Patients with Chromosome 11q-abberations are Characterized by a Combined Primary Immunodeficiency Involving Both B- and T-lymphocytes

Elisabeth Janneke Huisman (e.j.huisman@erasmusmc.nl)
Erasmus MC Sophia https://orcid.org/0000-0002-0700-2194
A. Rick Brooimans
University Medical Center Rotterdam
Samone Mayer
University Medical Center Rotterdam
Marieke Joosten
Erasmus University Medical Center
Louis de Bont
University Medical Center Utrecht
Mariëlle Dekker
Albert Schweitzer hospital
Elisabeth L.M. Rammeloo
Bernhoven Hospital
Frans J. Smiers
Leiden University
P. Martin Van Hagen
University Medical Center Rotterdam
C Michel Zwaan
University Medical Center Rotterdam
Masja de Haas
Sanquin Diagnostic Services
Marjon H. Cnossen
University Medical Center Rotterdam
Virgil A.S.H. Dalm
University Medical Center Rotterdam

Research Article

Keywords: chromosome 11q, Jacobsen syndrome, primary immunodeficiency, B-lymphocyte function, T-lymphocyte function, granulocyte function, hypogammaglobulinemia, children

Posted Date: August 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-740083/v1

Abstract

Disorders of the long arm of chromosome 11 (11q) are rare and involve various chromosomal regions. Patients with 11q-disorders, including Jacobsen syndrome, often present with a susceptibility for bacterial, prolonged viral and fungal infections partially explained by hypogammaglobulinemia. Additional T-lymphocyte or granular neutrophil dysfunction may also be present.

In order to evaluate infectious burden and immunological function in patients with 11q-disorders, we prospectively studied a cohort of 14 patients with various 11q aberrations. Clinically, 12 patients exhibited prolonged and repetitive respiratory tract infections, frequently requiring (prophylactic) antibiotic treatment (n=7), ear-tube placement (n=9) or use of inhalers (n=5). Complicated varicella infections (n=5), chronic eczema (n=6), warts and chronic fungal infections (n=4) were reported. Six patients were on immunoglobulin replacement therapy. We observed a high prevalence of low B-lymphocyte counts (n=8), decreased T-lymphocyte counts (n=5) and abnormal T-lymphocyte function (n=12). Granulocyte function was abnormal in 29% without an aberrant clinical phenotype. Immunodeficiency was found in patients with terminal and interstitial 11q-deletions and in one patient with 11q trisomy. Genetically, *FLI1* and *ETS1* are seen as causative for the immunodeficiency, but these genes were deleted nor duplicated in 5 of our 14 patients. Alternative candidate genes on 11q such as *ATM, CD3-cluster, CBL* and *THYN1* may have a role in immune dysregulation in our patients.

In conclusion, we present evidence that a combined primary immune deficiency may be present in patients with 11q-disorders leading to clinically relevant infections. Therefore, broad immunological screening and necessary treatment is of importance in this patient group.

Introduction

Patients with 11q-disorders are characterized by a partial deletion or partial trisomy of the long arm of chromosome 11. These chromosomal aberrations occur in various regions of 11q with deletion sizes ranging from < 5–20 Mb [1] and can be combined with abnormalities in other chromosomes. [2, 3] Breakpoints arising within or distal to subband 11q23.3 with a deletion extending to the telomere, give rise to Jacobsen syndrome (JS), alternatively referred to as 11q-terminal deletion disorder. [5, 6] The estimated incidence is 1:100.000 births with a male:female ratio of 1:2. [1] Clinical phenotype depends on the length, position and type of the chromosomal abnormality, and may include cognitive impairment, cardiac malformation, increased bleeding tendency and increased susceptibility for infections. [1] The latter is attributed to a humoral immunodeficiency with an abnormal B-lymphocyte development and low memory B-lymphocytes, resulting in hypogammaglobulinemia and an impaired response to immunization. [7–10] Although immunoglobulin replacement therapy (IgRT) significantly decreases the infectious burden [7], infectious complications still occur. [11] Some studies suggest that low T-lymphocyte counts [7–9, 12] or T-lymphocyte dysfunction [8, 9, 12] may be causative. We hypothesized that granular dysfunction of neutrophils may also play a role, because patients with JS are also known to suffer from increased bleeding tendency due to delta storage pool defects [13], or giant alpha granules in platelets leading to the Paris-Trousseau syndrome. [14] The combination of platelet en neutrophil dysfunction is seen in other syndromes, such as Hermansky-Pudlak type 2. [15]

Genetically, focus is on the transcription factors ETS1 and FLI1, both located on 11q24.3. For the *ETS1* gene, experimental evidence in mice with a homozygous deletion of *ETS1* shows that proliferation of B- and T-

lymphocytes is decreased, [16] and spontaneous apoptosis of T-lymphocytes is increased. [17][18][19] Somatic loss of 11q23.3 has been found in B-lymphocyte lymphoma (*MYC* negative), also indicating an effect on lymphocyte proliferation. [20] In humans, the role of *ETS1* was demonstrated in a recent case-report of a neonate with a germline pathogenic frameshift mutation in *ETS1* in whom a decreased number of total and naïve B-lymphocytes was found, as well as reduced presence of memory B-lymphocytes. T-lymphocyte number however was normal. [21]

