Total colonic aganglionosis and cleft palate in a newborn with Janus-cysteine 618 mutation of RET proto-oncogene: a case report

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Abstract

Background: Hirschprung disease, the most important congenital colonic dysmotility in children results from neural crest migration, differentiation, proliferation, or apoptosis defects where the rearranged during transfection (RET)-Protooncogene pathway has a central role. Although palatal and retinal anomalies in the context of chromosomopathies and some mono-/-oligogenic syndromes are reported associated with Hirschprung disease the role of inactivating RET mutations in these cases is not clarified.

Case presentation: We report on a dysmorphic newborn with cleft palate and palatal synechia, who showed intestinal obstruction after 24 h of life. Transient ileostomy and surgical biopsies were performed to diagnose aganglionosis of the colon and last ileal loop. No chromosomal anomalies or copy number variations were found. We identified a paternal heterozygous germline mutation c.1852 T > C, which results in the substitution of cysteine by arginine in the RET-receptor tyrosine kinase (p.C618R mutation). There was no family history of Hirschprung disease, but the father underwent surgery for medullary thyroid carcinoma and was affected by retinal dystrophy.

Conclusions: The occurrence of Hirschprung disease and carcinoma shows how a single mutation may be responsible for adverse effects: gain and loss of function of the same receptor. Furthermore, it would be interesting to study its dual role in face and retina embryology, and to extend targeted investigations of RET hotspots in these developmental abnormalities to facilitate counselling, follow-up, and tumor prevention. Complex surgical procedures and genetic testing as well as socio-economic impact are a challenge for familiar compliance.

Keywords: Case-report, RETarranged during Transfection, Neurocristopathy, Hirschsprung disease, Congenital digestive system abnormalities

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Background
Hirschsprung disease (HSCR, #142,623), a colonic dysmotility due to neural crest migration, differentiation, proliferation or apoptosis defects during intestinal development, occurs in approximately 1:5000 live births, more frequently in males (4:1), except for the long-segment-disease (1:1) [1, 2]. Especially the long-segment-disease (aganglionosis beyond the retrosigmoidal junction) is presenting during the first few days of life with features of intestinal obstruction and complications as perforation peritonitis or enterocolitis and has a poor outcome despite of timely surgical intervention [3].

As HSCR is a highly heritable neurocristopathy, genetic variation in the genomes of these patients must largely explain disease development. Inactivating RET rearanged during Transfection (RET, #164,761) mutations are implicated in approximately half the familial cases but also in 20% of sporadic cases, the majority of which were associated with long-segment-disease [2].

The gene RET encodes a transmembrane receptor tyrosine kinase, that is activated by a complex consisting of a soluble glial cell line-derived neurotrophic factor (GDNF) family ligand (GFL) and a glycosyl phosphatidylinositol-anchored co-receptor, GDNF family receptors alpha (GFRα). Four different GFLs, namely GDFN, neurturin, artemin and persephin, can bind to and specifically activate RET through their cognate coreceptors GFRα1–4, respectively [1, 4]. As a signal transducer of these four different ligand/co-receptor complexes, RET has many functions in different tissues and mediates signals through a range of pathways including RAS/ERK, p38MAPK, NF-kB, PI3/AKT, and JNK.

Chromosomopathies (Down syndrome, Cat-eye-syndrome) and some mono- or oligogenic syndromes (Bardet-Biedil syndrome, Cartilage-hair hypoplasia, Goldberg-Shprintzen syndrome, Ondine-Hirschprung syndrome, Mowat-Wilson syndrome, Smith-Lemli-Opitz syndrome, Waardenburg-Shah syndrome) occasionally include palate and retinal anomalies and are frequently associated with HSCR due to phenotype modifier effects of RET haplotypes [1, 4–6] (Table 1).

Orofacial clefts (cleft lip and/or cleft palate) are the result of tissues of the face not joining properly during development, occur in up to 1:690 live births, and are twice as frequent in males [8, 9]. Cleft palate alone occurs when midline fusion of the palatal shelves fails, has a frequency of 1:1500, and is prevalent in female neonates [9]. Advanced maternal age, some maternal medications, cigarette smoking and folate deficiency have been associated with the risk of isolated orofacial clefts in offsprings [9]. Although of complex heterogenetic origin, syndromic cleft palate might be associated with variants in a single gene or in a cluster of contiguous genes (copy number variants), such as van der Woude syndrome, 22q11-deletions syndrome, or chromosomopathies [9, 10]. Furthermore, in non-syndromic orofacial clefts more than 50 genes as well as additive gene-gene and gene-environment interactions, with modifier phenotypic effects, are postulated to play an etiologic role. We present herein a family in which the father manifested medullary thyroid carcinoma (MTC) and the infant not only HSCR but also cleft palate as a developmental abnormality by the loss-of-function nature of Janus-Cysteine 618 mutation of RET proto-oncogene.

