Case Report

**Discovering Genotype Variants in an Infant with VACTERL through Clinical Exome Sequencing: A Support for Personalized Risk Assessment and Disease Prevention**

Gloria Pelizzo ¹,2,*, Luigi Chiricosta ³,†, Emanuela Mazzon ³, Gian Vincenzo Zuccotti ²,4, Maria Antonietta Avanzini ⁵, Stefania Croce ⁵, Mario Lima ⁶, Placidio Bramanti ³ and Valeria Calcaterra ⁴,7

1 Pediatric Surgery Unit, Ospedale dei Bambini “Vittore Buzzi”, 20154 Milano, Italy
2 Department of Biomedical and Clinical Science “L. Sacco”, University of Milano, 20157 Milano, Italy; gianvincenzo.zuccotti@unimi.it
3 IRCCS Centro Neurolesi “Bonino-Pulejo”, 98124 Messina, Italy; luigi.chiricosta@irccsme.it (L.C.); emanuela.mazzon@irccsme.it (E.M.); placido.bramanti@irccsme.it (P.B.)
4 Department of Pediatrics, Ospedale dei Bambini “Vittore Buzzi”, 20154 Milano, Italy; valeria.calcaterra@unipv.it
5 Immunology and Transplantation Laboratory, Cell Factory, Pediatric Hematology Oncology Unit, Department of Maternal and Children’s Health, Fondazione IRCCS Policlinico S. Matteo, 27100 Pavia, Italy; ma.avanzini@smatteo.pv.it (M.A.A.); stefania_croce186@yahoo.it (S.C.)
6 Pediatric Surgery Unit, S. Orsola Hospital, University of Bologna, 40138 Bologna, Italy; mario.lima@unibo.it
7 Pediatrics and Adolescnetology Unit, Department of Internal Medicine, University of Pavia, 27100 Pavia, Italy
* Correspondence: gloriapelizzo@gmail.com
† These authors equally contributed to this work.

Abstract: Congenital anomalies may have an increased risk of noncommunicable diseases (NCDs) We performed a clinical exome analysis in an infant affected by “Vertebral, Anorectal, Cardiac, Tracheoesophageal, Genitourinary, and Limb” (VACTERL) malformation association to identify potential biomarkers that may be helpful for preventing malignancy risk or other chronic processes. Among the variants, six variants that may be linked with VACTERL were identified in the exome analysis. The variants c.501G>C on OLRI and c.-8C>G on PSMA6 were previously associated with myocardial infarction. The variants c.1936A>G on AKAP10 and c.575A>G on PON1 are linked to defects in cardiac conduction and artery disease, respectively. Alterations in metabolism were also suggested by the variants c.860G>A on EPHX2 and c.214C>A on GHRIL. In addition, three variants associated with colon cancer were discovered. Specifically, the reported variants were c.723G>A on CCN1 and c.-91T>A on AURKA proto-oncogenes as well as c.827A>C in the tumor suppressor PTTR. A further inspection identified 15 rare variants carried by cancer genes. Specifically, these mutations are located on five tumor suppressors (SDHA, RB1CC1, PTCH1, DMRT1, BCR) and eight proto-oncogenes (MERTK, CSF1R, MYB, ROS1, PCMI, FGFR2, MYH11, BRCC3) and have an allele frequency lower than 0.01 in the Genome Aggregation Database (GnomAD). We observed that the cardiac and metabolic phenotypic traits are linked with the genotype of the patient. In addition, the risk of developing neoplasia cannot be excluded a priori. Long-term surgical issues of patients with VATER syndrome could benefit from the clinical exome sequencing of a personalized risk assessment for the appearance of further disease in pubertal timing and adult age.

Keywords: VACTERL; exome sequencing; infant; healthcare; personalized; prevention; risk

1. Introduction

“Vertebral, Anorectal, Cardiac, Tracheoesophageal, Genitourinary, and Limb” (VACTERL/VATER) malformation association is a rare condition characterized by the presence of at least three of the following congenital malformations: vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula, renal anomalies, and limb abnormalities [1,2].
Other congenital anomalies (Cas) may also be present, including airway malformations. No major genetic risk factors are known to be involved in the etiology of VACTERL, and multifactorial pathogenesis has been proposed [1,2]. The management of patients with VACTERL association can be complex and can result in longer-term sequelae [1,2].

Cas represent one of the main causes of fetal death, infant mortality and morbidity, and long-term complications. Although the pathogenesis of Cas is still unknown, complex interactions between genes and the environment have been proposed. This multifactorial interaction modifies the normal embryo-fetal development, especially during the organogenesis phase [3]. The organism will retain memories of the insult immediately, and the adaptive response may result in pathology later on [4,5]. In particular, an increased risk of noncommunicable diseases (NCDs), including type 2 diabetes, cardiovascular disorders, and cancer, is also reported during childhood [4,5]. In patients with multiple congenital malformations, such as VACTERL, the risk of NDCs in pediatric and adult age is not defined but could be considered.

Given the recent advances in scRNAseq, its application in human diseases may enable a better understanding of pathological processes [4]. The identification of the gene expression patterns may be useful to predict the risk of developing chronic diseases and personalizing their prevention and treatment [4–8].

We performed a clinical exome analysis in an infant affected by the VACTERL association to identify potential biomarkers helpful for early detection of risk malignancy and chronic degenerative processes. A potential personalized prevention strategy could also allow personalized treatment.

2. Case Report

The patient was a six-month-old girl, born to Chinese nonconsanguineous parents. Diabetes and exposure to other environmental factors during pregnancy were not recorded. No previous pregnancies with congenital malformations were noted, and family history was also unremarkable. She was diagnosed with VACTERL association due to the presence of an imperforated anus with rectovestibular fistula, sacral vertebral anomalies and coccygeal agenesis, vesicoureteral reflux, and complex cardiovascular anomalies (double-outlet right ventricle and subaortic stenosis associated with ventricular septal defect). Cardiosurgery was performed at the age of four months. At six months, the baby was readmitted for treatment of the anorectal anomaly. Before general anesthesia induction for anorectal malformation repair, a severe and rare long-segment congenital tracheal stenosis was detected, and a slide tracheoplasty was subsequently performed. The postoperative course was uneventful.