The *FLI1* gene is a member of the E26 transformation specific (*ETS*) gene family, that shares a DNA-binding domain called *ETS*-domain, which is responsible for sequence-specific DNA recognition of target promotors. This *ETS* domain is known to have a role in cellular development of B and T-lymphocytes. [22] Experimental evidence comes from *FLI1*-knockout mice, constructed with deletion of N-terminal region of *FLI1 (*FLI1ΔNT). FLI1ΔNT-mice exhibited thymic hypocellularity. [23] This defect was not associated with a specific subpopulation of thymocytes or apoptosis, implicating the role of *FLI1* in prethymic T-lymphocyte progenitors. [23] The role of *FLI1* in B-lymphocyte development has also been demonstrated by *FLI1*-knockout mice lacking the CTA domain (FLI1ΔCTA). [22, 24] FLI1ΔCTA-homozygous mice show significantly less splenic follicular and more transitional and marginal zone B-lymphocytes. [22] Despite this fundamental evidence for a role of *ETS1* and *FLI1*, clinically a variable phenotype-genotype is seen. In two families with an identical microdeletion in 11q24.2-11q24.3, one family demonstrated persistent lymphopenia and low IgG, but the other did not. [25]

In order to evaluate immunological dysfunction in patients with 11q-disorders, we prospectively investigated 14 patients focusing on clinical features, and tested both B-lymphocyte, T-lymphocyte and granulocyte function. We correlate our results with monosomy or trisomy of *ETS1* and *FLI1*.

Patients And Methods

Patient population and study design

Patients were invited for this study by their treating physician or via the newsletter of the Dutch Chromosome 11 Network. Institutional Review Board approval was obtained (MEC-2013-026, Erasmus Medical Center, Rotterdam, The Netherlands) and the study was performed according to the Declaration of Helsinki. All patients were included after obtaining written informed consent according to local law and regulation.

Clinical data included number and type of infections, antibiotic treatment and/or prophylaxis, and IgRT which was derived from electronic patient files.

For laboratory tests a minimum of 0.5 mL of EDTA-blood, 0.5mL serum and 6mL heparinized blood was drawn by venepuncture. Cell count and indices were analyzed on Sysmex XN-9100 (Sysmex®). Total IgG, IgM and IgA were investigated on Cobas 8000 Modular Analyzer Series (Roche Diagnostics®, Basel). Heparinized whole blood was used for flowcytometric measurement of lymphocyte count (FACSCanto II, BD®, USA).

Detection of antigen-specific $CD4^+$ T-lymphocytes

Antigen-specific CD4 + T-lymphocytes were measured using a commercially available kit (Act-T4 Cell™, Cytognos, Spain) that is based on the so-called 'OX40 assay'. [26] Heparinized whole blood was diluted 1:1 with RPMI-1640 (Gibco, Paisley, UK) culture medium supplemented with 10% fetal calf serum (Gibco), L-glutamine 2mM

(BioWhittaker), and penicillin and streptomycin (100IU/mL; BioWhittaker). Then aliquoted into 500µL volumes in sterile capped 5mL polystyrene flow cytometry tubes. The following conditions were used for each assay: no exogenous stimulation (negative control), PHA (2.5 µg/mL), Staphylococcal enterotoxin B (SEB; Sigma-Aldrich) (0.5 µg/mL), and activation with tuberculin PPD (4µg/mL), Tetanus toxoid (1µg/mL), Diphteria toxoid (10µg/mL), Candida lysate (4µg/mL), CMV lysate (2µg/mL), HSV1 lysate (1µg/mL), HSV2 lysate (1µg/mL), and VZV lysate(1µg/mL). After 44–48 hour stimulation in a humidified atmosphere of 5% CO2, samples were vortexed and 100µL of each culture was stained with 20 µL of the 4-antibody mixture provided in the Act-T4 Cell™ kit (CD3-PerCP-Cy5.5, CD4-FITC, CD25-APC, and CD134-PE, Cytognos®) and incubated for 15minutes at room temperature. The assay was protected from light. After incubation, 1mL of erythrocyte lyse-non-wash solution provided in the Act-T4 Cell™ kit was added to each tube, mixed and incubated for 10minutes at room temperature, still protected from light. After lysis 10,000 CD3 positive events were acquired on flow cytometer (FACSCanto II) and analyzed with Infinicyt software (Cytognos®, Spain). Quantification of CD3 + CD4 + CD25 + CD134 + T-lymphocytes were calculated by setting gate coordinates on the negative control sample to equal 0,1% CD3 + CD4 + CD25 + CD134 + events as a percentage of all CD3 + CD4 + events. These gate coordinates were applied to subsequent antigen tubes to produce a percentage value of CD25 + CD134 + double positive events.

