J Clin Immunol (2015) 35:168–181
DOI 10.1007/s10875-015-0129-5

ORIGINAL RESEARCH

Infectious and Immunologic Phenotype of *MECP2* Duplication Syndrome

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Received: 21 April 2013 / Accepted: 12 January 2015 / Published online: 27 February 2015
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Abstract *MECP2* (methyl CpG binding protein 2) duplication causes syndromic intellectual disability. Patients often suffer from life-threatening infections, suggesting an additional immunodeficiency. We describe for the first time the detailed infectious and immunological phenotype of *MECP2* duplication syndrome. 17/27 analyzed patients suffered from pneumonia, 5/27 from at least one episode of sepsis. Encapsulated bacteria (*S.pneumoniae*, *H.influenzae*) were frequently

Electronic supplementary material The online version of this article (doi:10.1007/s10875-015-0129-5) contains supplementary material, which is available to authorized users.

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isolated. T-cell immunity showed no gross abnormalities in 14/14 patients and IFNy-secretion upon ConA-stimulation was not decreased in 6/7 patients. In 6/21 patients IgG_2-deficiency was detected – in 4/21 patients accompanied by IgA-deficiency, 10/21 patients showed low antibody titers against pneumococci. Supra-normal IgG_1-levels were detected in 11/21 patients and supra-normal IgG_3-levels were seen in 8/21 patients – in 6 of the patients as combined elevation of IgG_1 and IgG_3. Three of the four patients with IgA/IgG_2-deficiency developed multiple severe infections. Upon infections pronounced acute-phase responses were common: 7/10 patients showed CRP values above 200 mg/l. Our data for the first time show systematically that increased susceptibility to infections in *MECP2* duplication syndrome is associated with IgA/IgG_2-deficiency, low antibody titers against pneumococci and elevated acute-phase responses. So patients with *MECP2* duplication syndrome and low IgA/IgG_2 may benefit from prophylactic substitution of sIgA and IgG.

Keywords Xq28-duplication syndrome · methyl CpG binding protein 2 (MECP2) · *MECP2* duplication syndrome · primary immunodeficiency · intellectual disability · humoral immunodeficiency

Abbreviations

MECP2 Methyl CpG binding protein 2
XLID X linked intellectual disability

Introduction

MECP2 duplication on chromosome Xq28 (#300260) causes a severe form of X-linked intellectual disability (XLID). It was first clinically described as Lubs syndrome, the underlying genetic condition was identified in 2005 [1–3]. Up to now more than 200 patients have been described [1–50]. The exact prevalence of *MECP2* duplication syndrome is still unknown, but in synopsis of different screening attempts it is estimated that *MECP2* duplication syndrome may explain ~1 % of cases of severe XLID [51]. The core phenotype of *MECP2* duplication syndrome includes infantile hypotonia, mild dysmorphic features, developmental delay/severe to profound intellectual disabilty and absent to minimal speech [51]. Spasticity, ataxia and autism are facultative clinical signs that occur in different combinations and to different degrees. Since the description of the first individuals it became evident that besides the striking neurological phenotype patients with *MECP2* duplication syndrome are at increased risk for severe infections. About 70–75 % of the affected individuals are reported to develop recurrent infections, especially of the respiratory tract. Respiratory infections are the main cause of early death reported among the population [51, 52]. However a detailed description of the infectious phenotype and an investigation of the immunological phenotype based on a large cohort has been missing to date.

Suspecting an immune dysfunction in patients with *MECP2* duplication syndrome we assessed the infectious and immunological phenotype of a cohort of 30 patients. We here provide for the first time a detailed description of the type of infections, the pathogens isolated and the core cellular and

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humoral immunological phenotype in patients with *MECP2* duplication syndrome.

Materials and Methods

Patients The current study was conducted in accordance with the Helsinki Declaration, with informed consent obtained from each patient or the patient's family. Samples from the patients were used in this study with approval from the local ethics committee of the Charité (approval # EA2/063/12). All patients enrolled in this study show a genetically confirmed duplication of at least *MECP2* and *IRAK1*. The diagnostics were performed prior to this study and the results were submitted to us by the performing laboratories or geneticists. In patients P1, P2, P8, P9, P10, P11, P12, P13, P14, P17, P19, P20, P21, P25, P27, P28, P29 and P30 the duplication had been detected by use of array-CGH thus revealing the actual size of the duplication on Xq28. In Patient P15 quantitative PCR had been performed and in patients P3, P4, P5, P6, P7, P18, P23, P24 and P26 Multiplex-ligation probe amplification was used for the diagnosis of *MECP2* duplication and single neighbouring genes, e.g., *IRAK1*. So in patients P15, P3, P4, P5, P6, P7 and P18, P23, P24 and P26 the actual size of the duplication is unknown. The following patients have been described previously: P1, P2 in Bartsch et al. [21], P3, P4, P5, P6, P7 in Echenne et al. [18], P8 in Budisteanu et al. [31], P9 in Jezela-Stanek et al. [28], P10 in Mayo et al. [32], P12 in Grasshoff et al. [30], P13 in Bauters et al. [9], P15 in Meins et al. [2], P17 in Madrigal et al. [8], P20, P21 in Xu et al. [43], and P22, P27 in Vignoli et al. [40]. For detailed molecular analyses of these patients see the corresponding publications. Patients P11, P14, P16, P18, P19, P23, P24, P25, P26, P28, P29 and P30 have not been published before. For molecular characterization of these patients see supplementary table 1.

Blood Samples of the Patients Venous punctures were performed in parallel to routine blood tests. The blood samples were sent to our laboratory to ensure that all patients' samples were analyzed with the same methods and with the same laboratory equipment.

Assessment of the Infectious Phenotype A detailed questionnaire on the clinical phenotype was completed by the physicians caring for the patients with *MECP2* duplication syndrome and sent to two of the authors (MB and HVB) for thorough review.

Assessment of Immunoglobulin Levels and Antibody Titers Against Tetanus Toxoid and Pneumococci Total immunoglobulin levels and immunoglobulin subclasses of all patients' samples in our cohort were measured by ECLIA on a COBAS 6000 (Roche, Switzerland) in our laboratory. Antigen-specific IgG antibodies against Tetanus-Toxoid and PCP as well as anti-PCP-IgG_2 of all patients' samples in our cohort were measured by ELISA according to manufacturer's protocols (MK013, MK012 and MK010, The Binding Site) in our laboratory.