Case presentation
The boy was born at full term from non-consanguineous Caucasian parents and was spontaneously delivered without perinatal problems and with weight (3440 g) appropriate for his gestational age. His mother aged 36 years and his father 39 age, and both were originating from a small Greek-spoken southern Sicilian (Italy) village. He was referred to our Institution for mild hypotonia and bilateral cleft of the hard and soft palate (Veau-II cleft palate). Other clinical findings included prominent forehead, a filiform synechia between lingual frenulum and anterior hard palate and syndactyly of second and third toes of left foot. The single synechia was long enough for him to be able to open the mouth. The patient did not have other anomalies of the midline nor congenital lip pits or limb or skeletal malformations.

Enteral nutrition was replaced with parenteral nutrition after 24 h because of clinical evidence of bowel obstruction. The meconium did not pass, he had biliary emesis and abdominal distension. Radiographical investigations showed distended small bowel loops with a distal obstruction (Fig. 1). Laboratory and ultrasound studies were unremarkable. On third day of life, he underwent an explorative laparotomy and a double terminal ileostomy. Immunohistochemical examination showed in all colonic and distal ileal biopsies the absence of ganglion cells in the intestinal nerve plexus consisting with HSCR.

A laparoscopic assisted ileoanal endorectal pull-through and a palatoplasty and synechia release have been planned at 8 and 15 months of age, respectively. The synechia could be useful to provide additional tissue for the following surgical closure of soft palate.

The family history was negative for HSCR, but the father underwent a thyroidectomy at the age of 38 years for an accidentally diagnosed MTC and a germline RET proto-oncogene mutation (p.C618R).

The father presents also hypernasal speech and a not better investigated inherited retinal dystrophy.

Based on clinical presentation and history the newborn’s family followed genetic counselling and periodically check-up for onset of neural crest tumors. The infant’s cardiac, renal, neurodevelopmental, and
Table 1 Chromosomal loci with risks of Hirschsprung disease, modified [6, 7]

