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RESEARCH

22q11.2 Deletion-Associated Blood-Brain Barrier Permeability Potentiates Systemic Capillary Leak Syndrome Neurologic Features

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Abstract

We present a case study of a young male with a history of 22q11.2 deletion syndrome (22qDS), diagnosed with systemic capillary leak syndrome (SCLS) who presented with acute onset of diffuse anasarca and sub-comatose obtundation. We hypothesized that his co-presentation of neurological sequelae might be due to blood-brain barrier (BBB) susceptibility conferred by the 22q11.2 deletion, a phenotype that we have previously identified in 22qDS. Using pre- and post-intravenous immunoglobulins (IVIG) patient serum, we studied circulating biomarkers of inflammation and assessed the potential susceptibility of the 22qDS BBB. We employed in vitro cultures of differentiated BBB-like endothelial cells derived from a 22qDS patient and a healthy control. We found evidence of peripheral inflammation and increased serum lipopolysaccharide (LPS) alongside endothelial cells in circulation. We report that the patient's serum significantly impairs barrier function of the 22qDS BBB compared to control. Only two other cases of pediatric SCLS with neurologic symptoms have been reported, and genetic risk factors have been suggested in both instances. As the third case to be reported, our findings are consistent with the hypothesis that genetic susceptibility of the BBB conferred by genes such as claudin-5 deleted in the 22q11.2 region promoted neurologic involvement during SCLS in this patient.

Keywords Blood-brain barrier · 22q11.2 deletion syndrome · Systemic capillary leak syndrome · Immunology · T cells · Myeloid cells · Endothelial cells

Introduction

The deletion of the q11 region of chromosome 22 is the most common microdeletion disorder in humans, occurring in approximately 1 in 4000 live births [1]. This deletion confers the condition 22q11.2 deletion syndrome (22qDS), a highly variable phenotypic presentation encompassing aortic arch anomalies, congenital heart defects, thymic hypoplasia, hypocalcemia, facial deformities, as well as neuropsychiatric disorders [2–4]. The deleted region includes approximately 40 protein coding genes, including T-box protein 1 (*TBX1)*, which has been linked to cardiac defects, Catechol-O-methyltransferase (*COMT)*, which could be related to neuropsychiatric phenotypes, and 6 mitochondrial genes [5–7]. Another deleted gene encodes for claudin-5 (*CLDN5*), the tight junction protein that underlies the integrity of the blood-brain barrier (BBB). *CLDN5* is localized in the LCRA-B region, close to *TBX1* [7, 8], and hence is affected in essentially all individuals with the 22qDS. The BBB relies on an elaborate junctional network and unique metabolic properties to restrict interactions with

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the periphery and maintain the relative immune privilege of the central nervous system (CNS) [9, 10]. Recently, we and others have identified that the BBB is intrinsically compromised in 22qDS, potentially rendering the brain susceptible to additional insults or stressors [11–13]. Compromised BBB function has been linked to neurological and neuropsychiatric conditions ranging from stroke, multiple sclerosis, and schizophrenia [14, 15].

Here, we report a patient with 22qDS diagnosed with systemic capillary leak syndrome (SCLS, Clarkson's disease) who presented with neurological symptoms. SCLS is a rare, life-threatening condition caused by acute episodes of increased capillary permeability due to unknown triggers, although the cases are often preceded by a viral infection [16]. This leakage of plasma into the interstitial space causes hemoconcentration, hypoalbuminemia and edema followed by hypovolemic shock [16]. While several hundred cases have been reported in adults, only a handful of SCLS cases in children have ever been documented and none have been described in patients with the 22qDS [17, 18]. As brain involvement is a rare presentation of SCLS [19], here we explore the clinical and immunological phenotypes in a 22qDS patient with severe SCLS, and identify the BBB as a potential conduit by which SCLS impacted the brain in this patient.