Assessment of Lymphocyte Subsets Lymphocyte subsets of patients' EDTA-blood were stained with fluorescence-labelled monoclonal antibodies against CD14 (RMO52), CD56 (N901), CD16 (3G8), CD4 (SFCI12T4D11), CD19 (J3-119), CD8 (SFCI21Thy2D3), CD3 (UCH-T1), CD45 (J33), CD45R0 (UCHL1), TCRαβ (IP26A), TCRγδ (IMMU510) and CD45RA (2H4LDH11LDB9) (all Beckman Coulter, USA) for 15 min at room temperature and were measured and analyzed after erythrocyte lysis (Versa-Lyse, Beckman Coulter) on a NAVIOS-FACS (Beckman Coulter, USA) in our laboratory.

Lymphocyte Proliferation Assay Lymphocyte proliferative responses were tested according to standard protocols in heparinised whole blood and were stimulated with phytohemagglutinin (PHA, Sigma-Aldrich, L1668), plate bound anti-CD3 (3, 10, and 30 μg/ml; clone HIT3a, Becton Dicinson), Pokeweed-Mitogen (PWM, 1, 5, and 15 μg/ml, Sigma-Aldrich, L-9379), IL-2 (20 ng/ml, #200-02, PeproTech, Rocky Hill/NJ, USA) and Staphylococcus aureus Cowan-1 strain (SAC, Pansorbin, 1:1000, 1:10,000, Merck, Darmstadt, Germany) for 72 h and with Tetanus-Toxoid RT50 (40, 20, and 10 Lf/ml, Staten Serum Inst. Copenhagen), Candida albicans antigen (0.025, 0.25, and 2.5 μg/ml; CAN001-P; Biologo, Kronshagen, Germany) and Diphtheria-Toxoid (20 LF/ml, Chiron-Behring) for 120 h. After pulsing with 1 μCi [^{3}H]-thymidine per well during the last 12 h incorporated [^{3}H]-thymidine was measured on a β-scintillation counter (MicroBeta 1450 TriLux, PerkinElmer).

Assessment of IFNγ After Stimulation with ConcanavalinA (ConA) Heparinised blood was stimulated 1:5 diluted in RPMI with 50 μg/ ml ConA (Sigma-Aldrich, #7246) and IFNy was assessed by Th1/Th2-cytokine detection kit (Becton Dicinson Biosciences, #550749) according to the manufacturers protocol. Measurement was performed on a FACS-Navios (Beckman Coulter). For analysis FCAP-Array-Software (Soft Flow Hungary) was used. The normal range of IFNy-secretion upon activation with ConA after 4 h of activation was assessed based on the activation of 50 healthy donors (25 male and 25 female). The normal range of IFNy-secretion upon activation with ConA after 24 h of activation was assessed based on the activation of 10 healthy male donors.

Results

Cohort, Genotypes and Infectious Phenotype 30 patients with *MECP2* duplication syndrome from Germany, France, Italy,

Poland, Romania, Spain, The Netherlands, Belgium, USA and China were enrolled in the study (28 male patients and two female patients). All patients showed a duplication of *MECP2* and neighbouring *IRAK1* (Fig. 1). Patients' age ranged from 3 to 48 years. We had access to the history of 27 of the 30 patients with 343 cumulative patient-years. The occurrence of infections varied in our cohort of patients: In 9/27 patients (including the two girls) no severe infections were reported at all. 18/27 patients developed at least one severe infection requiring intravenous antibiotic treatment. Pneumonia occurred in 17/27 patients. Regarding the type of pneumonia, complete assessment was possible in patient P14 and in this patient all pneumonias were X-ray-proven bronchopneumoniae. 5/27 patients developed at least one episode of sepsis. Urinary tract infections were reported in 6/27 patients. Recurrent fever of unknown origin occurred in three patients. No meningitis was diagnosed in our cohort. The frequency of severe infections also varied with a maximum of 27 episodes requiring intravenous antibiotic treatment since birth in a 16-year old boy (P1). Two patients died of pneumonia during the course of the study (Table 1).

Of the 25 different pathogens detected in the patients, the majority were bacteria with 84 %. Only 3 viruses (12 %) and 1 fungus (4 %) were among the 25 pathogens. Of the 21 different bacteria, 7 bacteria were Gram-positive (33 %) and 14 bacteria were Gram-negative (66 %). Thirteen of the 21 isolated bacteria (61.9 %) are capable of building a capsule (*A.hydrophila* [53], *B.fragilis* [54–56], *E.coli* [57], *H.influenzae* [57], *K.oxytoca* [58], *K.pneumonia* [59], *P.mirabilis* [60], *P.aeruginosa* [59, 61], *S.aureus* [62–65], *S.epidermidis* [66, 67], *S.agalactiae* [57], *Streptococcus group A* [68–70], *S.pneumoniae* [57, 64]).

Pathogens were isolated in pharyngeal swab, sputum, bronchoalveolar lavage, bronchial secretion/aspirate, tracheal secretion, ear swab, urine, stool, peritoneal liquid and blood. When considering the isolation of pathogens in these biological materials altogether, the pathogens isolated in most patients were *S.pneumoniae* (5/27) and *H.influenzae* (5/27) - both are typical encapsulated bacteria [64]. In 4/27 patients *E. coli,* in 4/27 *C.albicans,* in 3/27 patients *S.aureus*, in 3/27 *P.aeruginosa*, in 2/27 *P.mirabilis* and in 2/27 *Streptococcus group A* were isolated. *S.agalactiae*, *Streptococcus group C*, *Streptococcus group F*, *S.epidermidis*, *K.pneumonia, K.oxytoca, M. tuberculosis, O.anthropi*, *A.hydrophila*, *C.freundii, B.fragilis, S.enteritidis, S.marcescens* and *S.plymuthica* were each detected in one patient. *Influenza A*, *Respiratory-Syncytial-Virus* and *Rhinovirus* were also each isolated in only one patient (Fig. 2a).