The parents of the patient provided informed consent for genetic testing and publication. We conducted an analysis of the clinical exome of the patient in triplicate. The gene panel TruSight One was retrieved by Illumina and sequenced on the MiSeq platform as paired-ends using reads 150 nucleotides in length. The final variant call format (VCF) included 4240 variants. All of the found variants passed all the filters. To be included in the final VCF, each variant had to be called at least two out of three times in the analysis. All the variants were associated with the up-to-date version of dbSNP (151) and ClinVar (at 16/07/2020). Among them, we found two heterozygous variants described as pathogenic (rs1566734, rs72474224), one heterozygous pathogenic/risk factor (rs696217), three homozygous (rs662, rs9344, rs2273535), and four heterozygous risk factors (rs751141, rs11053646, rs1048990, rs203462) (Table 1). We highlighted the sequence window of the variants in Figure 1.
Table 1. Pathogenic and risk factor variants included in the clinical exome of the patient.

| Chromosome | Gene   | Position | Rs ID   | Reference | Alternative | Variant Type   | Consequence     | HGVS.c | HGVS.p     | Condition                                                                 | Clinical Significance               |
|------------|--------|----------|---------|-----------|-------------|----------------|-----------------|---------|-------------|----------------------------------------------------------------------------|---------------------------------------|
| chr3       | GHRL   | 10331457 | rs696217| G         | T           | SNP HET        | Missense variant | c.214C>A | p.Leu72Met  | Metabolic syndrome, susceptibility to obesity, age at onset of                | Pathogenic, risk factor               |
| chr11      | PTPRJ  | 48415375 | rs1566734| A         | C           | SNP HET        | Missense variant | c.827A>C | p.Gln276Pro | Carcinoma of colon                                                           | Pathogenic                            |
| chr7       | PON1   | 94937446 | rs662   | T         | C           | SNP HOM        | Missense variant | c.575A>G | p.Gln192Arg | Enzyme activity finding coronary artery disease, susceptibility to (Coronary artery spasm 2, susceptibility to) | Risk factor                            |
| chr11      | CCND1  | 69462910 | rs9344  | G         | A           | SNP HOM        | Splice region variant and synonymous variant | c.723G>A | p.Pro241Pro | Von Hippel–Lindau syndrome, modifier of colorectal cancer, susceptibility to multiple myeloma, translocation 11,14 type | Risk factor                            |
| chr20      | AURKA  | 5486451  | rs2273535| A         | T           | SNP HOM        | Missense variant | c.91T>A  | p.Phe31Ile  | Colon cancer, susceptibility to multiple myeloma                            | Risk factor                            |
| chr8       | EPHX2  | 2703886  | rs751141| G         | A           | SNP HET        | Missense variant | c.860G>A | p.Arg287Gln | Familial hypercholesterolemia 1                                             | Risk factor                            |
| chr12      | OLR1   | 10313448 | rs1053646| C         | G           | SNP HET        | Missense variant | c.501G>C | p.Lys167Asn | Myocardial infarction                                                       | Risk factor                            |
| chr14      | PSMA6  | 35761675 | rs1048990| C         | G           | SNP HET        | 5 prime UTR premature start codon gain variant | c.-8C>G  | .           | Myocardial infarction                                                       | Risk factor                            |
| chr17      | AKAP10 | 19812541 | rs203462| T         | C           | SNP HET        | Missense variant | c.1936A>G | p.Ile646Val  | Cardiac conduction defect, susceptibility to                               | Risk factor                            |

The dot in HGVS.p column stands for no codon change caused by the variant substitution.
The bases from position-10 to position+10 are represented for each variant, and the genomic changes are highlighted in red.

**Figure 1.** Genomic sequence representation of the variants identified as pathogenic or a risk factor in the clinical exome. The bases from position-10 to position+10 are represented for each variant, and the genomic changes are highlighted in red.

Moreover, 2279 variants were associated with no clinical phenotype, and no variant was associated with VACTERL disease. In order to investigate the analysis in depth, we used the keyword “VACTERL” (Disease/Phenotype) on ClinVar to retrieve all the genes associated with the disease. The result showed 98 items on 8 genes (BAZ1A, FANCB, FANCL, HOXD13, PTEN, KLLN, SALL1, ZIC3). The only variant in our analysis affecting these genes is rs4614723, carried out by SALL1 but defined as benign. Given the predisposition of the patients suffering from VACTERL to develop pancreatic and esophageal cancers, we also inspected the genes associated with the keyword “pancreatic cancer” (Disease/Phenotype) and “esophageal cancer” (Disease/Phenotype) in ClinVar. The queries returned 250 items on six genes for pancreatic cancer and one item on a gene for esophageal cancer. The only genes having variants associated with the patient were BRCA2 and PALLD for pancreatic cancer, but they are clinically identified as benign. Furthermore, we extracted a list of proto-oncogenes and tumor suppressor genes from UniProtKB using the filters “Human” and “Reviewed” and selected only the variants carried on these genes. We then enriched the VCF file using ANNOVAR [9] to obtain its frequency for each variant. We identified nine rare variants on eight proto-oncogenes (MERTK, CSF1R, MYB, ROS1, PCM1, FGFR2, MYH11, BRCC3) and six rare variants on five tumor suppressors (SDHA, RB1CC1, PTCH1, DMBT1, BCR), Figure 2. Specifically, the allele frequency of these rare variants is lower than 0.01 in the Genome Aggregation Database (GnomAD) [10] and not reported in ClinVar as benign in the supported publications (Table 2). None of these genes were already associated with VACTERL in the literature.
Table 2. Rare variants associated with cancer genes, of which the allele frequency in the Genome Aggregation Database (GnomAD) is < 0.01.