Materials and Methods

Standard Protocol Approvals, Registrations, and Patient Consents

The subject and his family provided signed, informed assent/consent under a human subject's protocol approved by the Children's Hospital of Philadelphia (CHOP). In addition, healthy control subjects were enrolled in the same protocol and provided signed, informed consent.

Blood Samples

Paired blood and serum samples were collected prior to intravenous immunoglobulins (IVIG) treatment, during active symptom presentation, and again within 48 h after treatment had been initiated, coinciding with abatement of symptoms. Peripheral blood mononuclear cells (PBMCs) were isolated by density centrifugation, in parallel with an age-/sex-matched control PBMC donor. Serum lipopolysaccharide (LPS) was measured using Pierce™ Chromogenic Endotoxin Quantification Kit (Thermo).

Flow Cytometry

Circulating lymphocytes were isolated from peripheral blood and stimulated as previously described [20]. In brief, for assessment of T cell phenotypes, cells were stimulated (with PMA (20 ng/mL), ionomycin (1 µg/mL) and brefeldin (5 µg/mL) for 4 h prior to staining. Cells were stained with the Zombie Violet Fixable Viability Kit (Biolegend) and with antibodies for surface markers. Cells were fixed with 4% paraformaldehyde or eBioscience Intracellular Fixation and Permeabilization Buffer Set (Thermofisher) and stored at 4°C prior to analysis on LSRFortessa (BD Biosciences). Data was analyzed using FlowJo version 10 (BD Biosciences) as previously shown [21].

Induced BBB-like (iBBB) Model

Human iBBB cultures were derived from human induced pluripotent stem cell (iPSC) lines originating from one 22qDS patient and one age/sex matched healthy control. The human iPSC lines were generously provided by Dr. Sergiu P. Pașca, Stanford University, Stanford, CA. Human iPSCs were differentiated into iBBBs, stored, and cultured following established protocols [11, 22].

Transendothelial Electrical Resistance (TEER)

TEER was measured as previously published [23]. In brief, arrays were coated with a collagen/fibronectin mixture and iBBBs were cultured in human endothelial serum-free media (Thermo) containing B-27 Supplement without antioxidants (Thermo) as reported [11, 22]. Cells were treated with 25% serum for 72 h during continuous TEER monitoring at 250 Hz.

Results

Here, we report an 8-year-old male patient with a history of a *de novo* heterozygous 22q11.2 deletion (Diagnosis made by microarray analysis on day of life (DOL) #5. Breakpoints: chromosome 22: 18,916,842 – 21,798,907. Patient had a ventricular septal defect repaired at 6 months of age) and infliximab-responsive Crohn's Disease, who presented with acute onset of diffuse anasarca, respiratory failure and sub-comatose obtundation (Glascow Coma Score = 7) after several weeks of worsening colitis symptoms. After several weeks of no clinical improvement, the patient was diagnosed with SCLS, a condition characterized by leakage of plasma from the vasculature causing hemoconcentration, hypoalbuminemia and edema but rarely profound mental status changes. Despite daily fevers, infectious

and malignant SCLS triggers were not identified. Patient did not exhibit abnormal cytokine levels. The patient was treated with a high dose (2 g/kg) of IVIG to address SCLS symptoms followed by aggressive diuresis. Within days the patient's mental status returned to baseline, he no longer required respiratory support and his edema resolved. In the three years since hospital discharge, the patient received prophylactic IVIG at lower doses (500 mg/kg each month) and SCLS has not recurred.

Despite negative blood cultures that ruled out bacteremia, we found substantially elevated serum LPS (2.788 EU/mL) compared to two healthy controls (0.664 and 0.683 EU/ml). The increased in LPS in the 22qDS SLCS patient normalized following treatment (0.748 EU/mL), suggesting colitis-associated endotoxemia as a potential contributor to the condition. As cellular immunity has been linked with SCLS [24], we assessed the status of circulating immune cells. We found increased PBMCs relative to a control and observed increased percentage of monocytes and T lymphocytes (Fig. 1A-B). Upon IVIG treatment, there was a reduction in the expression of inflammatory markers on leukocytes (Fig. 1C). Due to the patient's history of Crohn's Disease and our findings of endotoxemia, we studied γδ T cells, a subset associated with gut barrier function [25]. γδ T cell frequencies were increased in the patient prior to IVIG administration and expanded further after treatment (Fig. 1D). Together, these findings suggest that increased translocation of gram-negative bacterial components during a Crohn's Disease exacerbation contributed to SCLS pathophysiology.