When considering only the isolates in biological material of the respiratory tract, the pathogens isolated in most patients were *H. influenzae* (4/27) and *C.albicans* (4/27) followed by *S.pneumoniae* (3/27) (Fig. 2a). The spectrum of pathogens isolated in blood included *S.pneumoniae*, *H.influenzae*, *S.aureus*, *S.epidermidis* and *C.albicans*, each only isolated in one patient (Fig. 2b). Pathogens isolated in urine were *P.mirabilis* (in 2/27 patients), *E.coli* (in 1/27 patients) and *S.pneumoniae* (in 1/27 patients) (Fig. 2b). Neither infections by *Pneumocystis jirovecii*, nor invasive infections by

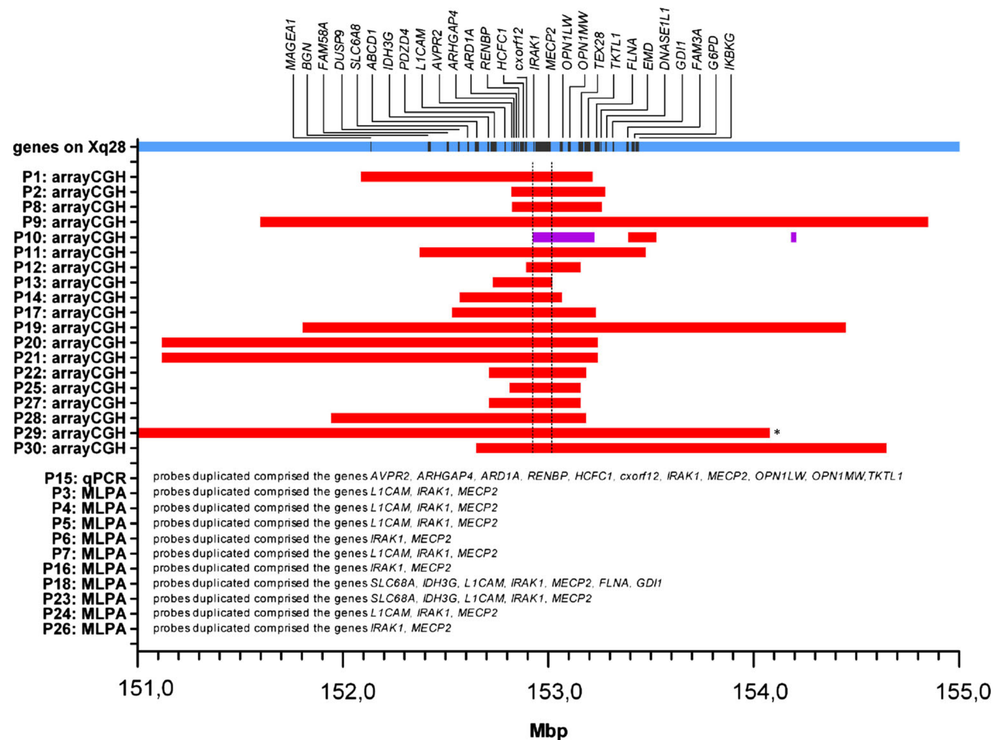

Fig. 1 Size of duplications in MbP on chromosome Xq28. The x-axis depicts positions on chromosome Xq28 between 151 and 155 Mbp. *Red bars* indicate duplicated segments, *purple bars* indicate triplicated segments in the individual patients. Positions on the X chromosome are based on the UCSC genome browser build hg18. (* Duplication of patient P29 extends the depicted area on Xq28 to the *left side* until position 147.384.720 bp)

Table 1 Infectious phenotype of patients with *MECP2* duplication syndrome

	P1	P14	P9	P29	P15	P23	P17	P16	P2	P24	P27	P18	P13	P22
Age	16years	15years	15years	7years	15years	8years	9years	3years	6years	15years	12years	9years	8years	18years
Sex	m	m	m	m	m	m	m	m	m	m	m	m	m	m
Outcome status	alive	†	alive	alive	†	alive	alive	alive	alive	alive	alive	alive	alive	alive
Number of infections requiring intravenous antibiotic treatment	27	18	13	10	8 (between 8y-15y)	7	3	3	3	3	3	2	2	2
Invasive infections														
Sepsis	2	0	3	1	1	0	1	0	0	0	0	0	0	0
Meningitis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Others	–	–	–	–	–	–	–	–	–	–	–	–	–	1x peritonitis
Noninvasive infections														
Pneumonia	48	14	43	8	50	7	2	3	2	3	3	3	2	1
Purulent otitis	3	2	n.a.	4	3	1	0	n.a.	1	n.a.	1	>8	>4	n.a.
Others	>1 urinary tract infection 1 x ARDS 1 x streptococcal toxic shock syndrome	5x urinary tract infection 2x pyelonephritis	1x lung tuberculosis 3x cystitis recurrent idiopathic fever episodes (~1 day lasting)	3 x pyelonephritis	–	1 x Tonsillitis	1x cystitis 1x salmonella gastroenteritis	–	1 x pyelonephritis >2 urinary tract infection	–	–	recurrent episodes of bronchitis 1 x severe gastroenteritis	–	–
Others														
Severe fever of unknown origin	0	0	>4	0	3	0	0	3	0	0	0	0	0	0

	P28	P26	P19	P11	P12	P10	P3	P4	P5	P6	P7	P20	P21	P8	P25	P30
Age	4years	10years	22years	20years	9years	11years	9years	28years	23years	5years	17years	19years	10years	10years	48years	18years
Sex	m	m	m	m	f	f	m	m	m	m	m	m	m	m	m	m
Outcome status	alive	alive	alive	alive	alive	alive	alive	alive	alive	alive	alive	alive	alive	alive	alive	alive
Number of infections requiring intravenous antibiotic treatment	2	2	1	1	0	0	0	0	0	0	0	0	0	n.a.	n.a.	n.a.
Invasive infections																
Sepsis	0	0	0	0	0	0	0	0	0	0	0	0	0	n.a.	n.a.	n.a.
Meningitis	0	0	0	0	0	0	0	0	0	0	0	0	0	n.a.	n.a.	n.a.
Others	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Noninvasive infections																
Pneumonia	2	2	30	0	0	0	0	0	0	0	0	0	0	n.a.	n.a.	n.a.
Purulent otitis	n.a.	n.a.	5	0	n.a.	0	0	0	0	0	0	0	0	n.a.	n.a.	n.a.
Others	–	–	3 x urinary tract infection	1x adenophlegmon laterocervical 2 x pharyngotonsilitis with high fever	>4x tonsillitis	–	–	–	–	–	–	–	–	–	–	–

Table 1 (continued)

	P28	P26	P19	P11	P12	P10	P3	P4	P5	P6	P7	P20	P21	P8	P25	P30
Others																
Severe fever of unknown origin	0	0	0	0	0	0	0	0	0	0	0	0	0	n.a.	n.a.	n.a.

Age, sex, number of infections requiring intravenous antibiotic treatment, occurence of invasive versus non-invasive infections and outcome. Order of patients runs from the most often infected to the least often infected

mycobacteria or *S.enteritidis* were observed. In summary patients with *MECP2* duplication syndrome show increased susceptibility for pneumonia and sepsis caused by bacteria that are mainly capable of building a capsule (Fig. 2 and supplementary table 2).