| Chromosome | Gene | Position | Rs ID   | Reference | Alternative | Variant Type | Consequence                     | HGVS.c            | HGVS.p            |
|------------|------|----------|---------|-----------|-------------|--------------|---------------------------------|-------------------|-------------------|
| chr2       | MERTK| 112766060| rs112541306 | C         | T           | SNP HET     | Splice region variant and intron variant | c.1960+8C>T       |                   |
| chr5       | CSF1R| 149456893| rs3829986 | C         | T           | SNP HET     | Missense variant               | c.835G>A          | p.Val279Met       |
| chr5       | SDHA | 256483   | rs112307877| CTT        | C           | DEL HET     | Frameshift variant             | c.1945            | 1946delTT         |
| chr6       | MYB  | 135518255| rs182817536| C         | T           | SNP HET     | Missense variant               | c.1360C>T         | p.Arg454Cys       |
| chr6       | ROS1 | 117717348| rs79119625 | T         | C           | SNP HET     | Splice region variant and intron variant | c.856+3A>G       |                   |
| chr8       | PCM1 | 17796382 | rs754721723| AC        | GT          | MNP HET     | Missense variant               | c.476_477delACinsGT | p.Asn159Ser       |
| chr8       | RB1CC1| 53543090 | rs770160515| TA        | T           | DEL HET     | Splice region variant and intron variant | c.4441-3delT     |                   |
| chr8       | RB1CC1| 53586746 | rs77653001 | C         | T           | SNP HET     | Missense variant               | c.661G>A          | p.Asp221Asn       |
| chr9       | PTCH1| 98270531 | rs143494325| C         | A           | SNP HET     | Missense variant               | c.113G>T          |                   |
| chr10      | DMBT1| 124329672| rs34118835 | TC        | CT          | MNP HOM     | Splice region variant and intron variant | c.92-6_92-5delTCinsCT |                   |
| chr10      | FGFR2| 123274705| rs772986332| T         | C           | SNP HET     | Missense variant               | c.1216A>G         | p.Lys406Glu       |
| chr16      | MYH11| 15808856 | rs79129097 | T         | C           | SNP HET     | Missense variant               | c.5717A>G         | p.Asn1906Ser      |
| chr16      | MYH11| 15831299 | rs201955317| C         | T           | SNP HET     | Splice region variant and intron variant | c.3314+7C>G      |                   |
| chr22      | BCR  | 23654017 | rs879255379| G         | A           | SNP HET     | Missense variant               | c.3316G>A         | p.Asp1106Asn      |
| chrX       | BRCC3| 154348267| rs2290069  | A         | G           | SNP HET     | Splice region variant and intron variant | c.800-7A>G       |                   |

The dot in HGVS.p column stands for no codon change caused by the variant substitution.
**Figure 2.** Genomic sequence representation of the rare variants identified as tumor suppressors or proto-oncogenes in the clinical exome. The bases from position-10 to position+10 are represented for each variant, and the genomic changes are highlighted in red.

### 3. Discussion

Structural birth defects occur in approximately 3% to 6% of all live births [11]. Most structural birth defects develop early in embryogenesis during the first 10 weeks of pregnancy [11], and the vast majority of birth defects are “nonchromosomal anomalies” characterized, as in VACTERL association, by multiorgan involvement. The mechanisms by which environmental or genetic insults disrupt fetal development are not fully understood. However, there is no doubt that an adverse environment in utero leads to permanent changes in the structure of organs and systems, which have a key role in “fetal programming.” Fetal adaptation is responsible for an increased risk of NCDs and other chronic diseases, such as obesity, which, itself, is a major risk factor for NCDs throughout the life-course [3,5].

In this sense, NGS analysis may be useful to discover genetic aberration and support clinicians, providing a link to the disease.

The clinical exome analysis of infants with VACTERL performed in our study highlighted several genetic variants (Figure 1).

The polymorphism c.501G>C on *OLR1* gene encodes for oxidized low-density lipoprotein receptor 1, and the c.-8c>G on *PSMA6* gene encodes for proteasome subunit alpha type-6. These two heterozygous variants are associated with myocardial infarction in ClinVar. Specifically, c.501G>C is a missense variant that causes a change in the protein sequence of oxidized low-density lipoprotein receptor 1 and may result in reduced interaction with ligands. Tatsuguchi et al. observed a group of patients suffering from myocardial infarction and suggested that this variant promotes atherogenesis and coronary artery...
disease [12]. On the other hand, several studies were performed on the 5′ UTR variant c.-8c>G, leading to a premature start.

In a Japanese population, Ozaki et al. found a significant association of this variant with myocardial infarction pathogenesis via the activation of inflammatory processes [13], and Hinoara et al. proposed a modest risk factor of the variant in coronary artery disease [14].

In addition, the heterozygous variant c.1936A>G on the AKAP10 gene, which encodes for A-kinase anchoring protein 10, is associated with susceptibility to cardiac conduction defect, and the homozygous variant c.575A>G on PON1, which encodes for serum paraoxonase and arylersterase 1, is reported with susceptibility to coronary artery spasm and artery disease.

Krammer et al. found a strong correlation with c.1936A>G and aging, which, interestingly, seems to alter heart functionality. Indeed, the PR interval of the electrocardiogram is reduced in subjects that carry the variant. In an in vitro experiment, the authors observed a change in the ability of A-kinase anchoring protein 10 to bind the isoform of protein kinase A (PKA-R,α), altering the signal mediated by cAMP [15]. Ito et al. suggested that oxidative stress may be not properly suppressed when c.575A>G polymorphism occurs, facilitating the genesis of coronary spasm [16].

