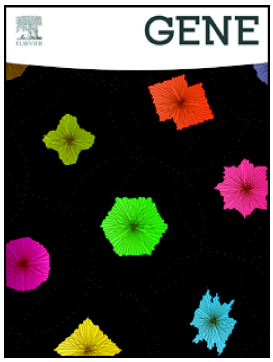

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Identification of Co-Occurrence in a patient with Dent's Disease and ADA2-deficiency by exome sequencing

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Keywords: exome sequencing, Dent's disease, ADA2-deficiency, co-occurrence

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Abstract

Patients with co-occurrence of two independent pathologies pose a challenge for clinicians as the phenotype often presents as an unclear syndrome. In these cases, exome sequencing serves as a powerful instrument to determine the underlying genetic causes.

Here, we present the case of a 4-year old boy with proteinuria, microhematuria, hypercalciuria, nephrocalcinosis, livedo-like rash, recurrent abdominal pain, anemia and continuously elevated CRP. Single exome sequencing revealed the pathogenic nonsense mutation p.(Arg98*) in the *CLCN5* gene causing the X-linked inherited, renal tubular disorder Dent's Disease. Furthermore, the two pathogenic and compound heterozygous missense variants p.(Gly47Ala) and p.(Pro251Leu) in the *CECR1* gene could be identified. Mutations in the *CECR1* gene are associated with a hereditary form of polyarteritis nodosa, called ADA2-deficiency. Both parents were carriers of a single heterozygous variant in *CECR1* and the mother was carrier of the *CLCN5* variant.

This case evidently demonstrates the advantage of whole exome sequencing compared to single gene testing as the pathology in the *CECR1* gene might have only been diagnosed after the occurrence of signs of systemic vasculitis like strokes or hemorrhages. Therefore, treatment and prevention can now start early to improve the outcome of these patients.

1. Introduction

In the last couple of years exome sequencing (ES) evolved from an exclusively research-related method to a diagnostic tool used in every-day clinical practice. Especially in pediatrics, it is often useful when trying to unravel a genetic cause for an unclear syndrome. Additionally, it can save patients from a vast number of invasive diagnostic procedures and therapy can be initiated early.

While ES has become an important instrument in diagnosing neurologic and metabolic diseases, the applications for the diagnosis of rheumatological diseases are still in early stages (Wiley et al., 2014). *CECR1* is one example of a gene that is thought to be associated with a hereditary form of polyarteritis nodosa (PAN) (Navon Elkan et al., 2014). It encodes the enzyme adenosine deaminase 2 (ADA2) and patients usually present in childhood with livedo reticularis which is a typical cutaneous manifestation of vasculitis and vasculopathies. Other clinical and laboratory features described with ADA2-deficiency are visceral involvement most commonly with gastrointestinal symptoms, followed by renal hypertension, renal proteinuria and hematuria. Neurologic symptoms affect the peripheral nervous system more often than the central nervous system. Cerebrovascular events like haemorrhages and ischemic strokes have been described. Fever, myalgia or arthralgia, elevated CRP and elevated transaminases are typical signs of this necrotizing, predominantly medium-sized arteries targeting vasculitis (Nanthapisal et al., 2016), (Zhou et al., 2014). Therapeutic options include immunosuppressive drugs like corticosteroids combined with intravenous cyclophosphamide or mycophenolate mofetil for childhood PAN, azathioprine, rituximab, and TNFα-antagonists. So far, there are about 20 families with a *CECR1*-associated ADA2-deficiency described in the literature (Navon Elkan et al., 2014), (Nanthapisal et al., 2016), (Zhou et al., 2014).

Dent's Disease is a X-linked recessive inherited, renal tubular disorder that is caused by mutations in *CLCN5* or *OCRL1* (Hoopes et al., 2005), (Lloyd et al., 1996). Patients usually present during childhood and findings mainly include asymptomatic low molecular weight proteinuria, hypercalciuria, hematuria, and nephrocalcinosis (Devuyst and Thakker, 2010). In 60% of the cases the molecular cause for the disease is the absence or alteration of a chloride channel that is encoded by *CLCN5*. Medullary nephrocalcinosis is a prominent feature in Dent's disease and plays a critical role in renal failure. Progression to end-stage renal disease occurs in 30-60% of patients, mostly in the fourth decade of life. Hypercalciuria can be treated with thiazides. Angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers may delay the development of end-stage renal disease. However, patients

with Dent disease are underdiagnosed due to the wide spectrum of clinical presentation (Copelovitch et al., 2007; Devuyst and Thakker, 2010).