Cellular and Humoral Immunological Phenotype Peripheral blood smears of 21 patients showed values of total white blood cells that were elevated in two patients (P9, P30). Monocytes were slightly elevated in patient P9 and P22. Neutrophilic granulocytes were increased in patient P9 and P15, and reduced in patient P14. Eosinophilic and basophilic granulocytes were within normal ranges (supplementary figure 1). More detailed immunophenotyping of lymphocytes from 14 patients revealed numbers of total T (CD3+) lymphocytes that were elevated in 1/14 patient. CD4+ T-cells were above age-dependent reference value in 1/14 (different) patient. Absolute numbers of CD8+ T-cells were elevated in 1/14 further patient. In 5 of 14 patients tested naïve CD4+ T-cells (CD4+CD45RA+) were elevated, while memory CD4+ T-cells (CD4+CD45RO+) were reduced. B-cells (CD19+) were reduced in 3 of the 14 patients tested and NK-cells (CD16+CD56+CD3-) were below age-dependent reference value in 1 of the 14 patients tested (supplementary table 3A and supplementary table 3B).

Lymphocyte proliferation upon stimulation with mitogens (PHA, PWM) was normal in 13 patients tested. Stimulation with IL-2 showed attenuated responses in 2/13 patients and stimulation with anti-CD3 revealed a lymphocyte response below reference value in 1/13 patients. When stimulating cells of the 13 patients with SAC, 3 patients showed reduced lymphocyte response. Attenuated lymphocyte proliferation was also observed upon stimulation with candida-antigen (3 of 13 patients tested), diphtheria-antigen (5 of 13 patients tested) and tetanus toxoid antigen (2 of 13 patients tested) (supplementary table 4).

We tested IFNy-secretion upon stimulation with ConA for 24 h in whole blood of 7 patients. We observed a normal production of IFNy in blood that was stimulated soon after the blood withdrawal ($n=1$). In blood that had travelled, 4 patients showed an IFNy-level after stimulation that was within the reference values of healthy controls. The IFNy-level of one patient was above the reference value and the IFNy-level of one patient was below reference values. So in total in 6/7 patients tested IFNy-production upon stimulation with ConA was not impaired (Fig. 3). In summary lymphocyte subsets and lymphocyte proliferation assays show no gross abnormalities and IFNy-production upon stimulation with ConA seems to be normal.

Of 30 patients enrolled we could assess immunoglobulin levels (IgG, IgA and IgM), and levels of IgG subclasses ($IgG_1 – IgG_4$) and specific antibodies against tetanus and pneumococci in 21 patients. This revealed six patients with IgG_2-

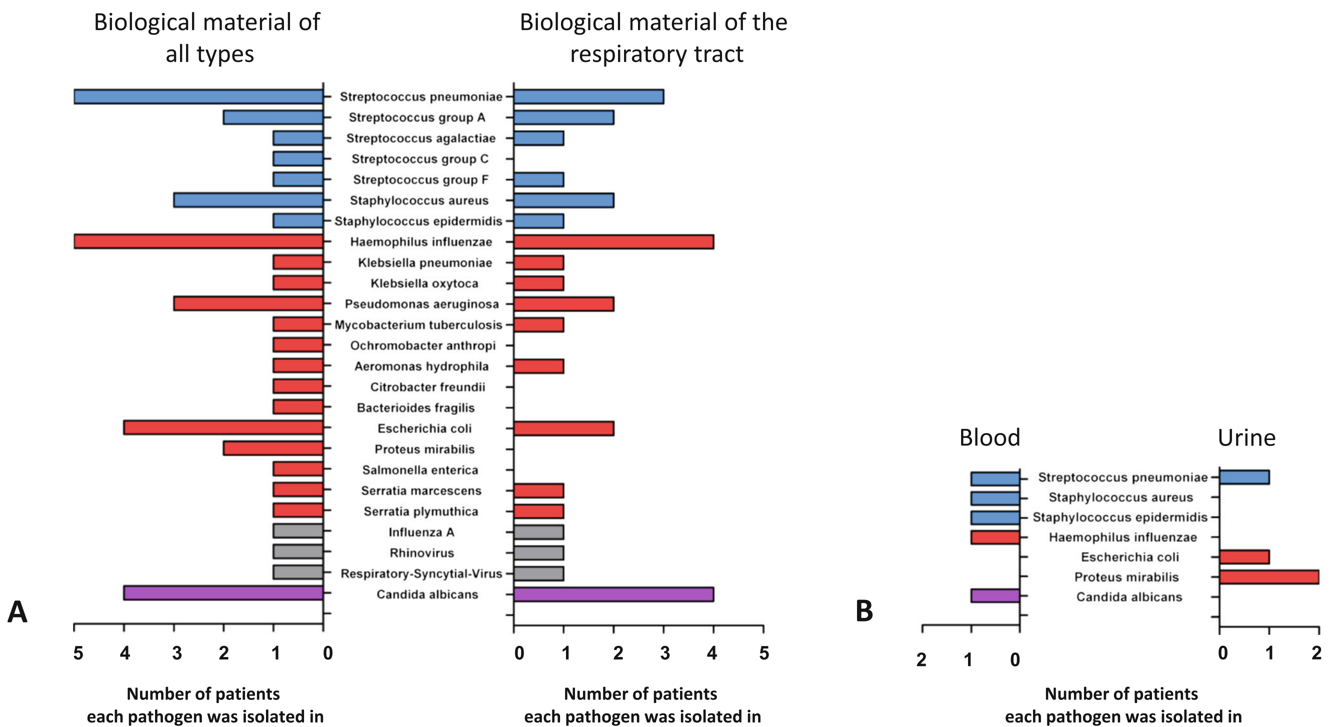

Fig. 2 Pathogens isolated in patients with *MECP2* duplication syndrome and number of patients each pathogen was detected in. **a**. All biological material and material of the respiratory tract **b**. Blood and Urine

deficiency – four of them with an additional IgA-deficiency. One of the four patients with IgA/IgG_2-deficiency had globalized low levels also for IgG, IgG_1, IgG_3, IgG_4 and IgM. Additionally IgG_2 was in low normal range in 3 further patients. IgG_4 was below reference values in 4 patients, all of which also had IgG_2-deficiency. IgG_1 was above age-dependent reference values in 11/21 and IgG_3 was above age-dependent reference values in 8/21 patients; 6/21 patients showed supra-normal values for IgG_1 and IgG_3 (Fig. 4a–g), IgG_2-antibodies against *S.pneumoniae* were below normal values in 4 patients, in whom also IgG_2 was low. Additionally *S.pneumoniae* IgG_2-antibodies were in the lower range of normal in 6 further patients. So in total 10/21 patients showed low antibody titers against pneumococci (Fig. 5). Of the 21 patients in whom we assessed the levels of *S.pneumoniae* IgG_2-antibodies, 8 patients had received vaccination against pneumococci, 8 patients had not received vaccination and in five patients information on vaccination status was not available. Interestingly *S.pneumoniae* IgG_2-antibodies were low even in the patients with vaccination against pneumococci (Fig. 5). Both pneumococcal polysaccharide vaccines and pneumococcal conjugate vaccines have been used in the 8 patients with pneumococcal vaccination and 7 of the 8 patients have received several pneumococcal vaccinations. The period between last vaccination and assessment of aPCP-IgG_2 ranged from 1 month up to 7 years and 6 month. It is noteworthy that even in the patient with the short interval of 1 month between last vaccination and ascertainment of antibody titers no appropriate antibody production against *S. pneumoniae* was determined after three vaccinations (Table 2). In contrast to this rather common impairement of IgG_2 formation against pneumococci IgG-antibodies against Tetanus-Toxoid were below age-dependent reference values in only 2/21 patients and low in normal range in 2 further patients, so normal in 19/21 patients (supplementary figure 2). In summary 6/21 patients show IgG_2-deficiency – four of them with an additional IgA-deficiency - and half of the patients (10/21) show antibodies against pneumococci either below normal range or in the low range of normal.

