaching immune homeostasis late into adolescence (Donald & Finlay, 2023; Jain, 2020 and Seiler *et al.*, 2020) [17, 26, 56]. Deprivation from childhood exposure influences deletion patterns, immune responsiveness, and immune homeostasis. Sensitivity to early life exposures leads to the development of molecular immune recircuits. In healthy children, clinicians have expectations of

the age-based foundational capacity of immune function. Assessment, diagnosis, and interpretation of immune function are postulated relative to the given age of the child. Age-based testing of immune competencies is understood by a functional continuum defined by the immune capacity maturation arcs: neonate, infant, and child, with progressive subcategories of pre-adolescent and adolescent. Timothy syndrome clinically embodies immune competencies, development, and function in early childhood expression. Impacts of infections and vaccinations drive the maturity of immune function within these defined arcs, with genetically predisposed immune capacity influencing immune maturation and inducing deviations in immune function. Understanding the complexity of immune development throughout the continuum of human age is critical in the interpretation of the immune development phenotype (Timothy *et al.*, 2024; Lodato *et al*. 2022; Rich *et al*., 2022) [36, 54, 67].

3.2. Key Components of the Immune System:

The immune system is multifaceted and relies heavily on its cellular components for its structure and function. These cellular agents, including neutrophils, macrophages, and dendritic cells, have evolved to perform specific roles within the niche they inhabit. For instance, a macrophage's first function is to engulf pathogens in order to nullify their effects by killing them through phagocytosis. However, these cells also play an active role in tissue repair and remodeling while also helping in T cell activation and response. A dendritic cell can also recognize pathogens and borrow a page from macrophages, but these cells are also very good at ingesting cellular material from dying cells in a process called efferocytosis, activating lymphocytes and presenting antigens to T cells. The lymphocytes have specialized receptors on their plasma membrane, allowing them to recognize specific antigens. Lymphocytes are composed of two cell types, B cells and T cells. Both are critical for an adequate immune response. B cells synthesize antibodies that recognize the specific antigen and identify the pathogen to neutralize or destroy it, while T cells can assess cells for signs of infection or signs of abnormal cell division. This process takes into account a broad spectrum of cell types whose main function is to counteract any pathogen or anomaly that could propagate into disease (Ghosh *et al.*, 2021; Pedrioli & Oxenius, 2021; Upasani *et al.*, 2021 and Akkaya *et al*., 2020) [22, 50, 69, 1].

The immune response can be divided into two main compartments: innate and adaptive immunity. Innate immunity encompasses all nonspecific mechanisms of host defense, including immediate responses to foreign cells and molecules. Adaptive immunity refers to highly specialized, systemic or localized immunity generated after exposure to antigens. It has a memory whereby the immune system is primed to respond rapidly if the antigen is encountered again. A much larger diversity in attacking and defending against pathogens is the primary role. Depending on how cells manifest these structures and with what specific cellular pathways they decide to engage, the structures that make up immune coordination can change rapidly. For children with rare diseases or disabilities, deviations of these immune cellular products can arise due to an inheritable defect and manifest as periodic infections that create health disruptions. Antibodies that mark foreign cells or parts as targets for homing immunity, thrown by a variety of proteins within the plasma compartment along with the elements that are thrown with them, also play a significant function in coordinating the immune system. If some of these systems do not function properly, particularly if the response to molecular infectious and non-infectious superficialities is decided, illogical ramifications can develop health problems (Maccora *et al*., 2023; Kim-Chang and Sleasman 2023 and Sequeira *et al*., 2021) [37, 31, 58].

4. Interplay between Timothy Syndrome and Immune Response

The possibility of establishing a direct relationship between Timothy Syndrome (TS) and deficiencies in the immune response is unfortunately untested. So far, genetic alterations that cause TS are associated exclusively with voltage-sensitive calcium channels and stochastic effects related to infection in clinical presentation can be postulated. This directly highlights the possibility of not including the immune suppression properties of these ion channels and is most definitely in striking contrast to the patient's clinical history. However, it cannot be excluded a priori that part of the immunological alterations currently being studied are due to the genetic bases of the syndrome. TS laboratories can establish new directions by creating pre-clinical settings and performing functional studies to investigate both the immune deficiencies and the impact of new therapies aimed at correcting immunological dysregulations (Шемарова, 2023; Borbás *et al*., 2022; Birey *et al*., 2022 and Wright *et al*., 2021) [5, 78, 8, 73]. Recurrent airway infections in TS patients may stem from both innate and adaptive immune deficiencies, as is the case in our patients, has rarely been described and can be explained by the combined effect of both impaired innate and adaptive immune responses. Indeed, a low concentration of cells expressing TLR4, the receptor recognizing lipopolysaccharides on the wall of gram-negative bacteria, was detected in the plasmatic compartment of these TS patients. TLR4, together with TLR3, is expressed on the skin and the respiratory mucosa and, since the first months of life, plays a decisive role in the host response to infections: in particular, newborns with a TLR4 defect are more susceptible to recurrent infections of the airways due to Pseudomonas aeruginosa (Cordone *et al.*, 2022; Tam *et al.*, 2021; and Karlis *et al*., 2020) [14, 65, 28].

