

Review Article

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Current Role of Molecular Genetics in Pediatric Surgery

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Abstract

Background: Molecular genetic techniques have greatly improved the understanding of pediatric illnesses. Based on our own experience, the aim of this review is to illustrate the importance of such molecular studies for the development of the pediatric surgery.

Methods: Various molecular techniques for the investigation of DNA, RNA, Chromosomes, gene sequences, genomic rearrangements and gene amplifications have been employed to answer the clinical and therapeutic questions. Suitable statistic methods allowed the comparison of the results between patients and controls whenever possible.

Results: We demonstrated that essential information for diagnostics and therapy of pediatric illnesses can be obtained through molecular genetic testing, including (but not limited to) translocations and consequent protein chimera expression in infantile lung tumors, the occurrence of genomic variants associated to neuroblastomas, the occurrence of microdeletions and insertions in target genes for autonomic diseases, the significantly higher occurrence of target gene mutations and polymorphisms in hypertrophic pyloric stenosis, the identification of a somatic gonosomal mosaicism and uniparental disomy in a complex disorder of sex differentiation and finally the simultaneous occurrence of embryonal tumors as early consequences of genomic instability.

Conclusions: The importance of molecular genetic research for the development of pediatric surgery is evident from the multiple findings hereby described, demonstrating that the application of molecular genetic techniques and the development of a "genetically oriented" thinking for diagnostic and therapy strategies may indeed broaden the expertise and knowledge of pediatric surgeons, ultimately resulting in a better quality of care and higher rate of success for the pediatric patients.

Keywords: Molecular genetics; Pediatric surgery; Genomic rearrangements; Mutations; Polymorphisms

Abbreviations: IHPS: Infantile Hypertrophic Pyloric Stenosis; NOS1, nNOS: Nitric Oxide Synthase (neuronal); HWE : Hardy-Weinberg Equilibrium; UTR: UnTranslated Region; LOH: Loss-Of-Heterozigosity; SNP: Single Nucleotide Polymorphism; NO: Nitric Oxide; PCR: Polymerase Chain Reaction; DNA: DeoxyriboNucleic Acid; NB: neuroblastoma; HSCR: Hirschsprung disease; MLPA: Multiplex Ligation-dependent Probe Amplification; CCHS: Central Congenital Hypoventilation Syndrome (Undine's curse); CCHS/ HSCR: Central Congenital Hypoventilation Syndrome associated with Hirschsprung Disease; MEC : mucoepidermoid carcinoma; cAMP: cyclic AdenosineMonoPhosphate; MCB: MultiColor Banding; RNA: RiboNucleic Acid; RT-PCR: Reverse-Transcriptase Polymerase Chain Reaction; CREB: cAMP Response Element Binding; OR: Odds Ratio; FISH: Fluorescent In-Sitz Hybridization.

Introduction

The complexity of disease spectra in the vast and multifaceted field of pediatric surgery requires ever-changing strategies for diagnosis, establishment of prognosis and for therapeutic strategies. To that effect, the combination of clinical skills and basic science research has proven particularly invaluable in the development of new standards for the management of pediatric surgical diseases.

In this context, the advent of techniques expanding the study of chromosomes, genes, transcription, translation and proteins have immensely broaden the comprehension of both common and rare pediatric diseases, to the point that molecular genetic investigations have become standard for the establishment of the diagnosis and/or prognosis in many diseases. There are countless examples, yet we have decided to include in this review the techniques that have encountered the greatest resonance in the investigation of the genetic background of tumors, congenital malformations, sexual differentiation and other frequent, "normal" diseases such as colonic aganglionosis (Hirschsprung disease, HSCR) and hypertrophic pyloric stenosis (IHPS). These techniques are:

- 1.a. Comprehensive chromosome analyses
- 1.b. DNA Sequencing and mutational analyses
- 1.c. Analyses of genomic rearrangements