In line with these considerations, it is interesting to observe the heterozygous variants c.860G>A on EPHX2 and c.214C>A on GHRL genes that expose the patients of this study to metabolic syndrome and familial hypercholesterolemia 1, respectively. Specifically, EPHX2 encodes for the soluble epoxidase hydrolase with lipid-phosphate phosphatase activity that regulates cardiovascular functions. Interestingly, Fornage et al. found a twofold greater risk of developing coronary artery calcification in young people carrying c.860G>A [17]. Moreover, Othoshi et al. suggested that this variant may lead to insulin resistance in the pathogenesis of type 2 diabetes [18]. GHRL encodes for the hormone ghrelin, which prepares food intake by the secretion of gastric acid and increases gastric motility [19]. Ghrelin regulates energy homeostasis, and Imaizumi et al. proposed this variant as a risk factor for obesity, leading to an increase in body mass index [20].

Childhood cancer risk in chromosomal anomalies has been described well, such as acute lymphoblastic leukemia in children with Down syndrome [21,22] or retinoblastoma in patients with chromosome 13q14 deletion syndrome [23]. More recently, Norwood et al. reported that any congenital anomaly, including nonchromosomal anomalies, was associated with an increased risk of cancer for several cancer types, including neuroblastoma, renal, hepatoblastoma, soft-tissue sarcoma, and germ cell tumors, during childhood [3].

The pathogenic mechanism of the link between congenital anomalies and cancer remains to be elucidated. Plausible theories include environmental exposures leading to both conditions, somatic mutations in developmental genes early in embryogenesis or overexpressed genes, or altered pathways, including both developmental and cancer predisposition genes [3].

Our analysis highlights the heterozygous variant c.827A>C on the tumor suppressor gene PTPRJ and the homozygous variants c.723G>A and c.91T>A on the proto-oncogenes CCND1 and AURKA, respectively. These variants are associated with colon cancer in ClinVar. Specifically, PTPRJ encodes for the receptor-type tyrosine-protein phosphatase eta, an enzyme that regulates angiogenesis, cell growth, proliferation, differentiation, and migration. Mita et al. observed a highly increased risk of developing colon cancer when c.827A>C is present simultaneously with p.Arg326Gln on the same gene [24]. Interestingly, the patient carries both variants.

Hryhomorowicz et al. studied c.723G>A and identified it as a risk factor for thyroid carcinoma even if with low penetrance, especially when it is in homozygosity, as in our study [25]. In a comparative study, Weinhold et al. found a significant association between this variant and the translocation of a portion of chromosome 11, resulting in a risk factor for multiple myeloma [26]. Moreover, Absenger et al. inspected the prognostic potential of the variant, suggesting it as a possible biomarker in colon cancer [27]. Ewart-Toland et al. studied the c.91T>A substitution in rat models, discovering an increased association
of the variant with aneuploidy in human colon tumors. Indeed, the variant strengthens the binding of aurora kinase A with the E2 ubiquitin-conjugating enzyme, altering the cell cycle progression [28].

In addition, we analyzed the tumor suppressors or proto-oncogenes BCR, BRCC3, CSF1R, DMBT1, FGFR2, MERTK, MYB, MYH11, PCMI, PTCH1, RB1CC1, ROS1, and SDHA, which carried very rare variants in our analysis (Figure 2). They have never been directly associated with VACTERL.

In detail, one heterozygous missense variant was identified on the BCR gene encoding for a guanine nucleotide exchange factor, whose activity is identified as a serine/threonine kinase [29]. When BCR genes fuse with the translocated ABL1 gene, they cause uncontrollable cell division in chronic myeloid leukemia [30]. Another heterozygous splice region variant is identified on the gene BRCC3 that encodes for the Lys-63-specific deubiquitinase BRCC36 protein, a subunit of the BRCA1-BRCA2-containing complex. It is involved in the DNA damage response and was associated with myeloid neoplasms [31,32]. CSF1R carries one heterozygous missense variant. CSF1R encodes for the tyrosine kinase transmembrane macrophage colony-stimulating factor 1 receptor. CSF1R regulates the survival, proliferation, and differentiation of macrophages, along with monocytes interacting with CSF1 and IL34 [33] and actin cytoskeleton reorganization, cell migration, and cancer cell invasion through the ERK1/2 and JNK pathways [34]. It is associated with pediatric-onset leukoencephalopathy and brain malformation when absent in the brain [35,36]. In addition, one homozygous splice region variant of two close nucleotides was identified on the DMBT1 gene. DMBT1 gene encodes for deleted in malignant brain tumors 1. It is a candidate tumor suppressor gene for colorectal, gastric, esophageal, lung, and brain cancers [37–40], probably influencing the immune system [41]. One heterozygous missense variant was discovered on the FGFR2 gene. FGFR2 encodes for fibroblast growth factor receptor 2, a tyrosine-protein kinase that regulates cell proliferation, differentiation, and apoptosis specifically during embryonic development [42–44], controlling lung morphogenesis and skeleton and skin development [45]. MERTK includes one heterozygous splice region variant. MERTK encodes for the tyrosine-protein kinase Mer, a member of the TAM receptor tyrosine kinases involved in cytokine release, cell proliferation, and migration. Mutations on MERTK are associated with autoimmune diseases, and expression alterations may have oncogenic potential [46]. The transcriptional activator Myb is encoded by MYB, which had a heterozygous missense variant in our analysis. It is a transcription factor mainly involved in the proliferation and differentiation of hematopoietic cells and plays an important role in breast and salivary adenoid cystic carcinoma [47,48]. MYH11 has one missense heterozygous and one heterozygous splice region variant. It encodes for Myosin-11 and is mainly involved in muscle contraction; however, when altered, it may contribute to intestinal [49], gastric, and colorectal [50] cancers and acute myeloid leukemia [51]. PCM1 carries a heterozygous missense mutation of two close nucleotides. It encodes for the pericentriolar material 1 protein, required for the assembly and functioning of the centrosome and to attach microtubules [52], and is involved in the chromosomal rearrangement in myeloid or lymphoid neoplasms [53,54]. PTCH1 has one homozygous missense variant. PTCH1 encodes for protein patched homolog 1, essential in embryogenesis [55]. Mutations of this gene have already been associated with holoprosencephaly [56] along with several cancers such as nevoid basal cell carcinoma syndrome and medulloblastoma [57,58]. RB1CC1 carries two heterozygous variants, one missense in the coding sequence and one deletion in an intron and splice region. This gene encodes for RB1-inducible coiled-coil protein 1, which plays a key role in the initiation of autophagy, the impairment of which increases cell death [59,60]. This protein also acts as a transcription factor for retinoblastoma 1 and seems to regulate the progression of various cancers [61]. ROS1 carries a heterozygous splice region variant. This gene encodes for the proto-oncogene tyrosine-protein kinase ROS, which is an integral membrane protein receptor that functions as a growth and differentiation factor via PI3K-mTOR, STAT3, or VAV3 signaling [62,63]. Its rearrangement is associated with lung cancer, glioblastoma, ovarian carcinoma, sarcoma, and cholangiocarcinoma [64,65].
SDHA has a heterozygous frameshift variant. It encodes for the succinate dehydrogenase flavoprotein subunit mitochondrial that in the inner membrane of mitochondria is involved in mitochondrial electron transport chain but seems to represent a link with hereditary tumors [66].