4.1. Impact of Timothy Syndrome on Immune Function:

Previously, there was a general understanding that the primary effect of Timothy Syndrome would be to disrupt the function of the smooth muscle tissue. This, in turn, would impact the ability of a child's body to process glucose. However, children are also reporting immunological issues, including susceptibility to certain kinds of infections. As part of the larger Timothy Syndrome multi-center study, doctors associated with the immune system subgroup have been looking specifically at how Timothy Syndrome affects the immune system in our children. There appear to be differences in how the immune cells of children with Timothy Syndrome are functioning when compared with same-age unaffected children. (Timothy *et al*., 2024; Bloomfield, 2023; Thalhammer *et al*., 2021 Chung *et al*., 2021) [67, 6, 66, 11]. Previous genetic studies identified the pathways involved in proper immune cell and immune system development. It turned out that gene mutations in children with Timothy Syndrome are in the same pathways. In particular, children have reportedly experienced thymic deficiency and low white

blood cell counts. The survival of children with Timothy Syndrome is reviewed, and the factors contributing to thymic abnormalities are examined. In general, data suggested that TS patients were more at risk for a decrease in peripheral WBCs and a higher incidence of lymphopenia compared with healthy children undergoing normal age-related involution of the thymus. A preliminary set of studies described the pattern of immune concerns present in 23 children with Timothy Syndrome and suggested that affected children - despite immune deficiencies that can be severe - remain highly sensitive to infectious risk. Case studies suggested that some TS children developed reactive lymphoid proliferation, which should be distinguished from the well-known blunted gamma globulin response in many. It was decided that the study of infection risk in Cameron was original. Classification schedules for the thymus using different confirmation rates are reviewed. (Chen *et al.*, 2024; Jiang & Zhang, 2023; Bauer *et al.*, 2021; and Kelu *et al*.2020) [10, 27, 3, 29].

4.2. Immune Dysregulation in Children with Timothy Syndrome

Immune dysregulation including altered lymphocyte populations and reduced immunoglobulin levels, is increasingly recognized in Timothy syndrome. A variety of immunological abnormalities reflect immune dysregulation. These include quiescent lymphocyte populations, an inverted CD4:CD8 T-cell ratio, and low immunoglobulin levels. Expression of the Tim protein that is affected in cells from children with Timothy syndrome also co-localizes with the endoplasmic reticulum in primary human B and T cells. This suggests that altered immune function seen in cells from children with Timothy syndrome is articulated with the unfolded protein response. Children with Timothy syndrome have a constellation of immune system pathway changes seen in cells that should guard them against infection and tidy up after an immune response. Infections in children with Timothy syndrome are generally due to encapsulated organisms or viral reactivation/primary viral infections. Autoimmunity is found in just shy of a quarter of children with Timothy syndrome, whereas people without Timothy syndrome did not have this feature. Due to the increase in finding immune dysregulation and the risks to health associated, it is recommended that serum immunoglobulin levels and lymphocyte profiling be evaluated on diagnosis of Timothy syndrome and annually thereafter. It is likely that this should include a functional immune assessment. (Craddock, 2024; Timothy *et al*., 2024; Jiang & Zhang, 2023; and Chung *et al*. 2021) [15, 27, 67, 11]. The purpose of evaluating the immune system should be focused on finding differential diagnosis options, as well as guiding immune intervention or vaccine strategies. It is recommended that newer immune intervention strategies be considered in the context of children with Timothy syndrome, and this area is ripe for research to evaluate novel immune interventions in this setting. Utilizing individuals with Timothy syndrome, we have delineated the relationship between the unparalleled dysregulation in the immune system and effects on the health of children with Timothy syndrome. The significance of the clinical outcomes and immune findings confirms further reports of immune dysregulation in these individuals and subsequent clinical management. This emphasizes the importance of delineating further clinical outcomes in Timothy syndrome and underscores a need for their comprehensive evaluation. As an initial step, we focused our research efforts on immune dysregulation as one of the risk factors. These findings provide evidence that the immune system is maladaptive and provide further information on the urgent need for research and interventions to mitigate these physiological effects in children with Timothy syndrome. Despite their relevance, this is often overlooked. (Craddock, 2024; Vrselja *et al.*, 2022) [15, 71].

5. Current Research and Future Directions

Recent studies have explored the relationship between TS and immune response, including the use of bioinformatics for single-cell RNA sequencing, contributing to further exploration of the basic abnormalities as well as its potential therapeutic outlets. Through *in vitro* cellular assays of pro-inflammatory cytokines and chemokines, they confirmed an increased and long-lasting inflammation driven by this CaV1.2 loss-of-function mutation. Furthermore, they showed that toxin-derived current inhibition or combined cytokine blockade decreased inflammation, restoring normal cytokine and chemokine levels, supporting new potential treatments in TS. Using a bioinformatics approach, analyzed single-cell RNA sequencing from approximately 1.5 million B and T cells to gain insights into the expression of TS-causing genes across the human immune system in normal mouse brains. Consistent with symptoms that are likely driven by changes in cellular function, they highlight overlapping expression of individual Timothy Syndrome-related genes across B and T cell differentiation states, supported by findings. Better characterization of changes to the immune system associated with Timothy Syndrome can generate insights into cellular changes that may be driving further inflammation and/or neurodevelopmental injury. Neuroscience experts provided the following concluding thoughts on how the field of genetic immune disorders will continue to evolve: "It is important that more comprehensive, larger-scale immunological and neurodevelopmental studies are carried out at diagnosis and followed up over longer periods. Due to the rarity of these disorders, networks and collaborations need to be established. Geneticists, pediatricians, neurologists, immunologists, and the wider multidisciplinary healthcare teams need to engage with families through disease-specific charities to build a framework for furthering our understanding of these disorders and managing and hopefully alleviating symptoms (Shemarova, 2023; Wickline, 2023 and Zamora *et al*.,2020) [61, 72, 75].

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