| Cytogenetic location/gene | Relative risk | Prevalence in HSCR | Mode of inheritance | Phenotype |
|---------------------------|---------------|---------------------|---------------------|-----------|
| 1p36.12/ECE1              | AR            | rare AD             | HSCR cardiac defects, autonomic dysfunctions |
| 1q43-q44/SDCC             | AR            | HSCR/Mowat-Wilson syndrome (MR, craniofacial dysmorphisms) |
| 2q22.3/2FHX1B (ZEB2)      | 3000-fold 6%  | AD                  | Bardet-Biedl syndrome-16 (like Bardet-Biedl syndrome-1) /Senior-Loken syndrome-7 |
| 2q31.1/BB5                | AR            | Bardet-Biedl syndrome-5 (like Bardet-Biedl syndrome-1) |
| 3p21                      | 4-fold        | HSCR6               |
| 3p21.32/CELSR3 (EGF11)    | AR            | Encephalopathy      |
| 3p21.31/LZTFL1            | AR            | Bardet-Biedl syndrome-17 (like Bardet-Biedl syndrome-1) |
| 3q11.2/ARL6               | AR            | Bardet-Biedl syndrome-3 (like Bardet-Biedl syndrome-1) |
| 4p13/PHOX2B               | 1000-fold 0.5%| AD                  | Ondine syndrome (hypoventilation, autonomic dysfunctions) |
| 4q27/BB5                  | AR            | Bardet-Biedl syndrome-7 (like Bardet-Biedl syndrome-1) |
| 4q27/BB52                 | AR            | Bardet-Biedl syndrome-12 (like Bardet-Biedl syndrome-1) |
| 4q31.3-q32.3              | 1.5-fold      | AD                  | HSCR9               |
| 5p13.2/GDNF               | rare          | AD                  | HSCR3/Ondine syndrome (hypoventilation, autonomic dysfunctions) |
| 7p14.3/PHB1               | AR            | Bardet-Biedl syndrome-9 (like Bardet-Biedl syndrome-1) |
| 7q21.11/SEMA3A/SEMA3D     | 25-fold 0.5%  | AR                  | Cartilage-hair hypoplasia (short limb dwarfism, hypotrichosis, immunodeficiency) |
| 8p12/NRG1                 | 1.2-fold      | AD                  | Schizoaffective disorders |
| 8q22.1/C8orf37            | AR            | Bardet-Biedl syndrome-21 (like Bardet-Biedl syndrome-1) |
| 8q24.3/DERDND1            | AR            | Bardet-Biedl syndrome-11 (like Bardet-Biedl syndrome-1) |
| 9p13.3/RMIRP              | 25-fold 0.5%  | AR                  | Bardet-Biedl syndrome-18 (like Bardet-Biedl syndrome-1) |
| 9p21.2/IPT74              | AR            | Bardet-Biedl syndrome-20 (like Bardet-Biedl syndrome-1) |
| 9p24.1                    | AR            | Bardet-Biedl syndrome-10 (like Bardet-Biedl syndrome-1) |
| 9q31/IKBKAP               | AR            | Bardet-Biedl syndrome-11 (like Bardet-Biedl syndrome-1) |
| 9q33.1/TRIM32             | AR            | Bardet-Biedl syndrome-11 (like Bardet-Biedl syndrome-1) |
| 10q11.21/RET              | 3000-fold 48% | AD                  | HSCR1 classical; HSCR/medullary thyroid carcinoma/multiple endocrine neoplasia syndrome-2A/−2B/ Ondine syndrome (hypoventilation, autonomic dysfunctions) |
| 10q22.1/C10orf27 (TBATA)  | AR            | Thymus, brain and testes associated (TBATA) alterations |
| 10q22.1/AAXA1279 (KIF18B) | AR            | Goldberg-Shprintzen megacolon syndrome (MR, craniofacial dysmorphisms, nervous system anomalies) |
| 10q22.2/VCL               | AR            | Myopathy |
| 10q23.1/NRG3              | AR            | Bardet-Biedl syndrome-18 (like Bardet-Biedl syndrome-1) |
| 10q25.2/BBIP1             | AR            | Bardet-Biedl syndrome-18 (like Bardet-Biedl syndrome-1) |
| 10q25.3/GFRA1             | rare          | HSCR               |
| 11p15.4/NUP98             | AR, DR        | myelodysplastic syndrome |
| 11q13.2/BB51              | AR, DR        | Bardet-Biedl syndrome-1 (MR, obesity, retinal degeneration, genitourinary anomalies, HSCR, polydactyly and other laterality defects) |
| 11q13.4/CHCR7             | 50-fold 1%    | AR                  | Smith-Lemli-Opitz syndrome (MR, craniofacial dysmorphisms, 2–3 toe syndactyly, multiple malformations, hypogonadism) |
| 12q21.2/BB510             | AR            | Bardet-Biedl syndrome-10 (like Bardet-Biedl syndrome-1) |
| 12q21.32/CEP290           | AR            | Bardet-Biedl syndrome-14 (like Bardet-Biedl syndrome-1)/Joubert-syndrome/Meckel
### Table 1  
Chromosomal loci with risks of Hirschsprung disease, modified [6, 7] (Continued)

| Cytogenetic location/gene | Relative risk | Prevalence in HSCR | Mode of inheritance | Phenotype                  |
|--------------------------|---------------|---------------------|---------------------|----------------------------|
| 12q23.2/ASCL1            |               |                     |                     | syndrome/Leber congenital amaurosis-10 |
| 13q22.3/EDNRB            | 1000-3700-fold| 5%                  | AD, AR              | HSCR2/ABCD syndrome/ Waardenburg-syndrome-4A (deafness, pigmentation defects) |
| 14q12.13/NSD2            |               |                     | AD                  | Nonmedullary thyroid carcinoma, brain-lung-thyroid syndrome, chorea |
| 14q31.2/EDNRB            |               |                     | AR                  | Bardet-Biedl syndrome-8 (like Bardet-Biedl syndrome-1) |
| 15q24.1/BS6              |               |                     | AR                  | Bardet-Biedl syndrome-4 (like Bardet-Biedl syndrome-1) |
| 16q12/4                   |               |                     | AR                  | Bardet-Biedl syndrome-13 (like Bardet-Biedl syndrome-1) |
| 16q22.2/FT27             |               |                     | XLR                 | L1 syndrome (MR, hydrocephalus due to congenital stenosis of aqueduct of Sylvius, adducted thumbs) |
| Trisomy 21               | 50–100-fold   | 8%                  | AD                  | Cat eye syndrome (coloboma, craniofacial dysmorphisms, anorectal and cardiac malformations) |
| Partial tetratagy        |               |                     |                     | - SRY competes for SOX10 binding site |
| 22q11                    |               |                     |                     | - Males have less ECE1 and EDN3 expression in bowel |