Determination of granulocyte phagocytic activity–Phagotest

The granulocyte phagocytic activity was measured in whole heparinized blood using a commercially available kit (Phagotest®, Glycotope Biotechnology, Germany) according to manufacturer's instructions. In short, FITC-labeled opsonized *E.coli* bacteria were added to whole blood and incubated for 10 min at 37°C (experimental tube) or 0°C (negative control tube). After incubation, the reaction was stopped, erythrocytes were lysed and the DNA staining solution was added. Fluorescence of samples was measured by flow cytometer (FACSCanto II) in < 60 min after the last reagent had been added. Data were acquired by FACSDiva software (BD Biosciences) and analyzed by Infinicyte software. The Phagotest is performed with the involvement of fluorescein-stained E.coli bacteria which are phagocytized by the cells. The test determines the percentage of granulocytes and their phagocytic activity, i.e. the number of bacteria absorbed by a single cell in terms of mean fluorescence intensity (MFI).

Determination of oxidative burst activity of granulocytes–Phagoburst®

A respiratory burst-assay was performed using a commercially available kit (Phagoburst®, Glycotope Biotechnology, Germany) according to manufacturer's instructions. Briefly, opsonized *E.coli* bacteria (experimental tube), or phorbol 12-myristate 13-acetate (PMA, positive control tube) or washing solution (negative control tube) were added to whole blood and incubated for 10 min at 37°C. Following incubation, dihydrorhodamine (DHR 123) was added for 10min, erythrocytes were lysed and DNA-staining solution was added. Dihydrorhodamine 123 becomes fluorescent when oxidized by reactive oxygen species, and its fluorescence was measured in less than 30 min after the last reagent had been added by flow cytometry, as described above. The test determines the percentage of active cells, as well as the respiratory burst intensity within a single cell in terms of MFI.

Statistical analysis

We used descriptive statistics to summarize baseline characteristics of the study population. In case of a skewed distribution, data are presented as median and interquartile range (IQR). In case of a normal distribution, data are presented as mean and standard deviation (SD) or range. Categorical data are presented as numbers with percentages and range of minimum and maximum value.

Reference values of healthy controls were available for the various antigens that were used as stimuli in the OX40 test. The results of the T-lymphocyte function tests were considered abnormal when the results were < p5 of healthy controls. Also for both granulocyte function tests obtained reference values of healthy controls were used. The phagocytic and oxidative burst capacity results were considered abnormal when the results were < p5 of healthy controls. Statistical data analyses were performed using SPSS version 21.0 (IBM, Armonk, NY,USA).

Results

Patient characteristics

Sixteen 11q-patients were identified, but one patient declined participation and one passed away before study initiation. We included 14 patients, with a mean age of 11,8 years (range 0–40), four males (ratio 1:3). Twelve presented with a partial 11q-deletion, two with partial 11q-trisomy. The extent and location of the involved regions varied widely, and in three patients additional chromosomal abnormalities were observed. During follow-up one patient (patient 7, (P7)) passed away (Table 1).

Clinically, all patients were immunized according to general recommendations of the Dutch National Institute for Public Health and the Environment (RIVM). Frequent infections were reported in the majority of patients, especially during childhood (12/14; 86%) and most often affecting upper and lower respiratory tract (RTI). These infections required frequent or prophylactic antibiotic treatment (7/12; 70%). Nine required ear-nose-throat (ENT)-interventions such as ear tubes or adeno-tonsillectomy (9/14; 64%). Five required (5/14; 36%) inhalation therapy with beta2-mimetica, and two (2/14; 14%) were hospitalized for oxygen supplementation as supportive measures in case of infectious complications. Six patients (6/14) were on intravenous or subcutaneous IgRT because of hypogammaglobulinemia. All six patients on IgRT reported less infections and an improved physical condition (Table 2).

Complicated viral infections leading to hospitalization were confirmed for respiratory syncytial virus (RSV), picornavirus, para-influenza virus and rhinovirus. Complicated and prolonged varicella zoster virus (VZV) infection in 5/14 (36%). Two of 14 patients (14%) suffered from recurrent gastro-enteritis, resulting in repeated hospitalization because of dehydration. Rotavirus was confirmed in two of these episodes. Fungal infections, most often in the diaper area or the nails were reported in 4/14 (29%), persistent warts by two patients (2/14; 14%), and chronic eczema by six patients of the 14 (43%), resolving in one patient after IgRT was initiated.

An overview of immunological laboratory results is presented in Table 3. Total leucocyte counts were low in two patients of Caucasian ethnicity (P2,7), 2,8 and 3,2 x10^{E}9/L respectively (normal range 3,8–9,8xE10^{9}/L). In five of 14 patients (36%) an absolute lymphopenia was observed (mean 0,78; absolute range 0,63 – 0,95, normal range 0,34 – 5,36x10E^{9}L) and in one a relative lymphopenia (P14, normal range 15–47%).