In VACTERL syndrome, clinical exome sequencing could be included as a transformative test for prenatal diagnosis. The prenatal multidisciplinary team approach could benefit from the more accurate detection of a large spectrum of dysmorphologies, described as being part of this complex malformation.

Early detection of the different aspects of the syndrome allows taking charge perinatally, with a potential improvement of the clinical outcome for the child. In addition, most of them, such as tracheal malformations or urogenital malformations, are prenatally unexpected and undetected.

Data from our report could be included in the fetal exome database for the completion of a broad diagnostic capability in pregnancy, with unexpected anomalies. Additionally, considering the possibility for the patient to develop any additional long-term sequelae, a specialist multidisciplinary team for strict clinical monitoring is recommended during childhood and adolescence. Due to the relationship between pubertal timing, growth, and adult health, auxological evaluations should be recommended at least twice a year. Starting from puberty, metabolic profile and cardiologic evaluation may also be annually useful for the early detection of cardio-metabolic risk factors, such as insulin resistance, hypertension, and dyslipidemia.

The current challenge for the future is to translate these approaches into clinical use for surveilling the development of different diseases.

4. Conclusions

VACTERL disease is associated with multiorgan impairment, but its etiology is still unclear. In this work, we discovered several variants in a six-month-old patient that could be responsible for the clinical complication of complex malformation. Six of them are related to cardiac dysfunction. c.501G>C (OLR1) and c.-8C>G (PSMA6) are specifically associated with myocardial infarction, c.1936A>G (AKAP10) with cardiac conduction defects, c.575A>G (PON1) with artery disease, and c.860G>A (EPHX2) and c.214C>A (GHR1) with metabolic syndrome. In addition, the proto-oncogenes CCND1 and AURKA, along with the tumor suppressor PTEN, carry the variants c.723G>A, c.91T>A, and c.827A>C, respectively, which are related to colon cancer. For the first time, we associated nine rare variants on eight proto-oncogenes (MERTK, CSF1R, MYB, ROS1, PCM1, FGFR2, MYH11, BRCC3) and six rare variants on five tumor suppressor (SDHA, RB1CC1, PTCH1, DMBT1, BCR) with VACTERL. Clinical exome sequencing could offer support for clinicians to combine the surgical treatment of VACTERL syndrome with a dedicated risk assessment for the prevention of further disease during adolescence and adult age.

Author Contributions: Conceptualization, G.P., E.M., and V.C.; methodology and formal analysis, L.C., M.A.A., S.C., and E.M.; software L.C.; writing—original draft preparation G.P., E.M., and V.C.; writing—review and editing, G.P., E.M., G.V.Z., M.L., P.B., and V.C. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Current Research Fund 2020, Ministry of Health, Italy.

Institutional Review Board Statement: The parents consent to the threat of anonymous data before to start the analysis for clinical research purposes, epidemiology, study of pathologies, and training, with the objective of improving knowledge, care and prevention. The study was conducted according to the guidelines of the Declaration of Helsinki.

Informed Consent Statement: The parents of the patient provided informed consent for genetic testing and publication.

Data Availability Statement: The data presented in this study are openly available in the NCBI Sequence Read Archive at BioProject accession number PRJNA660915.

Conflicts of Interest: The authors declare no conflict of interest.
References

1. Solomon, B.D. The etiology of VACTERL association: Current knowledge and hypotheses. Am. J. Med. Genet. C Semin. Med. Genet. 2018, 178, 440–446. [CrossRef] [PubMed]

2. Solomon, B.D. VACTERL/VATER Association. Orphanet J. Rare Dis. 2011, 16, 56. [CrossRef] [PubMed]

3. Baldacci, S.; Gorini, F.; Santoro, M.; Pierini, A.; Minichilli, F.; Bianchi, F. Environmental and individual exposure and the risk of congenital anomalies: A review of recent epidemiological evidence. Epidemiol. Prev. 2018, 42, 1–34. [PubMed]

4. Norwood, M.S.; Lupo, P.J.; Chow, E.J.; Scheurer, M.E.; Plon, S.E.; Danysh, H.E.; Spector, L.G.; Carozza, S.E.; Doody, D.R.; Mueller, B.A. Childhood cancer risk in those with chromosome and non-chromosome congenital anomalies in Washington State: 1984–2013. PLoS ONE 2017, 12, e0179006. [CrossRef] [PubMed]

5. Charles, M.A.; Delpierre, C.; Bréant, B. Developmental origin of health and adult diseases (DOHaD): Evolution of a concept over three decades. Med. Sci. 2016, 32, 15–20.