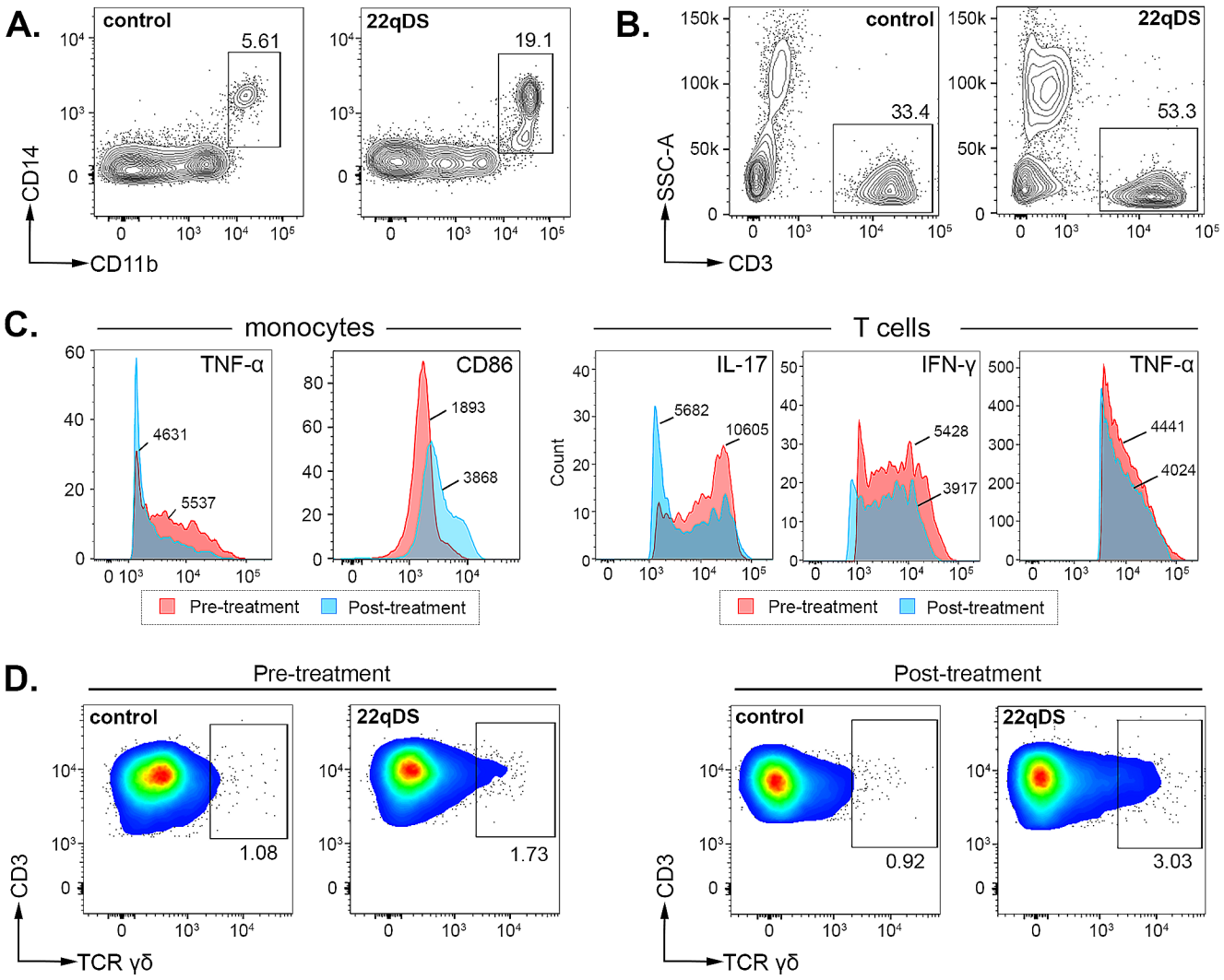

Fig. 1 Expansion and activation of peripheral leukocyte populations in a 22qDS patient with SCLS. Flow cytometry plot indicating expansion of circulatory (**A**) $CD11b^+$ monocytes and (**B**) $CD3^+$ T cells in the patient prior to IVIG treatment relative to a matched control. (**C**) Histogram comparing markers in blood monocytes and blood T cells prior to and following IVIG treatment. (**D**) Flow plots demonstrating increased γδ T cells in the 22qDS patient with SCLS compared to a matched control pre-treatment (left), and further expansion of this population post-treatment. Values in A, B and D indicate percentage and in C mean fluorescence intensity

We studied circulating endothelial cells and found an increase in number and inflammatory phenotype relative to a control (Fig. 2A), reinforcing the detrimental effect of SCLS on the vasculature. To address the potential role of 22qDS BBB dysfunction on the patient's severe, but reversible, neurologic impairment, we cultured induced-BBB-like