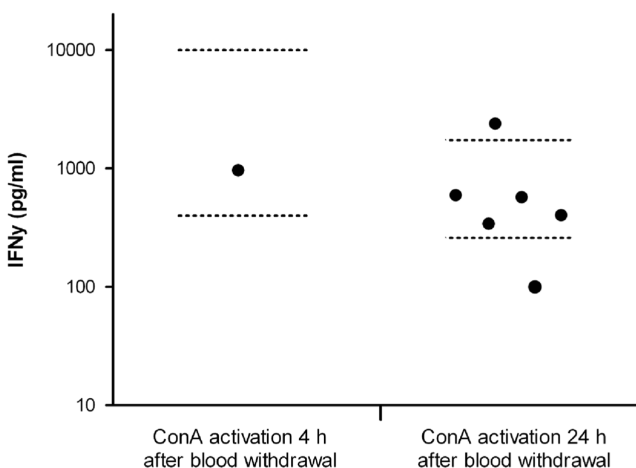

Fig. 3 Interferon-y secretion in whole blood upon stimulation with ConA. (Normal ranges are depicted as *dotted lines*)

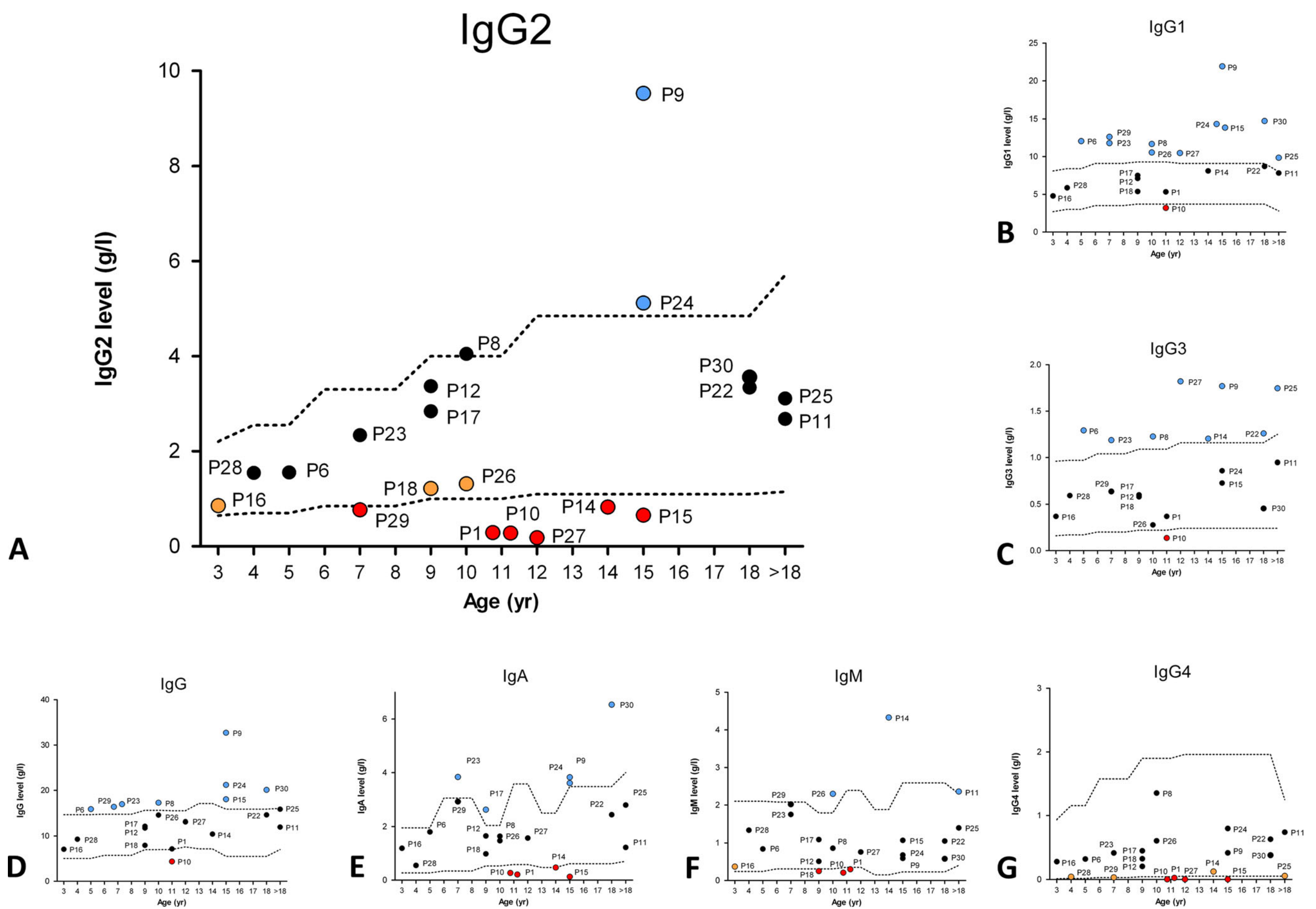

Fig. 4 Humoral immunological phenotype of patients with *MECP2* duplication syndrome. **a.** IgG_2 levels. **b.** IgG_1 levels. **c.** IgG_3 levels. **d.** IgG levels. **e.** IgA levels. **f.** IgM levels. **g.** IgG_4 levels. (Normal ranges are depicted as *dotted lines*)

Acute-Phase Responses As infections in patients with *MECP2* duplication syndrome have not only been reported for their high frequency but also for the severity of the single infectious episode we systematically assessed acute-phase responses in these patients. Data on acute-phase responses was available for 10 patients. 7/10 patients experienced non-invasive infections with maximal CRP values above 200 mg/l. In 4/10 patients CRP values above 300 mg/l were reported during non invasive infections. Only one patient (P16) showed no elevated CRP values upon non-invasive infections. The mean value of all reported maximal CRP values during non-invasive infections of all patients was 157,58 mg/l (Fig. 6a). The maximal temperature measured during non-invasive infections ranged from 38.5 to 41.6 °C and the mean value measured during non-invasive infections of all patients was 39.75 °C (Fig. 6b). 2/10 patients experienced non-invasive infections with leucocyte counts above 30/nl and 8/10 patients showed leucocyte counts above 20/nl during single infectious episodes. Mean of all reported maximal leucocyte values was 15.69/nl (Fig. 6c). 5/10 patients showed a neutrophil count above 15/nl during a non-invasive infection. The mean value of the maximal neutrophil count for all patients was 10.51/nl (Fig. 6d). Taken together these data indicate a strong acute-phase response in patients with *MECP2* duplication syndrome.