Total numbers of T-lymphocytes and B-lymphocytes were normal or elevated in four patients (4/14; 29%), all four had IgG-levels within the reference range. Lymphocyte counts were abnormal in 10/14 patients (72%). Total *B-lymphocyte* counts were low in eight patients (57%, normal reference > 0,20 x $10E^9$/L). In five (5/8, 63%) T-lymphocyte numbers were also low. Remarkably, all eight patients with low B-lymphocyte counts presented with clinically relevant and increased number of infections, regardless of IgG-levels (P1-3,7–10,14). The absence of an association between total number of B-lymphocytes and function is illustrated in P11, who had a hypogammaglobulinemia from infancy with normal but steadily decreasing B-lymphocyte count up to the age of 18 years (from 1,68 x $10E^9$/L at age 1, till 0,21 x $10E^9$/L at age 18).

Low IgG-levels were found in six patients (6/14; 43%), all corrected on IgRT. A concomitant low IgM (2/6) sometimes with combined low IgM and IgA (2/6) was seen in four (4/6, 67%). Only low IgM-levels were seen in three patients (3/14; 21%), of whom two were < 2 years of age at time of inclusion. One patient had a low combined IgA and IgM (P12).

Total *T-lymphocyte* counts were low in five patients (5/14, 36%, In one patient both CD4 + and CD8 + T-lymphocytes were decreased (1/5), in two only CD4 + and in two only CD8 + T-lymphocytes were decreased (normal reference CD4 + > 0.3 x $10E^9$/L, CD8 + > 0.2 x $10E^9$/L), resulting in an abnormal CD4:CD8-ratio in four (P1,6,8,9).

T-lymphocyte function was evaluated in 12 patients using an OX40-based test. We refer for details to Table 4. In general, only two patients showed a normal response to all stimuli (P6&13, 17%). T-lymphocytes from ten patients showed at least one abnormal response to the various stimuli (83%). For the viral agonists, T-lymphocytes of all patients on IgRT failed in CMV-response (5/5, 100%). Also, T-lymphocytes of three patients (not on IgRT, but with a history of frequent viral upper airway and ENT-infections) failed on CMV-response (P9-10,12). As this assay is a functional test, diminished responses are either due to an intrinsic T-lymphocyte defect, or due to the fact that a patient has not yet encountered CMV. Unfortunately, data of seroconversion were missing. Medical history was certain for 13 patients for VZV. Of these 13, three patients with a history of VZV-infection, demonstrated an abnormal T-lymphocyte response (P3,7,2; 3/12; 25%). Despite the fact that all patients were vaccinated, an impaired response to the conjugated vaccine against tetanus was observed in two patients (P1&2; 2/12; 7%). All responded well to diphtheria and also to tuberculin. For the bacterial agonists, five patients showed abnormal result after stimulation with PHA or SEB (P3,1,7,2,9; 4/12, 33%).

Neutrophil granulocyte counts were normal in all 14 patients. Neutrophil granulocyte function was abnormal in four patients (P13,7,2,9; 4/14, 29%). Two patients (P2,7), who responded with a lower than normal oxidative burst, presented with a history of frequent infections and antibiotic usage, but none of the patients had opportunistic infections with microbes that are pathognomonic for neutrophil dysfunction, e.g. invasive *S. Aureus, Aspergillus, Nocardia, Burkholderia* or *Serratia*. Both patients are currently on IgRT. The two other patients (P9,13) showed abnormal responses in chemotaxis, of whom patient 13 (P13) with a trisomy only reported chronic (diaper) dermatitis and ENT-infections in infancy, and patient 9 (P9) reported an increased infectious burden in ENT and RT, but is not on IgRT due to normal IgG-levels.

ETS1 and *FLI1* involvement did not correlate to patients with immunodeficiency of B- nor T-lymphocyte dysfunction in our cohort. See Table 5.

Discussion

We present 14 patients with a variety of 11q-disorders. We demonstrated an increased vulnerability to infections in almost all, but with varying clinical impact. Infections started in early infancy with recurrent and/or chronic ENT and airway infections, leading to high consumption of antibiotics, but also of inhalation therapy, hospitalization and the performance of ENT-surgery. Vulnerability for both viral and bacterial infections is illustrated by the finding that almost half of patients suffered from VZV infections with multiple skin lesions leading to secondary bacterial skin infections. Also, other skin manifestations, including eczema, (candida) dermatitis, fungal skin and nail infections, and warts, were encountered regularly.