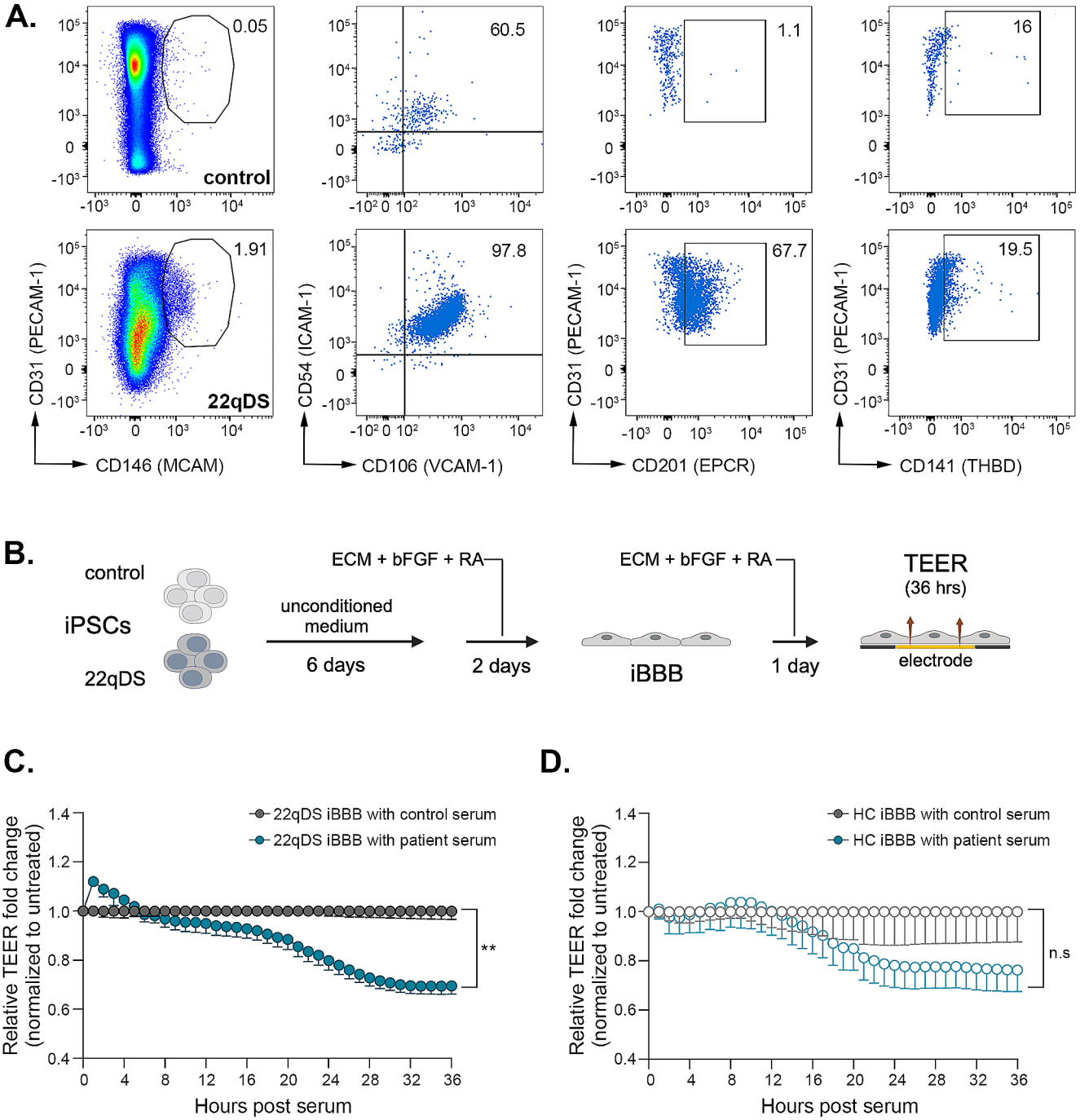

Fig. 2 Endothelial activation and BBB susceptibility associate with neurological symptoms. Flow cytometry plots of (**A**) circulating endothelial cells ($CD31^+$ $MCAM^+CD45^{neg}$) (left), expressing ICAM-1 (CD54), VCAM-1 (CD106), thrombomodulin (CD141) and EPCR (CD201) in the 22qDS SCLS patient (top) compared to the matched control (bottom). Values indicate percentage. (**B**) Human induced pluripotent stem cells (iPSCs) were differentiated into induced-BBB-like (iBBB) endothelial cells as previously described. ECM = endothelial cell media; bFGF = basic fibroblast growth factor, and RA = retinoic acid. Differentiated cells were grown in ECM + bFGF + RA for 1 day followed by 3 days of culture in ECM for transendothelial electrical resistance (TEER) analysis. Cartoon created with BioRender.com. (**C**) TEER of iPSC-derived iBBB cells from a 22qDS patient, treated with patient pre-treatment serum compared to control serum. (**D**) TEER of iPSC-derived BBB cells from a healthy control, treated with patient pre-treatment serum compared to control serum. ** $p < 0.01$ by two-way ANOVA

(iBBB) monolayers that were derived from either 22qDS, or control iPSC lines (Fig. 2B) [11] with the pre-IVIG treatment serum from the patient or a healthy control. The patient's pre-treatment serum significantly worsened 22qDS BBB integrity compared with control serum (Fig. 2C). In contrast, the impact of patient serum on control iBBB monolayers was muted (Fig. 2D). Together, these findings implicate 22qDS-associated BBB dysfunction in a uniquely severe neurological presentation of SCLS.

Discussion