Discussion

The duplication of *MECP2* on chromosome Xq28 leads to a rather common syndromic form of XLID (estimated ~1 % of x-linked patients). Besides the neurological phenotype it was soon noted that many of the patients suffer from recurrent infections [51, 52]. We here describe for the first time, systematically the infectious and immunological phenotype of *MECP2* duplication syndrome based on data derived from a standardized study-questionnaire filled out by the physicians together with the parents of 30 patients. Our findings show that patients with *MECP2* duplication syndrome are at an increased risk for infections by bacteria that are able to build capsules (61.9 % of isolated pathogens); in particular for infections by *S.pneumoniae* and *H.influenzae.* In our cohort we have not observed increased susceptibility to mycobacteria, pneumocystis spp, fungi and viruses. We

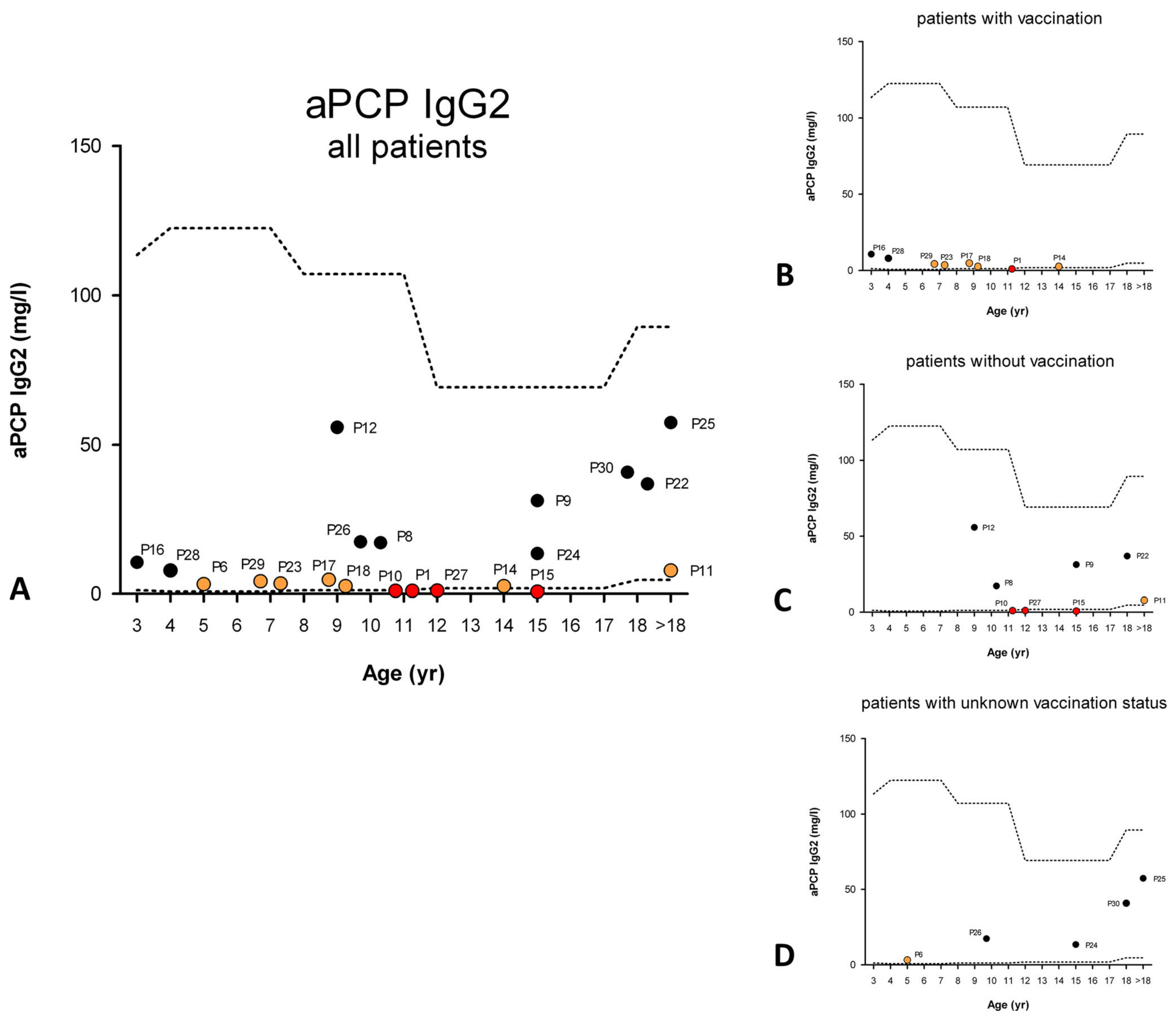

Fig. 5 Humoral immunological phenotype of patients with *MECP2* duplication syndrome**. a.** IgG_2 levels against *S. pneumoniae in all patients tested*. **b.** IgG_2 levels against *S. pneumoniae of patients with vaccination*. **c.** IgG_2 levels against *S. pneumoniae of patients without vaccination* **d.** IgG_2 levels against *S. pneumoniae of patients with unknown vaccination status (*Normal ranges are depicted as *dotted lines*)

could not identify a correlation between the size of the duplication (genotype) and the infectious or immunological phenotype. The predominance of respiratory and severe invasive infections caused by bacteria that are able to build capsules, suggested an impairment of humoral immunology.