We confirm B-lymphocyte dysfunction resulting in hypogammaglobulinemia in 64% (9/14) of patients in infancy or at older age, necessitating IgRT in 6/14, as well as low B-lymphocyte numbers (8/14). [7, 8] We underline the relevant finding that low IgG is not always accompanied by low total B-lymphocyte counts and vice versa, as demonstrated in patient 11 (P11). [7, 12]

We are the first to demonstrate that T-lymphocyte dysfunction occurs in the majority of patients with 11q-disorders. We showed that decreased T-lymphocyte counts or abnormal CD4/CD8-ratio occur in 43% of our cohort and abnormal *in vitro* T-lymphocyte activation in 12/13 patients (92%). Especially, the abnormal responses to stimuli such as PHA, SEB, Diphteria, CMV and VZV are clinically relevant. Unfortunately, we were not able to test seroconversion for CMV. The abnormal response may be due to naivety towards CMV, HSV1 or VZV in four patients, still giving an abnormal test result in 62%. Qualitative T-lymphocyte defects have been reported in cases before [8, 12, 27], but none of the applied stimulation tests in these studies was identical to the assays we used, which makes comparisons between results difficult. We conclude that a combined primary immunodeficiency regularly occurs, which was suggested in previous case-series. [7, 8, 28–31]

Finally, although we demonstrate a disturbed granulocyte function *in vitro*, these findings may be interpreted as mild or irrelevant as none of the patients experienced typical infections with e.g. *Aspergillus* or invasive *S. Aureus*. It therefore remains unclear whether the need for antibiotics due to a bacterial superinfection after VZV (P2,6,9,11,14; 56%) is due to hypogammaglobulinemia only, or combined with a potential granular dysfunction (only P2,11,14 are on IgRT). In a single patient on IgRT hospitalization for possible septicemia was documented. In this patient, an isolated abnormal response to NBT was found in granulocyte function analysis (P7). Our current results do not clearly support nor rule out the hypothesis that granulocyte dysfunction is responsible for the high incidence of infections, more research in this area is indicated .

Genetically, the role of *ETS1 nor FLI1* gene did not correspond with abnormal B nor T-lymphocyte number or function in our cohort. While monosomy of *ETS1* and *FLI1* was seen in nine patients (P1-9) and trisomy in one (P14), an abnormal B or T-lymphocyte number was only seen in seven. On the other hand, low numbers T-lymphocyte numbers were found in 1 patient and abnormal T-lymphocyte function in 3 patients, respectively, while *ETS1* and *FLI1* were not deleted in these patients (See Table 5). This variable phenotype-genotype may be due to incomplete penetrance, as is suggested in congenital microdeletions in two families with an identical microdeletion in 11q24.2-11q24.3. One family demonstrated persistent lymphopenia and low IgG, the other did not. [25] But, this variability may also be due to other genes of interest that are located on 11q. It could be hypothesized that a whole gene cluster is involved in cell proliferation and differentiation and could play a role in development of the combined immunodeficiency in 11q patients. Our two patients with interstitial deletions not

involving *ETS1* and *FLI1* also suffer from a combined immunodeficiency. Genes of interest to be considered as modifiers are *ATM, CD3, CBL* and *THYN1*.

More in detail, the *ATM* gene is located on 11q22.3 (OMIM #607585). Homozygous or compound heterozygous mutations cause ataxia-telangiectasia (AT). [32] AT is characterized by cerebellar ataxia, telangiectasia, predisposition to malignancy and immune defects, such as thymus hypoplasia, reduced IgG and IgA-levels, and sometimes accompanied by low IgM, lymphopenia and abnormalities in T-lymphocyte maturation. Besides the autosomal recessive AT-disorder, variant-AT with residual ATM protein expression and kinase-activity have been described as leading to a milder or more variable phenotype. Often, in variant-AT-patients only one truncating mutation in *ATM* is found, together with a compound missense, splice variant or leaky mutation. [33, 34] Most patients have elevated alpha-fetoprotein levels (AFP). No AFP-levels are known in 11q-disorder patients.

The cluster of CD3EAP, CD3D, CD3E, CD3G, CD3Z genes are all located on 11q23.2, and regulate the synthesis of T-lymphocyte antigen receptor chains: gamma, delta, epsilon and zeta. [35] The genes for the alpha and beta chains are located on other chromosomes. During development, the CD3 protein complex plays an important role in the transition of thymocytes from immature precursors to the final mature CD4 + or CD8 + T-lymphocytes. [36] Pathogenic variants in any of these genes may cause mild to severe blockage of this development at the stage of CD4+/CD8 + lymphocytes, resulting in reduced gamma-delta T- lymphocytes or even absent T-lymphocytes causing severe combined immunodeficiency. [36]

The CBL gene, located on 11q23.3 encodes for an E3 ubiquitin ligase acting as a regulator in the tyrosine kinase signaling pathway. [37] *CBL* plays a role in lymphocyte development and activation: in T-lymphocytes by regulating development of the thymocyte and thymic selection, in B-lymphocytes by regulating receptor signaling thresholds in order to stimulate B-lymphocyte maturation. [38, 39] Heterozygous pathogenic variants in patients may lead to juvenile myelomonocytic leukemia or Noonan-like syndrome with cardiac defects and lymphedema. Besides single nucleotide variants, Hanson et al. describe this entity as the result of uniparental isodisomy of 11q23 in this case, underlining the possible influence of this gene for the clinical phenotype of 11q-disorders. [40]

The THYN1 gene, located on 11q25, encodes for thymocyte nuclear protein 1, which is expressed in the thymus. [41] This gene may be involved in the induction of apoptosis or T-lymphocyte regulation. [42][43] As yet, we are not aware of diseases in humans caused by mutations this gene.