1.d. Comparative Genomic Hybridization and copy number variation analyses

Each of these techniques has been particularly useful in answering specific questions in pediatric surgery patients. For example, the analysis of chromosomal abnormalities has provided one of the first milestones for the genetic background in diseases like Turner syndrome and acute myeloid leukemia. Today, chromosomal analyses are important for almost all areas of pediatrics, particularly oncology, diseases of sexual differentiation (DSD), congenital malformations and innate metabolic disorders. As an example for the use of chromosome analysis in oncology, we have investigated a mucoepidermoid carcinoma (MEC) in a 9-year old female patient in search for a causal effect of chromosomal abnormalities with this particular tumor development, once MECs are the third most frequent primary malignancy of the lung in this age group and also the tumors most likely associated with chromosomal abnormalities [1,2].

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Similarly, chromosome analyses have been extremely important for the understanding of congenital abnormalities, such as the disorders of sex development (DSD, MeSH: D012734), which include a broad spectrum of genetic and endocrinological abnormalities profoundly impacting gender differentiation in affected children. The management of children with DSD is a challenge for all professionals involved, most importantly before life-changing decisions regarding genderdefinition and subsequent operative procedures are to be considered. DSDs possess a wide spectrum of chromosomal mosaic variants, whose phenotype may range from phenotypic female to phenotypic male with a gamut of alterations in internal and external genitalia in between [3]. However, in most cases, a diagnosis relying purely on blood samples does not reveal the underlying genetic variation responsible for the phenotype, especially when a somatic chromosomal variant is present. Further, cases of somatic mosaic as a result of acquired postzygotic uniparental disomy (UPD) during mitosis (i.e. the presence of two copies of a particular chromosome from the same parent, either maternal or paternal, and no copies of this chromosome from the other parent) may be associated with an increased risk of tumor development [4]. To illustrate the role of chromosome analysis in DSD, we have applied such techniques in the investigation of a phenotypically male, 2-year-old DSD patient with intersex, whose defects included a severe penoscrotal hypospadia, undescended testis, functional testicular but not ovarian tissue, a rudimentary vagina linked to an uterus, an open rudimentary fallopian tube and a streak-gonad on the right side, a small ductus deferens on the left side and finally no prostate.

DNA sequencing and mutation analyses have also yielded important results for the field of oncology, where it would be practically impossible to list all the gene variants that have been linked to a higher or lesser degree to tumor development. However, not necessarily all the hypothesized and/or confirmed mutations and polymorphisms observed in familial or syndromal cases (i.e. with a stronger genetic load) can be detected in the sporadic cases, which makes the causal association of mutations and tumors much more difficult to establish. One such case is the putative genetic background in the development of neuroblastomas (NB), the most common solid tumor in children [5]. NB are neuralcrest derived tumors whose clinical presentation, response to therapy and outcome of the treatment varies greatly, partially dependent on several genetic markers such as *n-myc* expression and 1p deletion [6]. Given the frequent association of NB with other neural-crest derived disorders such as a Hirschsprung diseases (HSCR), a congenital disorder characterized by the absence of enteric ganglia in a variable extent in the intestine of affected children, and central congenital hypoventilation syndrome (CCHS), an autonomous control disease resulting in hypoventilation, hypercapnia and hypoxia during quiet sleep, it has been suggested that common genes for HSCR and CCHS might play an important role in the development of NB. Specifically, the PHOX2b gene has been considered a serious candidate, since it was the first gene in which germline mutations were found in patients with NB [7,8].

However, sequencing studies are not only useful in oncology or in rare, seldom syndromes, but also in frequent diseases such as the infantile hypertrophic pyloric stenosis. IHPS is the most common indication for laparotomy in the first year of life, and still possibly fatal if left untreated [9,10]. A genetic background for the etiology of IHPS can be deduced both from the frequent familial occurrence of the disease and from recent research findings on nitric oxide (•NO) biology. Due to the essential role of •NO in the relaxation of the smooth muscle cells, one of the tentative candidate genes for the etiology of IHPS is the neuronal Nitric Oxide Synthase (*NOS1* or *nNOS*), one of the three subtypes of NOS responsible for •NO production, which also has warranted its investigation by our group.