IgG_2-deficiency was detected in 6/21 analyzed patients - in 4/6 patients accompanied by IgA-deficiency, while 15 out of 21 analyzed patients showed no IgG2 or IgA deficiency. IgG_2-deficiency in the 6/21 patients and low antibody titers against pneumococci in 10/21 patients suggest that among patients with *MECP2* duplication humoral impairement is more common than in the normal population. 5/6 patients with IgG_2-deficiency also developed severe infections. As 3/4 individuals with combined IgA/IgG_2-deficiency additionally suffered from a very high frequency of infections and developed the most severe infectious phenotype in our cohort there seems at least an association of IgA-/IgG_2-deficiency with severe infections. In line with this hypothesis only 2/15 patients without IgA or IgG_2 deficiency developed recurrent severe infections comparable to the phenotype observed in the patients with IgA/IgG_2-deficiency. One of them showed low IgG_2-antibodies against pneumococci even after vaccination indicating an inability to mount an appropriate antibody production although global levels of immunoglobulins are normal in this patient. The other patient (P9) with a severe infectious phenotype despite having an IgA-/IgG_2-deficiency showed massively elevated IgG (32 g/l). Given the fact that this patient has a persistently raised leucocyte count, it seems plausible that he additionally suffers from an unknown chronic inflammatory process. 7 of the 15 patients without IgA or IgG_2 deficiency

Table 2 Pneumococcal vaccination in patients with *MECP2* duplication syndrome

Patient	Type of vaccine	Number of vaccinations	Time between last vaccination and assessment of aPCP IgG2	Age at last vaccination
P16	PCV	5	3 months	3 years, 4 months
P17	PCV	2	7 years, 7 months	1 year, 8 months
P23	PCV	3	6 years, 10 months	10 months
P28	PCV	4	3 years, 4 months	1 year, 1 month
P29	PCV	4	5 years, 9 months	2 years
P1	PPV23	3	1 month	11 years, 1 month
P14	PPV23	2	1 year, 7 months	12 years, 11 months
P18	PPV23	1	7 years, 6 months	2 years, 3 months

Type of vaccine, number of vaccinations, time between last vaccination and assessment of aPCP IgG2, age at last vaccination

PCV polysaccharide conjugated vaccine, *PPV23* 23-valent pneumococcal polysaccharide vaccine

developed few infections and 2 of the 15 patients developed no infections. In 4 of the 15 patients without IgA or IgG2 deficiency the severity of the infectious phenotype could not be assessed as the patient was either still at young age or because the history could not be completed appropriately. The single patient who has to date not developed severe infections despite

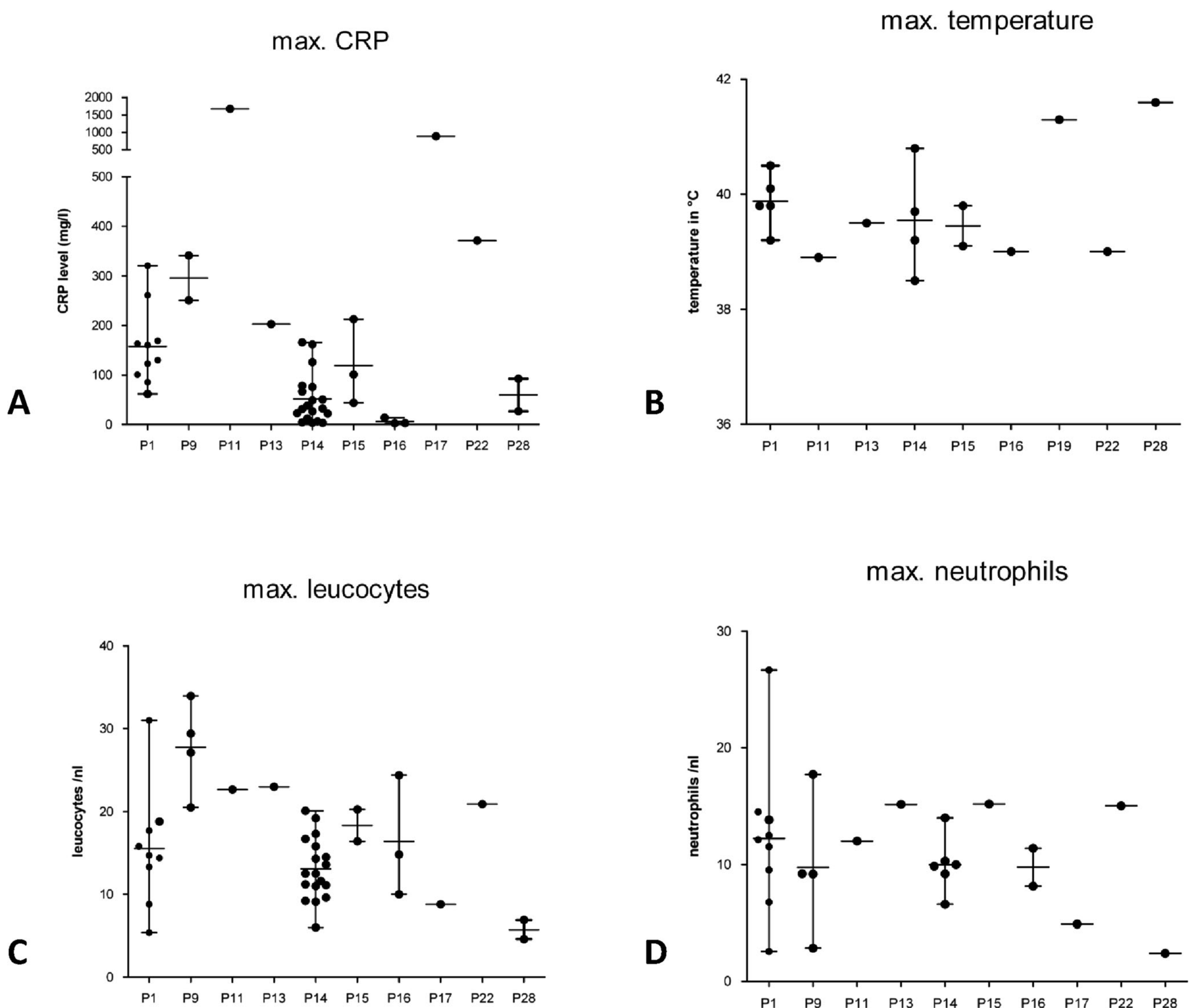

Fig. 6 Acute phase responses in patients with *MECP2* duplication syndrome. **a.** Maximal CRP-level during non-invasve infections. Upper reference value is 5 mg/l **b.** Maximal temperature during non-invasive infections **c.** Maximal leucocyte count during non-invasive infections. **d.** Maximal neutrophil count during non-invasive infections

IgA/IgG_2-deficiency is female. So in *MECP2* duplication syndrome not all patients develop humoral immunodeficiency but severe infections with potentially encapsulated bacteria are, at least in male patients, strongly associated with a lack of IgA and IgG_2.