Study limitations

Our study has several limitations. Firstly, we describe only a small cohort of 14 patients in whom the genetic defects on chromosome 11q vary considerably and concurrent chromosomal abnormalities occur. This reflects medical practice as 11q-disorders are a rare continuous gene syndrome, but makes extrapolation of results to all 11q-disorder patients more challenging. In our cohort, seven patients had the ‘classical’ Jacobsen syndrome (11q23.3-terminal deletion syndrome). Three patients had additional chromosomal abnormalities, of whom patient 14 (P14) had a combination of partial trisomy of 11q and partial trisomy of 22q, also referred to as Emanuel syndrome. No clear primary immune deficiency has yet been reported to occur in Emanuel syndrome, but frequent ENT-infections are reported in cases and are mentioned by the Unique patient organization to occur “in a minority”. [44][45] The trisomy of 11q23.3 in our patient may give rise to hypogammaglobulinemia and T-

lymphocyte dysfunction, but the 22q11 region can also cause T-lymphocyte dysfunctions or T-lymphocytopenia as is seen in other chromosome 22q abnormalities and in one case-report of a child with a 22q11.2 microduplication. [46] No hypogammaglobulinemia was seen in this child. [47, 48] We therefore feel that it is unclear whether the hypogammaglobulinemia and abnormal T-lymphocyte function in our patient (P14) are only due to terminal 11q-duplication or that the concurrent 22q11-duplication may also play a role. We decided to include this patient in our cohort, as the effect of 11q cannot be ruled out and it is essential to be aware that 11q-trisomy patients with Emanuel syndrome may be prone to a combined primary immune deficiency as well. More research in this specific patient group is needed.

Also, although a study design with a standardized protocol was followed, we were not able to avoid missing values with regard to clinical data and laboratory test results. The large volumes of blood necessary for laboratory tests certainly played a role in this regard. Still, we think that study results are applicable to other 11q-patients as specific clinical symptoms and immunological laboratory results were more frequently observed than expected in the general population.

Lastly, we were not able to organize serological testing for CMV infections retrospectively due to ongoing IgRT or lack of stored plasma. Therefore, it is more difficult to draw definite conclusions on the results of the OX40-based T-lymphocyte function test. We consequently recommend treating physicians to test EBV, CMV seroconversion before T-lymphocyte function is tested or before IgRT is started to enable more clear conclusions in the near future.

Overall, we conclude that patients with partial 11q-deletion or 11q-trisomy regardless of involvement of genes as *ETS1* or *FLI1*, have a risk of a combined primary immunodeficiency, consisting of both quantitative and qualitative defects. We therefore recommend regular immunological screening by testing IgG, IgA, IgM, response to immunization and B and T-lymphocyte counts in all patients with 11q-disorders. It is important to realize that lymphocyte dysfunction does not always correlate with B- and T-lymphocyte count. Immunological abnormalities may develop over time and need repetitive testing, or may present at birth. When newborn screening by T-lymphocyte receptor excision circle (TREC) is performed, T-lymphopenia should promptly lead to further investigation involving 11q-analysis. [49][50]. In case of a humoral immunodeficiency, prophylactic antibiotics and/or IgRT should be considered based on number and severity of infectious complications and potential end-organ damage, including bronchiectasis. In case of chronic fungal infections, especially affecting skin or nails, local treatment or systemic prophylaxis is indicated. Physicians should be aware that fungal infections and prolonged viral infections may still occur while on IgRT therapy.

As many questions remain in this intriguing immunological field, future research should focus on further pathophysiological understanding of this combined immunodeficiency in patients with 11q-disorders which can be seen as a model to identify modifiers of immunological function in various disorders. Focus should be on other candidate genes, that can influence the immunological dysfunctions seen in patients with 11q-disorders.

Declarations

Authorship Contributions

All authors contributed to this article. VD is the principal investigator of the study. VD and EH designed the study; EH and SM collected data; RB analyzed laboratory tests. EH, SM, and RB performed analyses; EH, MC and VD

wrote the manuscript. All authors critically revised the manuscript, agreed with its content and approved submission.