The analyses of genomic rearrangements play an important role in diseases affecting the development and the functionality of different organs and systems in children. Mutations in target genes have been associated with specific diseases, as seen before, yet in many patients these variants are found only in a limited percentage of the cases. An example is HSCR, which is transmitted in a complex pattern of inheritance, and the principal gene involved is located at 10q11.2, the RET proto-oncogene [11-14]. Although RET mutations resulting in either RET-protein truncation or functional inactivation have been associated with the HSCR phenotype, these are only identified in 15-20% of sporadic HSCR cases, and in only 50% of the familial cases [15,16]. Therefore, further genomic rearrangements in the coding sequence of the RET proto-oncogene as well as in other HSCR-associated genes may account for unexplained cases by mutation analysis alone, and are passive of investigation using Multiplex Ligation-dependent Probe Amplification (MLPA). Similarly, the main genetic variants detected in CCHS patients have been mutations in the PHOX2b gene, which in some series are present in up to 97% of isolated cases [17]. However, this disease is often associated to other neural crest pathologies, such as HSCR, which is linked to RET variants as mentioned above. Therefore, it is possible that microdeletions or amplifications in sensitive areas of RET or in any of the other associated genes may account for some of the concomitant CCHS and Hirschsprung phenotype (CCHS/HSCR) cases in which RET germline variants have not been identified. Again MLPA techniques are optimally suited for the investigation of these genomic rearrangements, particularly since syndromic cases of HSCR in association with variants of other genes have been reported: mutations in the ZEB2 (SIP-1) gene (located on chromosome 2q22.3) causing the Mowat-Wilson syndrome (or Hirschsprung disease-mental retardation syndrome, MIM235730) and mutations in the EDN3 gene (located on chromosome 20q13.32) resulting in the Shah-Waardenburg syndrome (MIM277580) [15]. Further mutations in genes such as EDNRB, GDNF, NRTN (NTN), SOX10 and ECE1 (either isolated or combined with a RET germline mutation) have been identified in up to 5% of HSCR cases, supporting a genetic heterogeneity for this disorder which may result from a cumulative effect of at least two mutations in different genes [18]. Furthermore, the role of microdeletions of RET may also be important in the attempt to elucidate the complex genetic background in HSCR, particularly those leading to allelic loss, which may result in loss-of-function due to protein shortening or inactivation, as observed in Mowat-Wilson syndrome [19].

Finally, a promising area of diagnostics in pediatric surgery is the comparative genomic hybridization and copy number variation analyses, particularly of oncogenes, since their overexpression or that from other genes may result in an additive effect contributing to tumorigenesis. This is particularly true in patients with chromosomal/ genomic instability and/or multiple tumors, such as in patients with Fanconi Anemia (FA) [20]. However, a former hypothesis that mutations in FA genes would be found in FA-typical neoplasms in the general population has not been confirmed, suggesting that not FA gene mutations alone but rather additional, more or less randomly occurring events such as amplification or deletion of oncogenes or tumor suppressor genes, respectively, must be involved in tumor development in FA patients [21]. We had the opportunity of addressing this question by analyzing somatic genomic alterations in simultaneously occurring nephroblastoma (Wilms Tumor, WT) and NB in a 2-yearold girl with FA-N, incomplete Vacter-L Association (VL) and extreme chromosomal instability. This female patient had a complex association of malformations (incomplete VL with sacral dysplasia and tethered cord at L3/L4, ventriculary septum defect (VSD), supra-levatory anal atresia, uterine bicorn dysplasia, vaginal atresia, fused and severely malformed thumbs and pelvic horseshoe kidney encroaching the aorta and inferior cava vein). In the familial history, there was a clustering of tumors (mostly carcinomas of the lung and breast) on the maternal side of the family. She subsequently developed a WT on the left kidney, which upon surgical resection also included a second tumor mass in the left adrenal, this time an NB. The patient eventually passed away one year later due to complications from a large intracerebellar tumor, possibly a medulloblastoma. The analysis of copy number variations has allowed the understanding of the unfortunate, rapid lethal course of the disease in this child.