6. Kim, D.; Kobayashi, T.; Voisin, B.; Jo, J.H.; Sakamoto, K.; Jin, S.P.; Kelly, M.; Pasieka, H.B.; Naff, J.L.; Meyerle, J.H.; et al. Targeted therapy guided by single-cell transcriptomic analysis in drug-induced hypersensitivity syndrome: A case report. Nat. Med. 2002, 26, 236–243. [CrossRef]

7. Sandhuc, C.; Qureshi, A.; Emili, A. Panomics for Precision Medicine. Trends Mol. Med. 2018, 24, 85–101. [CrossRef]

8. Manzoni, C.; Kia, D.A.; Vandrovocva, J.; Hardy, J.; Wood, N.W.; Lewis, P.A.; Ferrari, R. Genome, transcriptome and proteome: The rise of omics data and their integration in biomedical sciences. Brief. Bioinform. 2018, 19, 286–302. [CrossRef]

9. Wang, K.; Li, M.; Hakonarson, H. ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010, 38, e164. [CrossRef]

10. Karczewski, K.J.; Francioli, L.C.; Tiao, G.; Cummings, B.B.; Alföldi, J.; Wang, Q.; Collins, R.L.; Larricchia, K.M.; Ganna, A.; Birnbaum, D.P.; et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 2020, 581, 434–443. [CrossRef]

11. Hobbs, C.A.; Chowdhury, S.; Cleves, M.A.; Erickson, S.; Shaw, G.M.; Shete, S.; Witte, J.S.; Tycko, B. Genetic epidemiology and nonsyndromic structural birth defects: From candidate genes to epigenetics. JAMA Pediatr. 2014, 168, 371–377. [CrossRef] [PubMed]

12. Tatsuguchi, M.; Furutani, M.; Hinagata, J.; Tanaka, T.; Furutani, Y.; Imamura, S.; Kawana, M.; Masaki, H.; Kasanuki, H.; Sawamura, T.; et al. Oxidized LDL receptor gene (OLR1) is associated with the risk of myocardial infarction. Biochem. Biophys. Res. Commun. 2003, 303, 247–250. [CrossRef]

13. Ozaki, K.; Sato, H.; Iida, A.; Mizuno, H.; Nakamura, T.; Miyamoto, Y.; Takahashi, A.; Tsunoda, T.; Ikegawa, S.; Kamatani, N.; et al. A functional SNP in PSMA6 confers risk of myocardial infarction in the Japanese population. Nat. Genet. 2006, 38, 921–925. [CrossRef] [PubMed]

14. Hinohara, K.; Nakajima, T.; Sasaoka, T.; Sawabe, M.; Lee, B.S.; Ban, J.; Park, J.E.; Izumi, T.; Kimura, A. Replication studies for the association of PSMA6 polymorphism with coronary artery disease in East Asian populations. J. Hum. Genet. 2009, 54, 248–251. [CrossRef] [PubMed]

15. Kammerer, S.; Burns-Hamuro, L.L.; Ma, Y.; Hamon, S.C.; Canaves, J.M.; Shi, M.M.; Nelson, M.R.; Sing, C.F.; Cantor, C.R.; Taylor, S.S.; et al. Amino acid variant in the kinase binding domain of dual-specific A kinase-anchoring protein 2: A disease susceptibility polymorphism. Proc. Natl. Acad. Sci. USA 2003, 100, 4066–4071. [CrossRef] [PubMed]

16. Ito, T.; Yasure, H.; Yoshimura, M.; Nakamura, S.; Nakayama, M.; Shimasaki, Y.; Harada, E.; Mizuno, Y.; Kawano, H.; Ogawa, H. Paraoxonase gene Gln192Arg (Q192R) polymorphism is associated with coronary artery spasm. Hum. Genet. 2002, 110, 89–94. [CrossRef]

17. Fornage, M.; Papanicolaou, G.; Lewis, C.E.; Boerwinkle, E.; Siscovick, D.S. Common INSIG2 polymorphisms are associated with age-related changes in body size and high-density lipoprotein cholesterol from young adulthood to middle age. Metabolism 2010, 59, 1084–1091. [CrossRef]

18. Ohtoshi, K.; Kaneto, H.; Node, K.; Nakamura, Y.; Shiraiwa, T.; Matsuishi, M.; Yamashita, Y. Association of soluble epoxide hydrolase gene polymorphism with insulin resistance in type 2 diabetic patients. Biochem. Biophys. Res. Commun. 2005, 331, 347–350. [CrossRef]

19. Müller, T.D.; Nogueiras, R.; Andermann, M.L.; Andrews, Z.B.; Anker, S.D.; Argente, J.; Batterham, R.L.; Benoit, S.C.; Bowers, C.Y.; Broglio, F.; et al. Ghrelin. Mol. Metab. 2015, 4, 437–460. [CrossRef] [PubMed]

20. Inazumi, T.; Ando, M.; Nakatoki, M.; Yasuda, Y.; Honda, H.; Kuwatsuka, Y.; Kato, S.; Kondo, T.; Iwata, M.; Nakashima, T.; et al. Effect of dietary energy and polymorphisms in BRAP and GHRL on obesity and metabolic traits. Obes. Res. Clin. Pract. 2018, 12, 39–48. [CrossRef] [PubMed]

21. Carozza, S.E.; Langlois, P.H.; Miller, E.A.; Canfield, M. Are children with birth defects at higher risk of child- hood cancers? Am. J. Epidemiol. 2012, 175, 1217–1224. [CrossRef] [PubMed]

22. Hasle, H.; Clemmensen, I.H.; Mikkelsen, M. Risks of leukaemia and solid tumours in individuals with Down’s syndrome. Lancet 2000, 355, 165–169. [CrossRef] [PubMed]