Disclosure of Conflicts of Interest

None

References

1. Grossfeld, P.D., et al., *The 11q terminal deletion disorder: a prospective study of 110 cases.* Am J Med Genet A, 2004. **129A**(1): p. 51-61.
2. Hagen, A., et al., *Combined partial trisomy 11q and partial monosomy 10p in a 19-year-old female patient: phenotypic and genotypic findings.* Am J Med Genet A, 2011. **155A**(12): p. 3075-81.
3. Maruyama, K., T. Koizumi, and H. Ikeda, *Partial monosomy 10q with partial trisomy 11q due to paternal balanced translocation.* J Paediatr Child Health, 2001. **37**(2): p. 198-200.
4. Emanuel, B.S., E.H. Zackai, and L. Medne, *Emanuel Syndrome.* 1993.
5. Penny, L.A., et al., *Clinical and molecular characterization of patients with distal 11q deletions.* Am J Hum Genet, 1995. **56**(3): p. 676-83.
6. Su, J., et al., *de novo interstitial deletions at the 11q23.3-q24.2 region.* Mol Cytogenet, 2016. **9**: p. 39.
7. Dalm, V.A., et al., *The 11q Terminal Deletion Disorder Jacobsen Syndrome is a Syndromic Primary Immunodeficiency.* J Clin Immunol, 2015. **35**(8): p. 761-8.
8. Seppänen, M., et al., *Terminal Deletion of 11q with Significant Late-Onset Combined Immune Deficiency.* Journal of Clinical Immunology, 2013. **34**(1): p. 114-118.
9. Miller, C.D.E., A.R. Atkinson, and C.M. Roifman, *Immunodeficiency in Jacobsen syndrome.* Journal of Allergy and Clinical Immunology, 2003. **111**(2): p. S232.
10. Puglisi, G., et al., *11q terminal deletion disorder and common variable immunodeficiency.* Ann Allergy Asthma Immunol, 2009. **103**(3): p. 267-8.
11. Quinti, I., et al., *Effectiveness of immunoglobulin replacement therapy on clinical outcome in patients with primary antibody deficiencies: results from a multicenter prospective cohort study.* J Clin Immunol, 2011. **31**(3): p. 315-22.
12. Blazina, S., et al., *11q terminal deletion and combined immunodeficiency (Jacobsen syndrome): Case report and literature review on immunodeficiency in Jacobsen syndrome.* Am J Med Genet A, 2016. **170**(12): p. 3237-3240.
13. Mattina, T., C.S. Perrotta, and P. Grossfeld, *Jacobsen syndrome.* Orphanet J Rare Dis, 2009. **4**: p. 9.
14. Breton-Gorius, J., et al., *A new congenital dysmegakaryopoietic thrombocytopenia (Paris-Trousseau) associated with giant platelet alpha-granules and chromosome 11 deletion at 11q23 [see comments].* Blood, 1995. **85**(7): p. 1805-1814.
15. Wenham, M., et al., *Two patients with Hermansky Pudlak syndrome type 2 and novel mutations in AP3B1.* Haematologica, 2010. **95**(2): p. 333-7.
16. Bories JC, Willerford DM, Grévin D, Davidson L, Camus A, Marti P et al: Increased T-cell apoptosis and terminal B-cell differentiation induced by inactivation of the Ets-1 proto-oncogene, Nature

1995;377(6550):635-8.