In summary, the aim of the current review is to illustrate, using examples of our own practice, the importance of each of the above described molecular genetic studies for the field of pediatric surgery, associating the questions and the results obtained and emphasizing their importance in the clinical management of the cases, as well as the experience acquired with each project.

Methods

Comprehensive chromosome analyses

To investigate whether any chromosomal variants could have contributed to tumorigenesis in a 9-year-old girl with MEC, we have established primary MEC cell cultures from the resected tumor and analyzed the chromosomes using GTG- and GAG-banding, SKY and high-resolution multicolor-banding (MCB) techniques [22-25]. Concomitantly, mRNA from a putative chimeric fusion protein was obtained directly from peripheral blood leukocytes, tumor pellets, control cell cultures and from fresh tumor tissue, subsequently converted into cDNA by Reverse Transcriptase – PCR, which was then gel purified and sequenced using an automated DNA sequences. All results were confirmed by repeated testing.

In the case of the young boy with DSD, we have proceeded with the surgical removal of the female organs and the correction of the hypospadia using the Dukett technique, after which a thorough genetic analysis of was carried out using immunohistochemistry, banding cytogenetic analyses, single nucleotide polymorphism – comparative genomic hybridization (SNP-CGH) array analysis, genomic DNA Hybridization and finally Short Tandem Repeat (STR) analysis in blood and tissue samples from the patients as well as blood from the parents.

DNA Sequencing and mutational analyses

To investigate whether mutations and polymorphisms in the PHOX2b gene were associated to NB development, we studied 69 sporadic NB patients and sequenced PHOX2b DNA samples from tumors, peripheral blood leukocytes (to control for allele loss) and several NB cell lines as positive controls (Vi-856, Kelly, NGP, LAN-5, HD-N-16, NMB, Gimen1, SK-N-SH and LS1). 130 DNA samples from healthy, gender-matched blood donors served as normal controls. To evaluate whether identified polymorphisms conformed to Hardy-Weinberg equilibrium (HWE), the equivalence test proposed by Wellek was used (5% test-level) with ϵ = 0.1 separately in each group for establishing compatibility of an observed genotype distribution with HWE in the case of a biallelic locus [26]. Genotype frequencies between groups were compared using the Cochran-Armitage trend-test or the exact Fisher's test rendering nominal exact p-values and odds ratios (OR) with exact 95% confidence intervals. As for the association of variants of NOS1 with IHPS, we have sequenced of the complete coding region of this gene in 53 IHPS patients and compared the results to 48 gender-matched healthy controls and to tissue samples from 8 patients (as internal controls for allelic loss).

Analyses of genomic rearrangements

The investigation of the role of microdeletions and amplifications in HSCR and CCHS patients was carried out using MLPA in 80 Caucasian HSCR patients who fulfilled the histological and immunohistochemical criteria of HSCR, of which only 5 had a family history, 2 patients had also Down syndrome and another had concomitant Fallot's tetralogy, and in 38 CCHS patients, of which eleven (28.9%) had CCHS/HSCR. MLPA was performed as described by Schouten et al. [27] on genomic DNA isolated from peripheral blood leukocytes using MLPA test kit P169 lot 0106 (MRC-Holland, Amsterdam, The Netherlands) investigating for *RET, ZEB2, EDN3* and *GDNF*.