23. Motegi, T.; Kaga, M.; Yanagawa, Y.; Kadowaki, H.; Watanabe, K.; Inoue, A.; Komatsu, M.; Minoda, K. A recognizable pattern of the midface of retinoblastoma patients with interstitial deletion of 13q. Hum. Genet. 1983, 64, 160–162. [CrossRef] [PubMed]
24. Mita, Y.; Yasuda, Y.; Sakai, A.; Yamamoto, H.; Tooyooka, S.; Gunduz, M.; Tanabe, S.; Naomoto, Y.; Ouchida, M.; Shimizu, K. Missense polymorphisms of PTPRJ and PTPN13 genes affect susceptibility to a variety of human cancers. J. Cancer Res. Clin. Oncol. 2010, 136, 249–259. [CrossRef] [PubMed]

25. Hryhorowicz, S.; Ziemnicka, K.; Kaczmarek-Ryś, M.; Hoppe-Gołębiewska, J.; Plawski, A.; Skrzypczak-Zielińska, M.; Szukdarek, M.; Golab, M.; Budny, B.; Ruchala, M.; et al. CCND1 gene polymorphic variants in patients with differentiated thyroid carcinoma. Oncol. Lett. 2015, 9, 442–448. [CrossRef]

26. Weinhold, N.; Johnson, D.C.; Chubb, D.; Chen, B.; Försti, A.; Hosking, F.J.; Broderick, P.; Ma, Y.P.; Dobbins, S.E.; Hose, D.; et al. The CCND1 c.870G>A polymorphism is a risk factor for t(11;14)(q31;q32) multiple myeloma. Nat. Genet. 2013, 45, 522–525. [CrossRef]

27. Absenger, G.; Benhaim, L.; Szkandera, J.; Zhang, W.; Yang, D.; Labonte, M.J.; Pichler, M.; Stötz, M.; Samonigg, H.; Renner, W.; et al. Chronic myeloid leukemia with insertion- derived BCR-ABL1 fusion: Redefining complex chromosomal abnormalities by correlation of FISH and karyotype predicts prognosis. Mod. Pathol. [CrossRef]

28. Huang, D.; Nagata, Y.; Grossmann, V.; Przychodzen, B.; et al. BRCC3 mutations in myeloid neoplasms. Haematologica 2015, 100, 1051–1057. [CrossRef] [PubMed]

29. Meyer, T.; Jahn, N.; Lindner, S.; Röhner, L.; Dolnik, A.; Weber, D.; Scheffold, A.; Köpf, S.; Paschka, P.; Gaidzik, V.; et al. Functional characterization of BRCC3 mutations in acute myeloid leukemia with t(8;21)(q22;q22.1). Leukemia 2020, 34, 404–415. [CrossRef] [PubMed]

30. Wei, S.; Nandi, S.; Chitu, V.; Yeung, Y.G.; Yu, W.; Huang, M.; Williams, L.T.; Lin, H.; Stanley, E.R. Functional overlap but differential expression of CSF-1 and IL-34 in their CSF-1 receptor-mediated regulation of myeloid cells. J. Leukoc. Biol. 2010, 88, 495–505. [CrossRef] [PubMed]

31. Tamimi, R.M.; Brugge, J.S.; Friedman, M.L.; Miron, A.; Iglehart, J.D.; Colditz, G.A.; Hankinson, S.E. Circulating colony stimulating factor-1 and breast cancer risk. Cancer Res. 2008, 68, 18–21. [CrossRef]

32. Oosterhof, N.; Chang, I.J.; Karimiani, E.G.; Kuil, L.E.; Jensen, D.M.; Daza, R.; Young, E.; Astle, L.; van der Linde, H.C.; et al. Homozygous Mutations in CSF1R Cause a Pediatric-Onset Leukoencephalopathy and Can Result in Congenital Absence of Microglia. Am. J. Hum. Genet. 2019, 104, 936–947. [CrossRef]

33. Guo, L.; Bertola, D.R.; Takanohashi, A.; Sai to, A.; Segawa, Y.; Yokota, T.; Ishibashi, S.; Nishida, Y.; Yamamoto, G.L.; Franco, J.F.D.S.; et al. Bi-allelic CSF1R Mutations Cause Skeletal Dysplasia of Dysosteosclerosis-Pyle Disease Spectrum and Degenerative Encephalopathy with Brain Malformation. Am. J. Hum. Genet. 2019, 104, 925–935. [CrossRef] [PubMed]

34. Mollenhauer, J.; Wiemann, S.; Scheurlen, W.; Korn, B.; Hayashi, Y.; Wilgenbus, K.K.; von Deimling, A.; Pou tska, A. DMBT1, a new member of the SRCR superfamily, on chromosome 10q25.3-26.1 is deleted in malignant brain tumours. Nat. Genet. 1997, 17, 32–39. [CrossRef]

35. Holmskov, U.; Mollenhauer, J.; Madsen, J.; Litjens, E.; von Levine, L.; Torrini, M.; Tyrosine 769 of the keratinocyte growth factor receptor is required for receptor signaling but not endocytosis. Biochem. Biophys. Res. Commun. 2005, 327, 523–532. [CrossRef] [PubMed]
45. Kurosu, H.; Choi, M.; Ogawa, Y.; Dickson, A.S.; Goetz, R.; Eliseenkova, A.V.; Mohammadi, M.; Rosenblatt, K.P.; Kliweer, S.A.; Kuro-o, M. Tissue-specific expression of betaKlotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21. J. Biol. Chem. 2007, 282, 26687–26695. [CrossRef]

46. Linger, R.M.; Keating, A.K.; Earp, H.S.; Graham, D.K. TAM receptor tyrosine kinases: Biologic functions, signaling, and potential therapeutic targeting in human cancer. Adv. Cancer Res. 2008, 100, 35–83.