Here, we present the most in-depth analysis of an SLCS patient's circulating immune compartment to date. While the trigger for SLCS is unknown, our data suggest a role for circulating factors in the disease and generally support the hypothesis linking an immune-associated trigger to symptom onset [16]. For this reason, we addressed serum LPS, a pro-inflammatory mediator that can be found in circulation. In non-infectious pathological conditions, LPS is concentrated in gut gram negative commensals [26]. As the patient had a history of inflammatory bowel disease, our data that serum LPS was substantially elevated prior to treatment and attenuation following IVIG suggest that circulating LPS could have contributed to triggering this SCLS episode.

Neurological involvement in SCLS is a rare symptom and has only been reported in 2 pediatric SCLS patients [27, 28]. This is likely due to the specialized properties of the BBB, which renders the CNS less susceptible to peripheral insult [10, 29]. Family history was reported in one of the published cases [28], suggesting that genetic predisposition may contribute to neurological involvement in pediatric SCLS. However, causative genes or polymorphisms were not linked to those cases. Given that the integral BBB protein claudin-5 is in the 22q11.2 deleted region [12], coupled with our discovery that the BBB is affected in 22qDS [11], we hypothesized that genetic susceptibility (22qDS) contributed to the presentation of neurological symptoms in the case presented here. Indeed, our findings that the patient's pre-treatment serum significantly impairs barrier function in the 22qDS BBB in vitro supports the theory that the patient's BBB was more susceptible to peripheral drivers of capillary leakage compared to a control BBB.