Comparative genomic hybridization and copy number variation analyses

Our diagnostic methods in the study of a 2-year old girl with multiple tumors and FA comprised SNP-CGH array analysis from blood DNA of the patient and parents and DNA from tissue of the streak gonads, after which total genomic DNA was directly labelled against 500ng of Human male genomic reference DNA (Coriell Institute, Camden N.J. USA NA12891 male) and 500ng DNA of the patient using the Agilent Genomic DNA enzymatic labeling Kit (p/n 5190-0049) according to Agilent standard CGH+SNP protocol (version 4.0). The Sure print human genome CGH+SNP 180K microarrays (Agilent Technologies G4890A, Santa Clara, CA) were hybridized and washed using the Agilent aCGH Wash Kit (p/n 5188-5226) according to the manufacturers protocol, and scanned using an Agilent dual laser DNA microarray scanner. Data were extracted using Agilent Feature Extraction (v 9.5) software and analyzed using Agilent DNA-Analytics (v 4.0) software. Additionally, we performed Short Tandem Repeat (STR) analysis and Amelogenin: using DNA from the patient and his parents amplified in multiplex PCR reactions, which have been set up in a multiplex PCR with primers for STR-loci DXS8378, DXS6809, DXS10160, DXS8377, AMELX/Y and SRY. Primer sequences for the DXS-loci have been taken from the "ChrX-STR.org 2.0" database: http://xdb.qualitype.de/xdb/linkageTable.jsf, while Primer sequences for Amelogenin and SRY have been taken from the NCBI database. Amplifications for all primers have been performed with the Universal Multiplex Cycling Protocol for the QIAGEN Multiplex PCR Kit (cat 206143, Qiagen, Hilden, Germany) according to the manufacturers "Universal Multiplex Cycling protocol". Amplification products were analyzed with a genetic analyzer ABI 310 (Life Technologies, Carlsbad, CA) with Genscan software v3.7.1(additional specific methods for all techniques are available upon request).

Results and Discussion

Comprehensive chromosome analyses

Preliminary results in MEC cultured cells and in tumor samples showed a complex variant of the classical t(11;19) with a pericentric inversion of 11p11.2q21, interstitial deletion of 11q21q23.3 and terminal deletion of 11p11.2pter, but a retained translocation between bands 11q21 and 19p13. Further abnormalities were detected only after long-term cell culture: a supernumerary deleted copy of chromosome 10, del(10)(pter->q25.2:) and an unbalanced translocation between chromosomes 2 and 14, der(2)t(2;14)(?q1?;q?11). These results were confirmed by SKY and MCB analyses of the samples compared to the control cell line NCI-H292 (Figure 1).

Based on the t(11;19) translocation and the expression of both genes of interest *MECT1* and *MAML2* within the breaking points, the hypothesis of a fusion chimera from *MECT1-MAML2* was verified by sequence analysis of the RT-PCR fragments, confirming the presence of

oncogene MECT1-MAML2 fusion transcripts in both the pulmonary MEC tissue and in the positive control cell line NCI-H292. These observations allowed us to conclude that t(11;19) might have resulted in tumor development due to the expression of a fusion transcript originating from the two genes located at the breakpoints in both chromosomes [28], in this case either due to disruption of the Notch pathway (since this chimera MECT1-MAML2 was shown to activate in vitro the transcription of the Notch-target gene HES1 independently of both Notch-ligand and CSL binding sites) or due to increased expression of TORC1, a transducer of regulated cAMP response element binding (CREB) protein, which potently induces CREB1 target genes through a potent transcription activation domain [29,30]. Since low-grade tumors have a higher incidence of the translocation, it is also possible this t(11;19)(q21;p13) translocation might represent a diagnostic marker for low- to intermediate-grade MEC, and that the MEC-specific MECT1-MAML2 fusion product is associated with a subset of tumors that have a more favorable prognosis and a rather protracted clinical course [31,32]. The molecular classification of MEC based on the expression of the fusion transcript may indeed be useful as a marker of prognosis and prediction of the biological behavior [33,34].

As for the analysis of the DSD patient, the results showed a mosaic consisting of a cell line with a normal male karyotype (46,XY; observed in 85 metaphases) and of a cell line with monosomy X (45,X; represented by 9 metaphases), while in cultured skin fibroblasts the mosaic was represented by 153 metaphases with monosomy X and only 23 metaphases with a normal 46,XY karyotype. Similar results were seen by interphase FISH examination of both the gonadal structure and of the resected urethral plate. However, nuclei of spindle-cells in the uterine tissue were predominantly (90%) XX (same as in the epithelium of the fallopian tube) mixed with some XY-cells, possibly lymphocytes. In contrast, cells from the PLAP-positive segments of rudimentary testicular tissue were mostly (80-90%) XY, confirming a gonosome-mosaic in the streak-gonad. Interestingly, the highly atrophied urethral plate showed mixed XX and XY cells (Figure 2).