17. Muthusamy N, Barton K, Leiden JM. Defective activation and survival of T cells lacking the ETS-1 transcription factor. Nature 1995:377:639-642
18. Russell, L. and L.A. Garrett-Sinha, *Transcription factor Ets-1 in cytokine and chemokine gene regulation.* Cytokine, 2010. **51**(3): p. 217-26.
19. Garret-Sinha LA. Review of ETS1 structure, function, and roles in immunity. Cell Mol Life Sci 2013;70(18):3375-90
20. Salaverria, I., et al., *A recurrent 11q aberration pattern characterizes a subset of MYC-negative high-grade B-cell lymphomas resembling Burkitt lymphoma.* Blood, 2014. **123**(8): p. 1187-98.
21. Tootleman, E., et al., *Partial Jacobsen syndrome phenotype in a patient with a de novo frameshift mutation in the ETS1 transcription factor.* Cold Spring Harb Mol Case Stud, 2019. **5**(3).
22. Zhang, X.K., et al., *The transcription factor Fli-1 modulates marginal zone and follicular B cell development in mice.* J Immunol, 2008. **181**(3): p. 1644-54.
23. Melet, F., et al., *Generation of a novel Fli-1 protein by gene targeting leads to a defect in thymus development and a delay in Friend virus-induced erythroleukemia.* Mol Cell Biol, 1996. **16**(6): p. 2708-18.
24. Moussa, O., et al., *Thrombocytopenia in mice lacking the carboxy-terminal regulatory domain of the Ets transcription factor Fli1.* Mol Cell Biol, 2010. **30**(21): p. 5194-206.
25. Conrad, S., et al., *11q24.2q24.3 microdeletion in two families presenting features of Jacobsen syndrome, without intellectual disability: Role of FLI1, ETS1, and SENCR long noncoding RNA.* Am J Med Genet A, 2019. **179**(6): p. 993-1000.
26. Zaunders, J.J., et al., *High levels of human antigen-specific CD4+ T cells in peripheral blood revealed by stimulated coexpression of CD25 and CD134 (OX40).* J Immunol, 2009. **183**(4): p. 2827-36.
27. Lotz, D.R. and A.P. Knutsen, *Janus kinase 3 missense mutation in a child with jacobsen syndrome.* Annals of Allergy, Asthma & Immunology, 2010. **104**(6): p. 536-537.
28. Õunap, K., et al., *Girl with combined cellular immunodeficiency, pancytopenia, malformations, deletion 11q23.3 → qter, and trisomy 8q24.3 → qter.* American Journal of Medical Genetics, 2002. **108**(4): p. 322-326.
29. Fernández-San José, C., et al., *HYPOGAMMAGLOBULINEMIA IN A 12-YEAR-OLD PATIENT WITH JACOBSEN SYNDROME.* Journal of Paediatrics and Child Health, 2011. **47**(7): p. 485-486.
30. Puglisi, G., et al., *11q TERMINAL DELETION DISORDER AND COMMON VARIABLE IMMUNODEFICIENCY.* Annals of Allergy, Asthma & Immunology, 2009. **103**(3): p. 267-268.
31. Blazina, Š., et al., *11q terminal deletion and combined immunodeficiency (Jacobsen syndrome): Case report and literature review on immunodeficiency in Jacobsen syndrome.* American Journal of Medical Genetics Part A, 2016. **170**(12): p. 3237-3240.
32. https://omim.org/entry/607585
33. Saviozzi, S., et al., *A late onset variant of ataxia-telangiectasia with a compound heterozygous genotype, A8030G/7481insA.* J Med Genet, 2002. **39**(1): p. 57-61.
34. Verhagen, M.M., et al., *Presence of ATM protein and residual kinase activity correlates with the phenotype in ataxia-telangiectasia: a genotype-phenotype study.* Hum Mutat, 2012. **33**(3): p. 561-71.
35. https://omim.org

36. de Saint Basile, G., et al., *Severe combined immunodeficiency caused by deficiency in either the delta or the epsilon subunit of CD3.* J Clin Invest, 2004. **114**(10): p. 1512-7.
37. https://omim.org/entry/165360
38. Huang, F. and H. Gu, *Negative regulation of lymphocyte development and function by the Cbl family of proteins.* Immunol Rev, 2008. **224**: p. 229-38.
39. Rudd, C.E. and H. Schneider, *Lymphocyte signaling: Cbl sets the threshold for autoimmunity.* Curr Biol, 2000. **10**(9): p. R344-7.
40. Hanson, H.L., et al., *Germline CBL mutation associated with a noonan-like syndrome with primary lymphedema and teratoma associated with acquired uniparental isodisomy of chromosome 11q23.* Am J Med Genet A, 2014. **164A**(4): p. 1003-9.
41. https://omim.org/entry/613739
42. Jiang, X.Z., et al., *Anti-IgM-induced down-regulation of nuclear Thy28 protein expression in Ramos B lymphoma cells.* Apoptosis, 2003. **8**(5): p. 509-19.
43. Ji T, Wu Y, Wang H. et al. Diagnosis and fine mapping of a deletion in distal 11q in two Chinese patients with developmental delay. J Hum Genet 2010;55:486–489
44. https://www.rarechromo.org/media/information/Chromosome%2011/Emanuel%20syndrome%20FTNW.pdf
45. Carter, M.T., et al., *Phenotypic delineation of Emanuel syndrome (supernumerary derivative 22 syndrome): Clinical features of 63 individuals.* Am J Med Genet A, 2009. **149A**(8): p. 1712-21.
46. Traynor, R., et al., *Immunodeficiency in a Child with 22q11.2 Microduplication Syndrome.* J Clin Immunol, 2016. **36**(5): p. 418-9.
47. Bjork, A.H., et al., *Antibody deficiency in adults with 22q11.2 deletion syndrome.* Am J Med Genet A, 2012. **158A**(8): p. 1934-40.
48. Colarusso, G., et al., *Evans syndrome and antibody deficiency: an atypical presentation of chromosome 22q11.2 deletion syndrome.* Pediatr Rep, 2010. **2**(2): p. e13.
49. Puck, J.M., *Newborn screening for severe combined immunodeficiency and T-cell lymphopenia.* Immunol Rev, 2019. **287**(1): p. 241-252.
50. Dosanjh A: The Use of TREC Analysis as a Newborn Screening Test for Jacobsen Syndrome and T cell Deficiency. Int J Rare Dis Orphan Drugs 2(2): 1007

Tables

Due to technical limitations, Tables are only available as a download in the Supplemental Files section.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1MolecularDiagnosis.pdf
- Table2Symptomsandcomorbidities.pdf
- Table3Immunologicalanalysis.pdf

- Table4ResultsTcfunctionassay.pdf
- Table5ETS1andFLI1involvement.pdf