Conclusion

We report the first 22qDS patient diagnosed with SLCS. This 22qDS patient marks just the third documented pediatric case with neurological symptoms in SCLS. The patient's history of gut inflammation coupled with a peripheral inflammatory trigger could have contributed to the onset of SCLS. From a pathophysiological standpoint, the genetic susceptibility of the BBB due to the 22q11.2 deletion may have contributed to neurological manifestations in SCLS.

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Author Contributions AMC and HK: Carried out the experiments. AMC and JIA: Drafted the initial manuscript. NR and SJ: Provided clinical care. SA: Provided reagents and supervision to iPSC assays. JIA: supervised the study. All authors: Revised the manuscript critically for important intellectual content.

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Data Availability The raw data supporting the conclusions of this study will be made available by the authors, without undue reservation, to any qualified researcher.

Declarations

Ethics Approval and Consent to Participate Consent from the next of kin was obtained for the case presented here under a human subject's protocol approved by the Children's Hospital of Philadelphia (CHOP).

Consent for Publication The subject and his family were provided the opportunity to review the case report prior to submission and provided assent/consent for this work to be published.

Conflicts of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Gur RE, Bassett AS, McDonald-McGinn DM, Bearden CE, Chow E, Emanuel BS, et al. A neurogenetic model for the study of schizophrenia spectrum disorders: the International 22q11.2 deletion syndrome brain Behavior Consortium. Mol Psychiatry. 2017;22(12):1664–72.
2. Sullivan KE. The clinical, immunological, and molecular spectrum of chromosome 22q11.2 deletion syndrome and DiGeorge syndrome. Curr Opin Allergy Clin Immunol. 2004;4(6):505–12.
3. Tang SX, Yi JJ, Calkins ME, Whinna DA, Kohler CG, Souders MC, et al. Psychiatric disorders in 22q11.2 deletion syndrome are prevalent but undertreated. Psychol Med. 2014;44(6):1267–77.

