Genetic Diagnostic Elucidation of a Patient With Multiorgan Granulomas, Facial Peculiarities, and Psychomotor Retardation

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We report the case of a 19-years-old patient who presented with a perplexing variety of symptoms which included remarkable facial features, intellectual disability, granulomatous upper lip swelling (previously diagnosed as Melkersson–Rosenthal syndrome), Crohn’s-like disease, non-productive cough, and a granulomatous mass localized in the left lung. Chronic granulomatous disease (CGD) was diagnosed using dihydrorhodamine 123 assay that showed low levels of phagocytic NADPH-oxidase. DNA sequencing revealed a heterozygous mutation in the NCF-1 gene on chromosome 7. As remarkable facial features and psychomotor retardation are not associated with CGD, a more detailed genetic work-up using fluorescence in situ hybridization was performed. A microdeletion in 7q11.23 on one allele indicated Williams–Beuren syndrome (WBS). The NCF-1 gene and its two pseudogenes are part of a highly repetitive region within 7q11.23 and are prone to recombination events and deletions. Such deletions can involve both the WBS critical region and the NCF-1 wildtype gene, as was the case for our patient. The second allele of the NCF-1 gene was affected by the frequent c.75.76delGT mutation that stems from a recombination of the NCF-1 wildtype gene with one of its pseudogenes. In conclusion, patients with NCF-1-deficient CGD may also harbor microdeletions that result in WBS or other hereditary disorders; therefore, it is important to perform a thorough genetic analysis in order to initiate appropriate therapy for these patients.

Keywords: chronic granulomatous disease, lip, swelling, Williams–Beuren syndrome, microdeletion, compound heterozygosity, NCF-1, 7q11.23

BACKGROUND

Chronic granulomatous disease is a hereditary disorder that disrupts neutrophil activity. The disease is characterized by recurrent infections and a myriad of inflammatory complications (Holland, 2010). CGD is caused by mutations in genes responsible for the superoxide-generating phagocyte NADPH oxidase. This results in the absence of, or very low levels of enzyme activity

Abbreviations: CGD, chronic granulomatous disease; CT, computed tomography; e.g., exempli gratia; IFN, interferon; LCR, locus control region; MRS, Melkersson–Rosenthal syndrome; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NCF, neutrophil cytosolic factor; SVAS, supravalvular aortic stenosis; WBS, Williams–Beuren syndrome.
in neutrophils, increasing susceptibility to infection and allowing for the proliferation of systemic granulomatous disease. The incidence of CGD is rare with 1 in 200,000 live births (Heyworth et al., 2003). More than two-thirds of all cases are linked to defects in the CYBB gene acquired in an X-linked recessive manner. The remaining cases are autosomal recessive inherited diseases caused by defects in CYBA, NCF-1, and NCF-2 genes (Heyworth et al., 2003).

Williams–Beuren syndrome is a segmental aneusomy syndrome which results from a heterozygous deletion of numerous genes within the 7q11.23 region (Bayés et al., 2003). The phenotype of this syndrome includes growth retardation, facial dysmorphies, heart abnormalities, hyperacusis, infantile hypercalcemia, and abnormal gait. Cardiac abnormalities typically involve SVAS and peripheral pulmonary stenosis.
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FIGURE 4  |  Family pedigree: the patient (only child) is at the bottom. Paternal information is missing – father does not reside with the family. The NCF-1 gene was also heterozygous for the father. The mother’s sisters – marked with black dot – were both diagnosed with Crohn’s disease.

(Tassabehji, 2003). Patients have severe deficits in cognitive domains; personality is characterized by readiness to interact with strangers and overt friendliness. Approximately 70% exhibit attention deficit disorder (Tassabehji, 2003).

CASE PRESENTATION

A 19-years-old patient was transferred to our pediatric hospital for further diagnostic analysis from a clinic specialized in adult lung diseases. The afebrile patient suffered from a dry, non-productive cough. An intrathoracic inflammatory granulomatous mass was localized by radiogram and computer tomography (Figure 1). Serum levels of soluble interleukin 2 receptor, neopterin, and angiotensin converting enzyme were negative. Bronchoalveolar lavage fluid showed lymphocytosis. Transbronchial biopsy revealed chronic, partly granulomatous inflammation with no signs of necrosis (Figure 2). No mycobacteria were found and the results were not typical for sarcoidosis. Steroid and antibiotic (cotrimoxazol) therapy resulted in rapid resolution of the intrathoracic mass.

The patient’s past medical history included the diagnosis of a learning disability (patient currently attends special-needs school). Phenotypical dysmorphic facies had been noted including broad forehead, bi-temporal narrowing, strabismus, long philtrum, wide mouth, and large ear lobes (Figure 3). No further assessment of the patient’s cognitive abilities had been performed; however, clinically a mild intellectual disability was observed. MRS (OMIM 155900) and Crohn’s disease (OMIM 266600) had previously been diagnosed. MRS was histologically diagnosed at the age of 10 when the patient presented with swelling of the upper lip and granulomatous inflammation. However, typical features, such as lingua plicata or facialis paresis were not noted (Ang and Jones, 2002). Intense therapy with dapsone, infliximab, azathioprine, and steroids led only to intermittent improvement. Crohn’s disease was diagnosed by screening at the age of 17, as two aunts had previously been diagnosed with Crohn’s disease (Figure 4). The patient had neither diarrhea nor significant gastrointestinal complaints. Colonoscopic biopsies revealed epithelial cell granulomas compatible with Crohn’s disease (see Table 1).