2. Case report

A four ½ year old boy of German origin was referred for further follow-up after a screening test had revealed microscopic hematuria and proteinuria. The parents reported recurrent episodes of abdominal pain and the presence of a bilateral rash at both lower legs, which were first noticed nine months prior to admission.

Clinical examination revealed a height at the third percentile, a body weight at the tenth percentile, and a head circumference at the twenty-fifth percentile. The neurologic development was unremarkable. At both shins, mild livedo-like rash was observed (Fig. 1a). Laboratory examinations showed a mixed proteinuria (glomerular and tubular origin; 727mg/m²/d), microscopic hematuria, hypercalciuria, elevated CRP (max. 16 mg/dl), and microcytic hypochromic anemia due to iron deficiency (ferritin 36 µg/l). The search for a focus of infection (low-dose PET/CT) did not reveal signs of a neoplastic condition or focal inflammation. A kidney biopsy suggested thin basement membrane nephropathy. One arteria arcuata was visible in the renal biopsy and did not show specific changes. Endoscopy of the esophagus, stomach and intestines was unremarkable. Biopsy samples of the gastrointestinal tract and the skin did not show signs of vasculitis. As the thorough clinical examination did not lead to a definite diagnosis, whole-exome sequencing was performed.

Both parents were tested negative for hematuria and proteinuria. The family history was unremarkable.

3. Material and methods

Blood samples from the patient and his parents were collected after written informed consent. Exome sequencing was only performed in the patient. DNA fragments were enriched using the SureSelect Human All Exon Kit (Agilent, 50Mb V5). The prepared libraries were subsequently sequenced as 100 bp paired end reads on a HiSeq2500 (Illumina) to an average 182-fold coverage with >98% of target sequences being covered >20-fold. Rare variants and their allele frequency were detected using an in-house database containing almost 9,000 exomes, 1000Genomes, dbSNP, and the Exome Aggregation Consortium (ExAc)-Browser. Pathogenicity of the variants was determined by searching the databases ‘Online Mendelian Inheritance in Man’ (OMIM), ‘Human Gene Mutation Database’ (HGMD), MutationTaster,

HomoloGene, and ClinVar. Furthermore, the exome sequencing also included analysis of copy number variations (CNVs) and mtDNA. CNV analysis was performed with ExomeDepth and custom pearl scripts. CNVs with a size larger than 100kb were considered for further evaluation which were not identified in the present case. mtDNA variants were filtered for nonsynonymous variants with a heteroplasmy >50% and MITOMAP frequency of less than 0.1%. No variants passed these filtering steps.

After identification of variants in *CLCN5* and *CECR1* in the index patient we performed confirmation and segregation analysis in the parents by using Sanger sequencing. Primer sequences are available upon request.

4. Results

Exome sequencing was performed and a search for homozygous, compound heterozygous and hemizygous variants with a minor allele frequency (MAF) of less than 0.1% identified variants in 13 genes. Of these genes only variants in *CLCN5*, *CACNA1F,* and *CECR1* were associated with mendelian disorders. Hemizygous mutations in *CACNA1F* cause X-linked cone-rod dystrophy. As the variant is predicted to be tolerated in silico and there were no clinical signs of visual deficits we did not consider this variant as disease causing. The variants in *CLCN5* (NM_001127899.3) and *CECR1* (NM_017424.2), however were reported as pathogenic in the literature.

In *CLCN5*, the previously described hemizygous nonsense mutation c.292C>T, p.(Arg98*) was identified (Fig. 1b) (Lek et al., 2016). This mutation has already been associated with the X-chromosomal inherited Dent's disease (OMIM: #300009) (Hoopes et al., 1998).

Additionally, in the *CECR1* gene the compound heterozygous missense mutations c.140G>C, p.(Gly47Ala) and c.752C>T p.(Pro251Leu) have been identified (Fig. 1c/d). Mutations in this gene have previously been reported causing autosomal recessive inherited childhood onset polyarteritis nodosa (OMIM #615688) (Zhou et al., 2014), (Nanthapisal et al., 2016). Both mutations could only be found in heterozygous state in our in-house- and the ExAC database.

Sanger sequencing confirmed all identified mutations. Segregation analysis showed compound heterozygosity for the mutation in *CECR1* (Fig. 1c/d) and the patient's mother as heterozygous carrier for the mutation in *CLCN5* (Fig. 1b). According to the guidelines of the ACMG the variants detected in both genes were classified as pathogenic (Richards et al., 2015).

