Clinical Immunology (2014) **155**, 108–117

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

A novel FcγRIIa Q27W gene variant is associated with common variable immune deficiency through defective FcγRIIa downstream signaling

Thijs W.H. Flinsenberg, Willemijn J. Janssen, Eszter Herczenik, Peter Boross, Maaike Nederend, Lieneke H. Jongeneel, Rianne C. Scholman, Jaap-Jan Boelens, Coen Maas, Marielle E. van Gijn, Joris M. van Montfrans, Jeanette H. Leusen, Marianne Boes*

Laboratory of Translational Immunology, University Medical Center Utrecht, Wilhelmina Children's Hospital, Utrecht, The Netherlands

Received 25 June 2014; accepted with revision 7 September 2014
Available online 19 September 2014

KEYWORDS
CVID;
Children;
Fc gamma receptor;
Immune cell activation;
Susceptibility gene;
Receptor signaling

Abstract We identified a novel Q27W FcγRIIa variant that was found more frequently in common variable immunodeficiency (CVID) or CVID-like children. We analyzed the possible functional consequence of the Q27W FcγRIIa mutation in human cells. We used peripheral blood mononuclear cells from Q27W FcγRIIa patients and healthy controls, and cultured cells that overexpress the Q27W and common FcγRIIa variants. The Q27W FcγRIIa mutation does not disrupt FcγRIIa surface expression in peripheral blood mononuclear cells. Mononuclear cells express multiple FcγR, precluding careful analysis of Q27W FcγRIIa functional deviation. For functional analysis of FcγRIIa function, we therefore overexpressed the Q27W FcγRIIa and common FcγRIIa variant in IIA1.6 cells that are normally deficient in FcγR. We show that FcγRIIa triggering-induced signaling is obstructed, as measured by both decrease in calcium flux and defective MAPK phosphorylation. In conclusion, we here describe a novel Q27W FcγRIIa variant that causes delayed downstream signaling. This variant may contribute to CVID.

Abbreviations: FcγR, Fc gamma receptor; CVID, common variable immunodeficiency; ITAM, immunoreceptor tyrosine-based activation motif; BCR, B cell receptor; DC, dendritic cells; MAPK, mitogen-activated protein kinase; PBMC, peripheral blood mononuclear cells; ADCC, antibody-dependent cellular cytotoxicity; EGFR, epidermal growth factor receptor; WT, wild type.

* Corresponding author at: Department of Pediatric Immunology, Laboratory of Translational Immunology, University Medical Center Utrecht, Wilhelmina Children's Hospital, Lundlaan 6, P.O. Box 85090, KC.01.069.0, 3584 EA Utrecht, Netherlands. Fax: +31 88 75 55350.
E-mail address: mboes@umcutrecht.nl (M. Boes).

http://dx.doi.org/10.1016/j.clim.2014.09.006
1521-6616/

1. Introduction

Common variable immunodeficiency (CVID) is a heterogeneous disease characterized by defective antibody production [1,2]. Although more gene mutations are recently being described [3], the disease origins remain unknown in 90% of CVID patients [4–6]. Most of the mutations described directly involve the B cell receptor (BCR, CD19) complex involving additional molecules like CD20 [7]. However, other receptors or pathways expressed on B-cells and other immune cells are also associated with CVID. For example, TNF receptor defects such as the transmembrane activator and CAML interactor (TACI) [7,8] and the B-cell activating factor receptor (BAFFR) [9–11] or Fc receptor (FcR) defects are described to be associated with CVID [12]. FcR defects or polymorphisms are of particular interest, since these receptors bind immunoglobulins, or antibodies, which are typically reduced in CVID [1].

Blood-borne soluble antigens are readily opsonized by circulating immunoglobulins, thereby forming antigen immune complexes (IC). The capture and internalization of IC by immune cells is a well-studied process. Internalization exerts through the family of Fc Receptors (FcR), receptors that are widely expressed throughout the immune system. IC-FcR engagement elicits a range of cell type-specific effector mechanisms that include antibody-dependent cell-mediated cytotoxicity in neutrophils and peptide/MHC presentation by dendritic cells. Several isoforms for FcγRs exist that are often co-expressed on immune cell subsets, hampering functional studies of individual FcγR types. FcγRI, FcγRII (a, b, and c) and FcγRIII (a and b) [13] are expressed in varying numbers and ratios on neutrophils [14], monocytes/macrophages [14], B cells [15,16], dendritic cells (DCs) [17] and thrombocytes [14,18]. Our study focuses on the FcγR member FcγRIIa. FcγRIIa is a unique FcγR, since it harbors a signaling immunoreceptor tyrosine-based activation motif, or ITAM, within its ligand binding chain. Elicitation of signaling therefore does not require multimerization with signaling chains such as the γ-chain, known to associate with other FcR isoforms [19]. Moreover, studies performed in transgenic mice pinpoint the FcγRIIa as central mediator of inflammation in humans [20,21]. In this current study, we explore the function of FcγRIIa in immune activation, assisted by analysis of an FcγRIIa gene variant found in two unrelated immunodeficiency patients.