47. Yang, R.M.; Nanayakkara, D.; Kalimutho, M.; Mitra, P.; Khanna, K.K.; Dray, E.; Gonda, T.J. MYB regulates the DNA damage response and components of the homology-directed repair pathway in human estrogen receptor-positive breast cancer cells. Oncogene 2019, 38, 5239–5249. [CrossRef]

48. Xu, L.H.; Zhao, F.; Yang, W.W.; Chen, C.W.; Du, Z.H.; Fu, M.; Ge, X.Y.; Li, S.L. MYB promotes the growth and metastasis of salivary adenoid cystic carcinoma. Int. J. Oncol. 2019, 54, 1579–1590. [CrossRef]

49. Alhopuro, P.; Phichith, D.; Tuupanen, S.; Sammalkorpi, H.; Nybondas, M.; Saharinen, J.; Robinson, J.P.; Yang, Z.; Chen, L.Q.; Orntoft, T.; et al. Unregulated smooth-muscle myosin in human intestinal neoplasia. Proc. Natl. Acad. Sci. USA 2008, 105, 5513–5518. [CrossRef]

50. Jo, Y.S.; Kim, M.S.; Yoo, N.J.; Lee, S.H. Somatic Mutations and Intratumoral Heterogeneity of MYH11 Gene in Gastric and Colorectal Cancers. Appl. Immunohistochem. Mol. Morphol. 2018, 26, 562–566. [CrossRef]

51. Shoumariyeh, K.; Hussung, S.; Niemöller, C.; Bleul, S.; Veratti, P.; Follo, M.; Riba, J.; Philipp, U.; Palmer, J.M.; Pfeifer, D.; et al. Blastoc transformation of BCR-ABL1 positive chronic myeloid leukaemia through acquisition of CBFB-MYH11 and mutant KIT. Br. J. Haematol. 2020. [CrossRef] [PubMed]

52. Dammermann, A.; Merdes, A. Assembly of centrosomal proteins and microtubule organization depends on PCM-1. J. Cell Biol. 2002, 159, 255–266. [CrossRef] [PubMed]

53. Baer, C.; Muehlbacher, V.; Kern, W.; Haferlach, C.; Haferlach, T. Molecular genetic characterization of myeloid/lymphoid neoplasms associated with eosiophilia and rearrangement of PDGFRα, PDGFRB, FGFR1 or PCM1-JAK2. Haematologica 2018, 103, e348–e350. [CrossRef] [PubMed]

54. Lee, J.M.; Lee, J.; Han, E.; Kim, M.; Kim, Y.; Han, K.; Kim, H.J. PCM1-JAK2 Fusion in a Patient With Acute Myeloid Leukemia. Ann. Lab. Med. 2018, 38, 492–494. [CrossRef] [PubMed]

55. Torroja, C.; Gorfinknel, N.; Guerrero, I. Patched controls the Hedgehog gradient by endocytosis in a dynamin-dependent manner, but this internalization does not play a major role in signal transduction. Development 2004, 131, 2395–2408. [CrossRef]

56. Ribeiro, L.A.; Quiezi, R.G.; Nascimento, A.; Bertolacini, C.P.; Richieri-Costa, A. Holoprosencephaly and holoprosencephaly-like phenotype and GAS1 DNA sequence changes: Report of four Brazilian patients. Am. J. Med. Genet. A 2010, 152A, 1688–1694. [CrossRef]

57. Zhou, J.; Zhang, G.; Shi, M.; Liu, Z.; Xiao, M.; Fu, S.; Gong, X.; Shi, X. A novel splicing mutation of PTCH1 in a Chinese family with nevoid basal cell carcinoma syndrome. Med. Mol. Morphol. 2019, 52, 235–237. [CrossRef]

58. Jones, D.T.; Jäger, N.; Kool, M.; Zichner, T.; Hutter, B.; Sultan, M.; Cho, Y.J.; Pugh, T.J.; Hovestadt, V.; Stütz, A.M.; et al. Dissecting the genomic complexity underlying medulloblastoma. Nature 2012, 488, 100–105. [CrossRef]

59. Morrelli, E.; Shen, S.; Ruckenstuhl, C.; Bauer, M.A.; Mariño, G.; Galluzzi, L.; Criollo, A.; Michaud, M.; Maituri, M.C.; Chano, T. p53 inhibits autophagy by interacting with the human ortholog of yeast Atg17, RB1CC1/FIP200. Cell Cycle 2011, 10, 2763–2769. [CrossRef]

60. Bae, H.; Guan, J.L. Suppression of autophagy by FIP200 deletion impairs DNA damage repair and increases cell death upon treatment with anticancer agents. Mol. Cancer Res. 2011, 9, 1232–1241. [CrossRef]

61. Kontani, K.; Chano, T.; Ozaki, Y.; Tezuka, N.; Sawai, S.; Fujino, S.; Saeki, Y.; Okabe, H. RB1CC1 suppresses cell cycle progression through RB1 expression in human neoplastic cells. Int. J. Mol. Med. 2003, 12, 767–769. [CrossRef] [PubMed]

62. Charest, A.; Wilker, E.W.; McLaughlin, M.E.; Lane, K.; Gowda, R.; Coven, S.; McMahon, K.; Kovach, S.; Feng, Y.; Yaffe, M.B.; et al. ROS fusion tyrosine kinase activates a SH2 domain-containing phosphatase-2/phosphatidylinositol 3-kinase/mammalian target of rapamycin signaling axis to form glioblastoma in mice. Cancer Res. 2006, 66, 7473–7478. [CrossRef]

63. Feng, R.; Sachdev, P.; Yan, L.; Chan, J.L.; Trenkle, T.; McClelland, M.; Welsh, J.; Wang, L.H. Vav3 mediates receptor protein tyrosine kinase signaling, regulates GTPase activity, modulates cell morphology, and induces cell transformation. Mol. Cell. Biol. 2000, 20, 2351–2358. [CrossRef] [PubMed]

64. Birchmeier, C.; Sharma, S.; Wigler, M. Expression and rearrangement of the ROS1 gene in human glioblastoma cells. Semin. Oncol. 2014, 41, 110–125. [CrossRef] [PubMed]

65. Davies, K.D.; Doebele, R.C. Molecular pathways: ROS1 fusion proteins in cancer. Clin. Cancer Res. 2013, 19, 4040–4045. [CrossRef]