5. Discussion

Our case highlights the valuable diagnostic benefit of early ES for patients with complex unclear diseases.

The 4-year-old patient presented with glomerular and tubular proteinuria, hematuria and hypercalciuria, recurrent abdominal pain, anemia due to iron deficiency and livedo reticularis. Comprehensive clinical investigations were unable to identify a singular specific diagnosis explaining all symptoms. As the kidney biopsy suggested the presence of thin basement membrane nephropathy, genetic testing was initiated. Mutations in two genes could be identified that explained almost all symptoms of the patient. The tubular proteinuria, hematuria as well as the hypercalciuria are caused by the deleterious hemizygous *CLCN5* variant that is responsible for Dent's disease (Hoopes et al., 1998). The glomerular proteinuria, cutaneous rash, abdominal pain and the elevated CRP are a result of the ADA2-deficiency due to *CECR1* mutations causing polyarteritis nodosa in childhood (Zhou et al., 2014; Nanthapisal et al., 2016). Anemia due to iron deficiency has not yet been described in either of the two diseases, although pancytopenia was previously reported in patients with ADA2-deficiency (Zhou et al., 2014). Since Dent's disease is X-chromosomal inherited and ADA2-deficiency follows an autosomal-recessive inheritance, re-occurrence rates are 50 % for Dent's disease in male descendants and 25 % for ADA2-deficiency sex-independent.

At this point, we additionally want to emphasize the advantages of exome sequencing compared to a single gene approach. If the latter had been chosen, genetic diagnostics would likely have been stopped after the diagnosis of Dent's disease. The patient would then have been subjected to intensive – presumably also invasive – examinations if experiencing severe disease associated symptoms like ischemia and necrosis of the fingers and toes, coronary or mesenteric artery aneurysms and stenosis, strokes, intracranial hemorrhages or hepatopathy (Zhou et al., 2014). Our experience is that there are a significant number of patients with genetic co-pathologies in whom and only recently, a comprehensive study showed a co-occurrence of two pathologies in 5% of patients referred to exome sequencing (Posey et al., 2016).

Reviewing the literature describing patients with mutations in *CECR1* revealed genotype-phenotype correlation: Patients with the mutation p.(Gly47Ala) developed significant cerebral vasculopathy, resulting in ischemic or hemorrhagic strokes as well as hepatopathy with elevated transaminases (Zhou et al., 2014). However, patients with the substitution p.(Pro251Leu) did barely develop any described cerebrovascular pathologies (Nanthapisal et al., 2016). If the absence of the enzyme activity would be the only pathomechanism one

would expect a similar phenotypical presentation in all patients. As all studies have demonstrated a lack of ADA2 activity, this – and the fact that no patients with biallelic loss-of-function variants have been described yet – might imply that there is an additional function of the Adenosine Deaminase 2 protein.

Unfortunately, there is only a small number of families with a *CECR1* related vasculopathy and there are no reports concerning the successfulness of therapeutic attempts. Most of the patients were treated with immunosuppressive agents such as corticosteroids, cyclophosphamide, azathioprine or mycophenolate mofetil. TNF-α blockade, adalimumab and rituximab are additional therapeutical options possibly combined with acetylsalicylic acid (Nanthapisal et al., 2016)(Eleftheriou and Brogan, 2016). As most patients so far have only been diagnosed after the first irreversible cerebral defects, the early identification of the disease causing mutations in this patient might hopefully influence the progression of the condition by early administration of disease modifying agents.

The presented patient was treated with pulsed methylprednisolone, followed by prednisolone weaning over 6 months plus mycophenolate mofetil (1200 mg/m2/d). The inflammatory signs (elevated CRP) normalized within days and the gastrointestinal symptoms disappeared completely.

6. Conflict of interest

The authors declare no conflict of interest.