The interaction of Ig immune complexes with FcγRIIa induces signal transduction via its ITAM-motif, which appears to be triggered by receptor dimerization [13,19]. Genetic variants in FcγRIIa have been described [22] that link FcγRIIa to several immune diseases, including SLE, atherosclerosis and ulcerative colitis [12,23–27]. One of these FcγRIIa polymorphisms is found at position 131 and results in altered affinity to mouse IgG1 or human IgG2 (FcγRIIa 131H and 131R). FcγRIIa-131H is the only receptor that binds human IgG2 [22]. These polymorphisms result in variations in susceptibility to the development of auto-immune diseases. Structural studies on FcγRIIa predict that ligand binding induces FcγRIIa signaling through dimeric complexes [28,29]. Indeed, a dimer of FcγRIIa would place the two ITAM-containing chains in close proximity, suitable for propagating downstream signals. Studies in live cells from animals or patients to confirm the FcγRIIa crystallography studies have however not yet been performed.

From a genetic screen in a cohort of antibody deficiency patients we identified two patients with a homozygous FcγRIIa gene variant resulting in a Q27W change in the extracellular domain of FcγRIIa (NM 001136219.1). These two patients do not suffer from autoimmune diseases, suggesting a different mode of FcγRIIa immune deviation than IgG2-related disease-predisposition seen in FcγRIIa-131H patients. The FcγRIIa Q27W variant falls near the residues T23 and R30, which were shown to be important in receptor pairing within crystals and in their native context in membranes [29]. This FcγRIIa variant allowed us to study FcγRIIa signaling in human cells. We here describe functional consequences of the FcγRIIa Q27W variant in a model system. We show that the FcγRIIa Q27W variant yields reduced calcium mobilization and delayed mitogen-activated protein kinase (MAPK) phosphorylation.

2. Material and methods

2.1. Next generation sequence and patient selection

Next generation sequencing was performed as previously described [30]. In short: sample preparation was done using SOLiD compatible truncated adapters and barcoded primers. Enrichment was performed using the Agilent SurePrint G3 1M Custom CGH Microarray. Subsequently, emulsionPCR, bead enrichment and sequencing were performed according to the manufacturers' instructions using an AB SOLiD 5500XL sequencer (Applied Biosystems, Bleiswijk, The Netherlands). Sequencing reads were mapped against the hg19 reference genome using Burrows–Wheeler Aligner. The study was approved by the local institutional review board. All known and newly diagnosed CVID (-like) patients as well as multiple healthy controls were tested using NGS. At the time of writing this manuscript, 48 CVID or CVID-like patients were analyzed using NGS. CVID was diagnosed according to the European Society for Immunodeficiencies criteria. Several patients did not completely meet these CVID criteria and were therefore given a diagnosis of CVID-like disorder, which was defined as selective antibody deficiency (defined as the inability to produce protective titers of specific antibodies on vaccination to polysaccharide, to recall antigens, or both) combined with low IgA, low IgM, and/or low IgG subclass levels or a solitary decreased IgG level. These patients had recurrent infections and an inadequate response to prophylactic antibiotic treatment, which was defined by more than 4 breakthrough infections per year. Secondary immunodeficiencies (e.g., iatrogenic or caused by enteral protein loss) were ruled out in all patients. Previous studies have shown that pediatric patients with CVID-like disorders, as described above, are clinically and immunologically (e.g., B- and T-cell phenotype characterization) comparable with patients with definite CVID [5].

2.2. FcγRIIa mRNA by real-time PCR

Primary human neutrophils, monocytes, B cells, BDCA1$^+$ and BDCA3$^+$ DCs were isolated from Q27W patient or healthy donor blood. PBMC were separated by ficoll isopaque density gradient centrifugation (GE Healthcare Bio-Sciences AB). Next, cells were labeled with anti-human antibodies (Ab) (CD1c, CD11c, CD14, CD19, CD141, HLA-DR) and sorted by the FACS aria II (BD Biosciences) into 5 mL tubes (Thermo).