The STR-Analysis showed only one maternal allele for all X-chromosome markers, and SRY-testing confirmed a reduction



Figure 1: High resolution multicolour banding (MCB) analyses of the long-term tumor cells (chromosome 11 – A; chromosome 19 - B) and of the positive control cell line NCI-H292 (chromosome 11 – C; chromosome 19 - D). Note the translocation t(11;19) in the control cell line (karyotype: t(11;19) (11qter>11q23.3::11p11.2-11q21::19p12->19pter), which was identical to the translocation detected in the tumor of the patient.



Figure 2: FISH analysis of the gonadal structure and of the resected urethral plate. X-chromosomes are stained in green, Y-chromosomes in red. (A): Uterine tissue with predominantly (90%) XX in the spindle-cells mixed with some XY-cells, probably lymphocytes. (B): PLAP-positive segments of rudimentary testicular tissue with mostly (80-90%) XY cells, confirming the gonosome-mosaic in the streak-gonad. The red arrows show the Y chromosomes, the green arrows mark the X chromosomes (C): highly atrophied urethral with mixed XX and XY cells.

of peak height to 25% of the Y-chromosomal signal in the gonadal probe, which further confirmed the mosaicism of the gonosomes. Accordingly, the PCR amplification of the Amelogenin loci of the X and Y chromosomes revealed a Y to X chromosome signal ratio of 1:4 in the gonad sample, confirming the STR analysis. Finally, the SNP-Array analysis of peripheral blood from the child and both parents showed a total of 1860 SNPs in the X-Chromosome, of which 857 could be identified. All of these had only a monoallelic signal without any indication of a second maternal allele, confirming the Uniparental Disomy (UPD), possibly due to a trisomic rescue (Figure 3).

In this particular case, the decision regarding gender-definition and subsequent operative procedures based simply on the phenotype of the internal and external genitalia and/or peripheral blood analysis would have been grossly insufficient. In all DSD cases with mosaics or chimeras, SNP-Array analyses have been recommended to establish the underlying mechanism by identifying the origin of the gonosomes inherited [3]. Since 46,XY/46,XX mosaics are more common in Caucasians and present in ca. 10% of all DSD patients considered "true hermaphrodites" [35,36], the STR analysis plays a role in confirming the origin of the gonosomes and most importantly, the mechanism involved in the origin of the defect, which in this case was a "trisomic rescue" of a trisomy of postzygotic origin, constituting an UPD. [37,38]. The importance of UPD is the increased risk for tumor development due to homozygosity, which is more frequent in cases of acquired and not constitutional UPD, and mostly in autosomes. Specifically in regards to maternal UPD with X-chromosome isodisomy, as observed in our patient, there is an increased risk of sporadic basal-like breast cancer [4], which definitely warrants an adequate genetic counselling for the patient and the family. Therefore, it is clear that complex genetic somatic variants in patients with DSD and ambiguous genitalia require functional approaches with selected genes and chromosomes to better understand the biology of DSD, since chromosome analyses in peripheral blood lymphocytes alone might not be sufficient. Most importantly, many DSD patients may have an UPD which increases significantly the risk of tumor development. Such patients must undergo life-long, regular follow-ups and preventive cancer diagnostics in order to minimize the potentially lethal effects of the UPD [39].

DNA Sequencing and mutational analyses

The results of the sequencing of PHOX2b in 69 sporadic NB are



Figure 3: (A) STR- Analyses using probes DXS6809, DXS10160, DXS8377 and SRY showing a marked decrease of the Y signal in the gonads in comparison to blood and to samples from each parent. (B) Amplification of the X and Y chromosomes from blood and gonad samples of the patient in comparison to his parents using Amelogenin. A pathological Y to X chromosome signal of 1:4 is clearly evident in the probes from the gonads of the patient.