7. Acknowledgements

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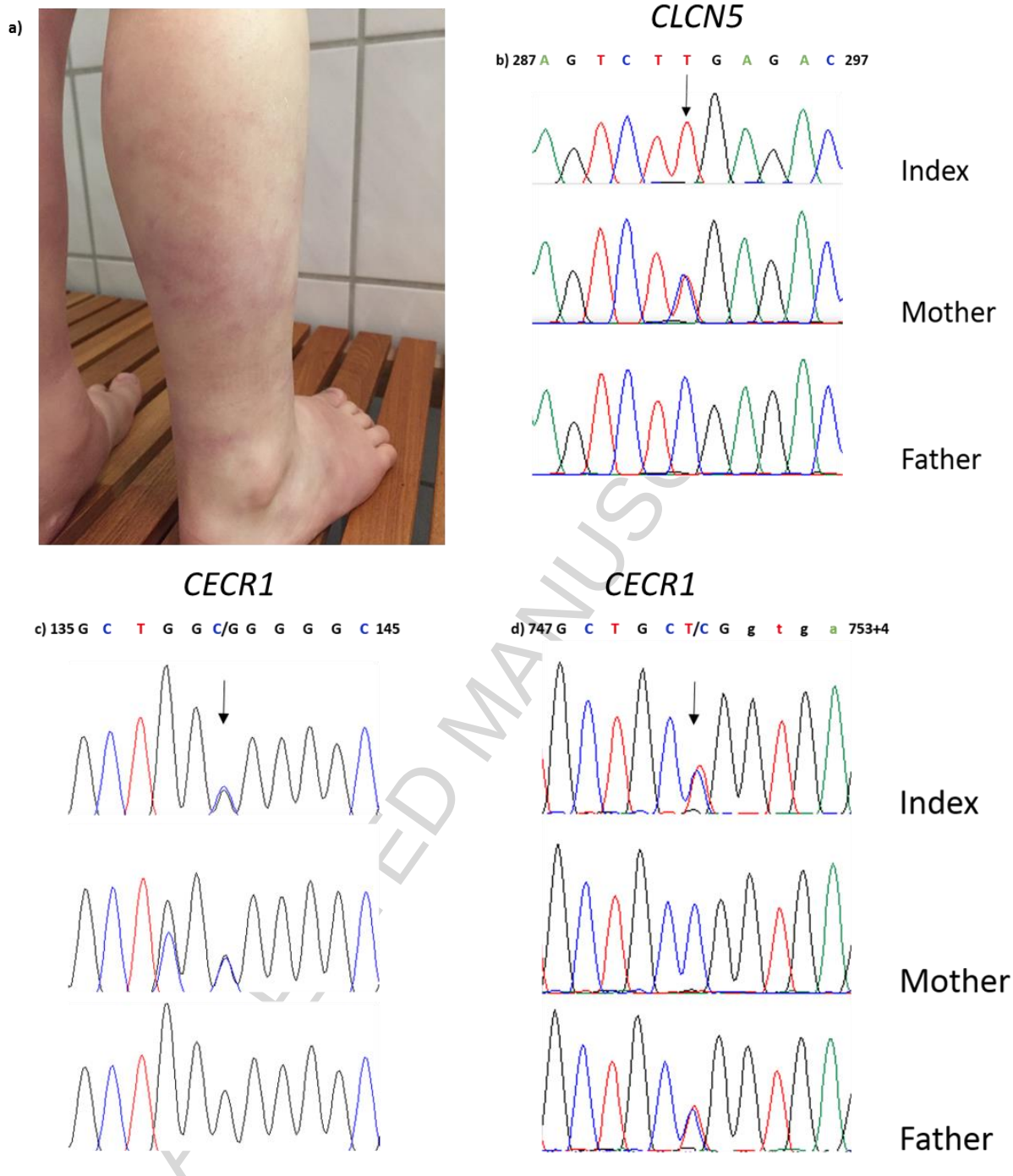
Figure 1



Legend to the figures

Fig. 1. a) Livedo reticularis rash of the 4-year old patient. **b)** Partial nucleotide sequence of exon 5 of the *CLCN5* gene from the patient showing the hemizygous mutation c.292C>T, p.(Arg98*). **c)** and **d)** Partial nucleotide sequence of exon 2 of the *CECR1* gene from the patient showing the heterozygous mutation c.140G>C, p.(Gly47Ala) and of exon 4 showing the heterozygous mutation c.752C>T p.(Pro251Leu). Note, that the mother is also a heterozygous carrier of the common polymorphism rs7289141.

Abbreviations list:

ACE	Angiotensin-converting enzyme
ADA2	Adenosine deaminase 2
CECR1	Cat Eye Syndrome Chromosome Region, Candidate 1
CLCN5	Chloride Voltage-Gated Channel 5
CRP	C-reactive Protein
ES	Exome sequencing
OCRL1	Oculocerebrorenal Syndrome Of Lowe
OMIM	Online Mendelian Inheritance in Man
PAN	Polyarteritis nodosa
TNFα	Tumor necrosis factor α

Highlights

- A young patient presented with an renal-inflammatory phenotype
- Two independent genetic pathologies were detected by exome sequencing
- Exome sequencing is a powerful method for diagnosis of co-pathologies