We further investigated whether patients with *MECP2* duplication syndrome showed stronger acute phase responses, which we could confirm in 7/10 patients in terms of elevated CRP values above 200 mg/l during non-invasive infections, mainly pneumoniae. This observation raises the question if the genetic alterations in patients with *MECP2* duplication syndrome lead to a hyper-inflammatory immune response. Because all patients show a duplication on Xq28 that includes *MECP2* and neighbouring *IRAK1* and given the fact that these two genes were assumed to be the minimal critical region leading to the phenotype of *MECP2* duplication syndrome until recently [15, 17, 51], it seems plausible to hypothesize a role of *IRAK1* in the pathogenesis of strong acute-phase responses. *IRAK1* encodes for a protein in the TLR/IL1R-signalling pathway and it could be assumed that its overexpression leads to stronger or constantly triggered NFκB-mediated pro-inflammatory signalling and to a hyper-inflammatory immune response. In our opinion recently published results suggesting normal TLR-mediated signalling in PBMCs of patients with *MECP2/IRAK1* duplication do not sufficiently falsify this hypothesis as these experiments were performed in PBMCs (without granulocytes) and as it remains unclear whether LPS-stimulation was performed with or without serum [44]. So it seems at least possible that the previously described similar production of cytokines upon activation with TLR-agonists in patients and controls in these experiments is due to the insufficient stimulation of cells or a lack of stimulation of granulocytes or both. So to date it remains an open question whether the duplication of *IRAK1* renders patients with *MECP2* duplication prone to strong inflammatory responses.

It has been published that *MECP2* duplication causes a lack of TH1-differentiation, elevated levels of CD4+CD45RA+ naïve/ lowered levels of CD4+CD45R0+ memory T-cells and impaired IFNγ-secretion by T-cells in mice and in patients with *MECP2* duplication. It was concluded that this lack of TH1 differentiation/ IFNγ-secretion is the major cause for the increased susceptibility for infections in patients with *MECP2* duplication [44]. These observations are partially in line with ours. Also in our cohort 5/14 patients showed elevated levels of CD4+CD45RA+ naïve/ lowered levels of CD4+CD45R0+ memory T-cells. The observed ratio of CD4+CD45RA naïve versus CD4+CD45R0+ memory T-cells in our cohort is >1 in 11/14 patients and < 1 in 3/14 patients. However our data do not fully confirm the hypothesis of an impaired IFNγ-secretion as sufficient explanation for the increased susceptibility to infections: 1. Upon ConA-stimulation we observed a normal IFNγ-production in 5/7 patients, an even stronger IFNγ-production in one patient and only a slightly impaired IFNγ-production in one patient compared to healthy controls (Fig. 3); 2. If the observed reduction of IFNγ-secretion in patients with *MECP2* duplication was causative for severe infections one would expect disseminated and/or invasive infections with atypical mycobacteria and *S.enteritidis* as described for molecular precisely–defined partial and complete defects in at least 9 genes leading to the impairment of IFNγ-receptor signalling or IFNγ-secretion [71–74]; 3. Conversely during a total of 343 patient years in our study only one patient (P9) developed a single, non-complicated infection by *M.tuberculosis*, which remained restricted to the lung and was successfully treated by standard therapy. In a patient with a strongly relevant impairement of IFNγ-secretion an infection with *M.tuberculosis* would have taken a severe course [71–74]. Interestingly however 13/21 patients developed either supra-normal values of IgG_1 of IgG_3 or of both, IgG_1 and IgG_3. This observation suggests a direct or indirect effect of *MECP2* duplication on the expression of IgG_3 and IgG_1 at the $\gamma3/\gamma1$ locus and of IgA, IgG_2 and IgG_4 at the $\alpha1/\gamma2/\gamma4$ locus. It has been shown that IFNy stimulates the expression of the IgG_{2a}-isotype and inhibits the production of IgG_3, IgG_1, IgG_{2b} and IgE in mice [75]. So a deficiency in IFNy-secretion may lead to alterations of immunoglobulin levels in humans with *MECP2* duplication although the role of IFNy in the regulation of IgG_3 is controversial [76, 77]. We did not observe a generally impaired IFNy secretion in our cohort. However previously published results on irregularities in IFNy secretion should be taken into account as being responsible for altered immunoglobulin levels in patients with *MECP2* duplication syndrome [44].

Besides the classical encapsulated bacteria *S. pneumoniae* and *H.influenzae* several other bacteriae were also isolated in the patients of our cohort, that are indeed able to build capsules but are not clearly linked to capsule-related virulence. So it remains unclear if humoral immunodeficiency is causative also for infections by these facultative-encapsulated pathogens or if additional cellular immune deficits also account for the broad spectrum of observed pathogens in patients with *MECP2* duplication syndrome.

In summary we here show for the first time systematically that patients with *MECP2* duplication syndrome are at increased risk for in particular non-invasive but also for invasive infections with potentially encapsulated bacteria, that this increased susceptibility to infections may be associated with IgG_2-subclass deficiency/ low titers against pneumococci and elevated acute-phase responses, while the precise role of T-cell immunity and in particular the extent of impaired IFNγ-secretion and its role for the observed infectious phenotype is still to be defined. Based on our observation we hypothesize a multifactorial pathogenesis of infections in patients with *MECP2* duplication syndrome: Pulmonary infections may be triggered by frequent aspirations due to the severe

neurological phenotype and favoured due to humoral immunodeficiency. The observed alterations in the IgG subclass composition (high IgG_1/IgG_3 and low $IgA/IgG_2/IgG_4$) may be caused directly by interaction of MECP2 with the respective loci or indirectly by spatially impaired IFNγ-secretion. An increased susceptibility to hyper-inflammation either caused by *IRAK1* duplication or other direct or indirect effects of *MECP2* duplication may aggravate infections. Based on the compiled immunological and infectious findings we recommend to vaccinate patients with *MECP2* duplication against pneumococci and to evaluate post-vaccination titers. If post-vaccination titers are not sufficient, several boosts might be required. In patients with *MECP2* duplication syndrome, suffering from recurrent infections and with an IgG_2-subclass deficiency and/or low post-vaccination titers against pneumococci prophylactic substitution of IgG seems a plausible strategy to prevent infections – eventually in combination with the use of prophylactic antibiotics.

Acknowledgments We thank the patients and their families for participating in this study and Margret Oberreit, Petra Ellensohn (deceased 01/2011), Christine Seib, Kristin Neuhaus, Anne-Hélène Lebrun and Karoline Strehl for discussions, critical reading of the manuscript and technical assistance. This work was supported by the German Research Foundation (DFG BE 3895/3-1), the Federal Ministry of Education and Research, Germany (BMBF PID-NET 01GM1111D/TP-A5) and the Sonnenfeld Stiftung, Berlin.

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