4. McDonald-McGinn DM, Sullivan KE, Marino B, Philip N, Swillen A, Vorstman JA, et al. 22q11.2 deletion syndrome. Nat Rev Dis Primers. 2015;1:15071.
5. Arinami T. Analyses of the associations between the genes of 22q11 deletion syndrome and schizophrenia. J Hum Genet. 2006;51(12):1037–45.
6. Cioffi S, Martucciello S, Fulcoli FG, Bilio M, Ferrentino R, Nusco E, et al. Tbx1 regulates brain vascularization. Hum Mol Genet. 2014;23(1):78–89.
7. Karayiorgou M, Gogos JA. The molecular genetics of the 22q11-associated schizophrenia. Brain Res Mol Brain Res. 2004;132(2):95–104.
8. Greene C, Hanley N, Campbell M. Claudin-5: gatekeeper of neurological function. Fluids Barriers CNS. 2019;16(1):3.
9. Abbott NJ. Inflammatory mediators and modulation of blood-brain barrier permeability. Cell Mol Neurobiol. 2000;20(2):131–47.
10. Cheslow L, Alvarez JI. Glial-endothelial crosstalk regulates blood-brain barrier function. Curr Opin Pharmacol. 2016;26:39–46.
11. Crockett AM, Ryan SK, Vásquez AH, Canning C, Kanyuch N, Kebir H et al. Disruption of the blood-brain barrier in 22q11.2 deletion syndrome. Brain. 2021.
12. Guo Y, Singh LN, Zhu Y, Gur RE, Resnick A, Anderson SA et al. Association of a functional Claudin-5 variant with schizophrenia in female patients with the 22q11.2 deletion syndrome. Schizophr Res. 2019.
13. Li Y, Xia Y, Zhu H, Luu E, Huang G, Sun Y et al. Investigation of neurodevelopmental deficits of 22 q11.2 deletion syndrome with a Patient-iPSC-Derived blood-brain barrier model. Cells. 2021;10(10).
14. Alvarez JI, Saint-Laurent O, Godschalk A, Terouz S, Briels C, Larouche S, et al. Focal disturbances in the blood-brain barrier are associated with formation of neuroinflammatory lesions. Neurobiol Dis. 2015;74:14–24.
15. Greene C, Kealy J, Humphries MM, Gong Y, Hou J, Hudson N, et al. Dose-dependent expression of claudin-5 is a modifying factor in schizophrenia. Mol Psychiatry. 2018;23(11):2156–66.
16. Druey KM, Parikh SM. Idiopathic systemic capillary leak syndrome (Clarkson disease). J Allergy Clin Immunol. 2017;140(3):663–70.
17. Bozzini MA, Milani GP, Bianchetti MG, Fossali EF, Lava SAG. Idiopathic systemic capillary leak syndrome (Clarkson syndrome) in childhood: systematic literature review. Eur J Pediatr. 2018;177(8):1149–54.
18. Hsu P, Xie Z, Frith K, Wong M, Kakakios A, Stone KD, et al. Idiopathic systemic capillary leak syndrome in children. Pediatrics. 2015;135(3):e730–5.
19. Günes AR, Berlit P, Weber R. Severe cerebral involvement due to idiopathic systemic capillary leak syndrome. Clin Case Rep. 2016;4(4):429–31.
20. Benallegue N, Kapoor R, Kebir H, Crockett AM, Li C, Cheslow L, et al. The hedgehog pathway suppresses neuropathogenesis in CD4 T cell-driven inflammation. Brain. 2021;144(6):1670–83.
21. Crockett AM, Kebir H, Benallegue N, Adelman P, Gur RE, Sullivan K, et al. Immune status of the murine 22q11.2 deletion syndrome model. Eur J Immunol. 2023;53(1):e2249840.
22. Neal EH, Marinelli NA, Shi Y, McClatchey PM, Balotin KM, Gullett DR, et al. A simplified, fully defined differentiation Scheme for producing blood-brain barrier endothelial cells from human iPSCs. Stem Cell Rep. 2019;12(6):1380–8.
23. Alvarez JI, Dodelet-Devillers A, Kebir H, Ifergan I, Fabre PJ, Terouz S, et al. The hedgehog pathway promotes blood-brain barrier integrity and CNS immune quiescence. Science. 2011;334(6063):1727–31.
24. Percik R, Nethanel A, Liel Y. Capillary-leak syndrome: an unrecognized early immune adverse effect of checkpoint-inhibitors treatment. Immunotherapy. 2021;13(8):653–9.
25. Nielsen MM, Witherden DA, Havran WL. γδ T cells in homeostasis and host defence of epithelial barrier tissues. Nat Rev Immunol. 2017;17(12):733–45.
26. Severance EG, Gressitt KL, Stallings CR, Origoni AE, Khushalani S, Leweke FM, et al. Discordant patterns of bacterial translocation markers and implications for innate immune imbalances in schizophrenia. Schizophr Res. 2013;148(1–3):130–7.
27. Simonin M, Corbeau P, Durand P, Rosenzwajg M, Filleron A, Tran TA. A possible role for IL-17 in Clarkson's disease. Eur J Paediatr Neurol. 2016;20(6):953–6.
28. Sion-Sarid R, Lerman-Sagie T, Blumkin L, Ben-Ami D, Cohen I, Houri S. Neurologic involvement in a child with systemic capillary leak syndrome. Pediatrics. 2010;125(3):e687–92.
29. Profaci CP, Munji RN, Pulido RS, Daneman R. The blood-brain barrier in health and disease: important unanswered questions. J Exp Med. 2020;217(4).