Abbreviations: AD Autosomal dominant, AR Autosomal recessive, ARL6 ADP-ribosylation factor-like protein 6 gene, ASCL1 Achaete-scute homolog 1, BBP1 BBSome interacting protein 1 gene, BBS Bardet Biedl syndrome gene, CERS3 Cadherin EGF LAG seve-pass G-type receptor 3 gene, CEP290 Centrosomal protein gene, C8orf37 Chromosome 8 open reading frame 37, C10orf27 Chromosome 10 open reading frame 27, DENND3 DENN domain-containing protein 3 gene, DHCR7 7-dehydrocholesterol reductase gene, DR Digenic recessive, EDNRB Endothelin receptor type b gene, EDN3 Endothelin 3 gene, GDNF Glial cell line-derived neurotrophic factor gene, GFRα1 GDNF family receptor alpha-1, HSCR Hirschsprung disease, IRF Intracellular transport, IRX4AP Inhibitor of kappa light polypeptide gene enhancer gene, IRAK1279 Kinesin binding protein, LTF1 Leucine zipper transcription factor like 1, L1CAM L1 cell adhesion molecule gene, MKK5 McKusick-Kaufman syndrome gene, MKS1 Meckel syndrome-1 gene, MR Mental retardation, NCLN Nicalin, NFR1 Neurourolin 1 gene, NRG1 Neuregulin 1, NRP2 Neurturin, NUCP8 Nucleoporin 98, PHOX2B Paired-like homeobox 2B gene, PTHB1 Parathyroid hormone-responsive B1 gene, RET Rearranged during transfection protooncogene, RMPR RNase mitochondrial RNA processing gene, SDCCAG8 Serologically defined colon cancer antigen 8 gene, SEMA Semaphorin, SOX10 Sry-box 10 gene, TRITC8 Tetratricopeptide repeat domain 8, TRIM32 Tripartite motif containing 32, VCL Vinculin, XLR X-linked recessive, ZFHX1B Zinc finger homeobox protein 1b gene
ophthalmological examinations were unremarkable at 7 months of age.

Laboratory investigations in the newborn were performed. High-resolution GTG-banding karyotype excluded aneuploidies. Deoxyribonucleic acid (DNA) extraction from lymphocytes (QIAamp DNA blood Midi Kit, Qiagen) was followed by an array comparative genomic hybridization (a-CGH) using the whole genome 8x60K. Scanned images of the arrays were processed and analyzed using Feature Extraction software and Genomic Workbench software (Agilent Technologies) with the statistical algorithm Aberration detection method-2 (ADM-2) and a sensitivity threshold of 6.0 as recently benchmarked [7]. This genetic analysis did not reveal cryptic chromosomal anomalies associated with HSCR or cleft palate.

Polymerase chain reaction amplification of the RET proto-oncogene (#164,761) on 10q11.2 followed by bi-directional direct sequencing (GenBank NM_020975.4) identified the paternal heterozygous germline mutation c.1852T>C on Exon 10 also in the newborn, which results in the known substitution of cysteine by arginine in the RET receptor (p.C618R). Informed parents refused to proceed with further studies involving other genes.

**Discussion and conclusion**

We observed an association of total colonic aganglionosis and cleft palate with palatal synechia in a newborn with paternal Janus-Cysteine 618 mutation of RET proto-oncogene and familial history of MTC.

Actually, HSCR cannot be diagnosed in utero, but it could be suspected if positive family history and early complications as meconium peritonitis occur. It is commonly diagnosed shortly after birth by image-based and non–image-based clinical techniques and specific laboratory tests to detect ganglion cells and nerve fibers on suction biopsies. These biopsies stained with hematoxylin and eosin and/or acetylcholinesterase are showing absent submucosal ganglion cells and an increase in nerve fibers in the submucosa and an increase in nervous filaments in the lamina propria. Combined immunohistochemical markers, as calretinin, S-100 protein, peripherin, neuron-specific enolase, cathepsin D, BCL-2 (B-cell lymphoma 2) and RET have been described as adjunctive diagnostic tests in HSCR. Genetic markers in familial forms could be helpful also for prognosis.

Ligand-independent activating mutations of RET cause neural crest proliferation defects in single (MTC, #155, 240) or multiple sites (Multiple endocrine neoplasia type 2A, #171,400; Multiple endocrine neoplasia type 2B, #162,300), while 2–5% of patients with HSCR also develop MTC [2, 11]. In case of this rare co-segregation, dual Janus mutations in exon 10 (codons 609, 611, 618, and 620) are implicated affecting the Cysteine-rich region of RET receptor by homodimerization. The closer mutations are located to the transmembrane domain of RET, the higher their tumorigenicity in thyroid parafollicular cells and adrenal chromaffin cells, and the lower the density of RET on the cell surface leading to apoptosis in precursor neurons in the developing enteric nervous system [12, 13].