A multisystem granulomatous disease was suspected; therefore, the patient was tested for CGD using a dihydrorhodamine 123 (DHR) assay. This assay revealed a considerable decrease in NADPH-oxidase activity. Subsequent Sanger sequencing identified the most common mutation within the NCF-1 gene (OMIM 608512: c.75.76delGT; p.Tyr26HisfsX26, chromosome 7, 7q11.23). The mutation was confirmed as heterozygous for the father, but surprisingly not for the mother. Unfortunately, symptomatic data for the father are unknown, as he does not reside with the family (Figure 4). The patient was administered the recommended prophylactic therapy of cotrimoxazol and itraconazol.

Nevertheless, neither learning disability nor facial dysmorphism are associated with CGD. The dysmorphism raised suspicion of WBS despite the absence of cardiac symptoms. Upon further genetic analysis, fluorescence in situ hybridization revealed microdeletions within 7q11.23 [46,XY.ish del(7)(q11.23q11.23)(ELN-)](OMIM 194050) with locus specific deletions; this confirmed the diagnosis of WBS.

DISCUSSION

The patient presented with granuloma formation in three different sites: lung, colon, and upper lip. This led to the differential diagnosis of CGD confirmed by DHR assay and NCF-1 gene analysis. The clinical course of our patient differed from most CGD patients, due to the fact that NCF-1- (p47phox-) deficient CGD is generally associated with residual NADPH activity. This may explain why our patient had no history of life-threatening infection even while under immunosuppressive therapy aimed at treating previously suspected MRS. However, although NCF-1-deficient CGD generally follows a milder course than CGD lacking residual activity, some infectious manifestations can be equally as severe and life threatening as those contracted during the course of classical CGD, e.g., invasive aspergillus infections of the lungs (Heyworth et al., 2003).

The diagnosis of CGD alone did not account for all of our patient’s symptoms; instead, some underlying features such as facial dysmorphism and cognitive disability pointed toward WBS. We therefore expanded the genetic analysis and were able to...
confirm WBS. Our patient could then be treated appropriately for this disorder; treatment includes physical, developmental, and speech therapy (Morris, 1999; Bayés et al., 2003; Tassabehji, 2003). WBS usually occurs sporadically and is the result of heterozygous deletions of contiguous genes located close to the gene locus of CGD at 7q11.23. In the majority of patients, approximately 30 genes are missing (Bayés et al., 2003; Heyworth et al., 2003).

The region depicted in Figure 5 is highly repetitive already in non-human primates – compared to other mammals – and even more so in humans. Three large region-specific segmental duplications (centromeric, medial, and telomeric LCRs) with genetic variations have been identified. Each segmental duplication is composed of three differentiated blocks designated as “A,” “B,” and “C” (Bayés et al., 2003; Heyworth et al., 2003). Several genes and their unprocessed highly similar pseudogenes such as NCF-1 are located centromeric and telomeric to the critical WBS region (see Merling et al., 2016). Depending on gene arrangement, several disorders may

TABLE 1 | Differential diagnosis of CGD and fundamental differences to other diagnoses.

| Diagnosis                        | Site of symptoms | Recurrent infections | Pathogen spectrum | Impaired neutrophil respiratory burst | Other findings in contrast to CGD |
|----------------------------------|------------------|----------------------|-------------------|--------------------------------------|----------------------------------|
| Chronic granulomatous Disease    | Systemic         | Yes                  | Opportune         | Yes                                  | –                                 |
| Cystic fibrosis                  | Lung (infections)| Yes                  | Opportune         | No                                   | Infection limited to lung, bronchiectasis |
| Hyperimmunoglobulin E syndrome   | Lung (infections)| Yes                  | Staphylococci     | No                                   | Characteristic facies, elevated IgE |
| G6PD deficiency                  | Systemic         | Yes                  | Bacterial         | Yes                                  | Hemolytic anemia                  |
| Glutathione synthetase deficiency| Systemic         | Yes                  | Bacterial         | Yes                                  | Hemolytic anemia, acidosis, ment. retard. |
| Crohn’s disease                  | Bowel            | No                   | –                 | No                                   | CGD has no extraintestinal symptoms |
CONCLUSION
It is important to be aware of possible clinical indications suggestive of WBS in patients with NCF-1-deficient CGD. The identification of disease causing mutations using in-depth genetic analysis will facilitate the initiation of appropriate therapy for patients who are afflicted by these and other hereditary disorders.

ETHICS STATEMENT
Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editor of this journal. The ethic committee approval has been obtained by institutional ethic committee of the medical faculty of the University Duisburg-Essen under file number 18-8226-BO and a copy of the approval is available for review by the editor of this journal.

AUTHOR CONTRIBUTIONS
DS made substantial contributions to conception and design and involved in drafting the manuscript. AK and JR made substantial contributions to the acquisition, analysis, and interpretation of the data. LP, ME, and MO involved in critically revising the manuscript for important intellectual content, and gave final approval of the version to be published.

FUNDING
All licenses to use were bought by Universitätsklinikum Essen.

ACKNOWLEDGMENTS
We would like to thank the patient and his family for allowing us to publish this case. We would also like to thank Mrs. Klco-Brosius for the English corrections made in this article.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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