While RET is crucial during embryogenesis, since it is expressed in all neural crest-derived cells and renal epithelium, its inactivating mutations could explain cleft palate (propositus) and retinal dystrophy (father) [13]. To our knowledge, this association with RET mutations has never been reported.

Many genetic factors contributing to cleft palate formation have been identified for some syndromic cases. However, many clefts run in families even though in some cases there does not seem to be any identifiable syndrome present [14].

A large number of genes are involved in nonsyndromic forms of orofacial clefts, including above all growth factors (CLPTM1, FGFR1, TGFA, TGFB3), genes related to nutritional metabolism (GADD, LRP6, MTHFR) and transcription factors (GRHL3, IRF6, MSXI, TBXI, TBX22, TP63) [10, 14, 15]. Unfortunately, we could not

![Fig. 1 Plain abdominal radiogram shows distended small bowel loops, distal obstruction, and absent rectal gasification, consisting with total colonic aganglionosis. There are no peritoneal free fluid or air and no associated skeletal anomalies or maturation defects, except for the still absent first coccygeal ossification center.](image)
further investigate these genes because the parents refused any further genetic examination. However, clinically and genetically we could exclude the most frequent monogenetic or copy-number variations associated with palatal and retinal anomalies and HSCR [6, 7, 15]. Congenital intraoral synechiaae associated with an oral cleft are exceedingly rare, described as clinical conditions as cleft palate lateral synechiaae (CPLS) syndrome or cleft palate and congenital alveolar synechia (AS) syndrome [14, 16]. However, and association with HSCR without lower lip pits has not reported.

Up to date the etiology is unclear and interposition of the tongue between the palatal shelves explain the cleft palate, while close contact between the floor of the mouth and the palate could predispose to the formation of the subglossopalatal membrane a precursor of intraoral synechiaae. We have been able to exclude the most frequent syndromic forms. Patient did not resemble the autosomal-dominant van der Woude syndrome because the pathognomonic characteristics of lower lip pits or pyramidal-shaped skin above the big toe were missing in the neonate and his parents [14]. He did also not have any other reported phenotypic features of oro-faciodigital syndromes, or extensive webbing behind the knee characteristic for autosomal-dominant popliteal pterygium syndrome, or any musculoskeletal or thoracic anomalies described in autosomal-recessive Fryns syndrome [9, 13].

Since HSCR most often presents shortly after birth, features of correlated syndromes may not be reported at the time of diagnosis. We could exclude consanguinity associated with hypothetical rare recessive disorders; however, oblivious additional heterozygosity conditions could be presumed since parents originate from the same small Greek-spoken Sicilian territory (Siceliotis). Furthermore, just the p.C618R mutation is considered a founder mutation in this Northern Southwest Asian J2 haplogroup which is spreading also among Greek Cypriots [17, 18]. Gene-environment and gene-gene interactions, as epigenetic modifiers, are the hallmark of inconstant penetrance, parent-of-origin effects, and more insidious disease in males than females in HSCR and MTC but could also open to future therapeutic perspectives [4–6, 18, 19].

In the interests of cost-effectiveness, targeted investigations of RET hotspots in HSCR patients should facilitate counselling, follow-up, and tumor prevention.

Complex surgical procedures and genetic testing as well as socio-economic impact are a challenge for familiar compliance.

**Abbreviations**

a-CGH: Array-comparative genomic hybridization; ADM-2: Aberration detection method-2; DNA: Deoxyribonucleic acid; GDNF: Glial cell line-derived neurotrophic factor; GFL: Glial cell line-derived neurotrophic factor family ligand; GFRα: Glial cell line-derived neurotrophic factor family receptors alpha; HSCR: Hirschsprung disease; MTC: Medullary thyroid carcinoma; RET: Rearranged during transfection

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**Authors’ contributions**

IAMS contributed in all parts of the study, concepted, and wrote the paper. MC performed surgical consulting and revised the manuscript. MG collected the patient data and revised the literature. OMA revised the literature and critically revised the manuscript. VA performed genetical consulting, coordinated and supervised all part of the study. EP performed data analysis and interpretation, and critically revised the manuscript. The authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

Parent’s informed written consent was provided.

**Consent for publication**

Not applicable.

**Competing interests**

Not applicable.

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