Polymorphism	Genotype Distribution									
		Control			NB	Statistics				
	WW (%)	Wp (%)	рр (%)	WW (%)	Wp (%)	pp (%)	OR	<i>p</i> -value		
IVS1 -114 <u>G</u> >A	62 (47.7)	61 (46.9)	7 (5.4)	38 (55.1)	30 (43.5)	1 (1.4)	0.705 (0.401-1.219) 0.497 (0.161-1.486)	0.1973		
c.552 <u>C</u> >T (S184S)	128 (98.5)	2 (1.5)	0 (0)	68 (98,6)	1 (1,4)	0 (0)	0.941 (0.016-18.837)	1.000		
c.733_747del 15 (in-frame deletion)	126 (96.1)	4 (3.1)	0 (0)	69 (100)	0 (0)	0 (0)	0.000 (0.000-2.845)	0.3003		
c.639 <u>C</u> >G (G213G)	130 (100)	0 (0)	0 (0)	68 (98.6)	1 (1.4)	0 (0)	+ inf (0.048 - +inf)	0.3467		
c. 870 <u>C</u> >A (P290P)	130 (100)	0 (0)	0 (0)	67 (95.7)	3 (4.3)	0 (0)	+ inf (0.789 - +inf)	0.0405		

Table 1: Genotype distributions (percentages in parenthesis) of *PHOX2b* polymorphisms in 69 neuroblastoma patients (NB) and in 130 controls; the wild-type alleles (W) have been underlined as opposed to varient alleles (p). Genotype statistics present odds ratios (OR), exact 95% confidence intervals (in parenthesis) and nominal p-values from Cochran-Armitage trend-test (for IVSI-114G>A) and Fisher's exact test (for the other polymorphism). The corresponding amino acid varients for each mutation are shown in parenthesis. Note that all mutations were silent, with teh exception of c. 733_747del 15, which leads to a protein truncation.

summarized in Table 1. Briefly, we observed a deletion in exon 3 (c.721_740del20) in one NB cell line (SK-N-SH), while in the cell line Vi-856 the in-frame polymorphism c.733_745del_15 was detected. A silent mutation in codon 290 (c.870C>A, P290P) was present in 3 of 69 (4.3%) tumor samples. Additionally, a polymorphism in intron 1 (IVS1-114 G>A) was detected. The IVS1-114 homozygous wild-type genotype (WW) was present in 55.1% of the tumors, while the heterozygous variant and the homozygous variant genotype (Wp, pp) were present in 44.9%. These frequencies did not significantly deviate from those of the controls (OR: 0.705 (0.401-1.219), *p* = 0.1973) and did not conform to HWE in either cases or controls. Other observed polymorphisms of the PHOX2b gene [c.552C>T (S184S); c.733_747del_15; c.639C>G (G213G)] did not show any significant variation among tumor samples, peripheral blood DNA probes and NB cell lines when compared to the control population. Other previously described mutations and polymorphisms were not observed either in the control or in the tumor populations and there was no allelic loss (Figure 4) (Table 1).

Contrasting with the indisputable association of *PHOX2b* mutations and CCHS [23], the prevalence of mutations in NB can be considered small at best, possibly excluding this gene as causative of the tumor [40]. Accordingly, we identified only three silent mutations (S184S, G213G, P290P) of *PHOX2b* in 69 patients and one frameshift mutation in a NB cell line, of which none can be considered pathogenic. Despite a very high frequency of the IVS1-114A allele in the NB cell lines, the overall analyses does not suggest a significant role for *PHOX2b* polymorphisms in the genesis of NB. [41]. In studies were *PHOX2b* mutations were found in NB patients with concomitant CCHS, these variants were most likely associated with the latter and not the former. Clearly, the genetic background of hereditary and sporadic NB is rather complex and does not seem dependent on single-gene mutations, rather suggesting an oligogenic model of disease transmission which involves more loci in



(below). Note that the most of the polymorphisms and mutations are located in Exon 3. Silent mutations are in bold. Further classification according to origin [(T): tumor; (P): peripheral blood DNA; (C): cell lines] and associated diseases [(\dagger): CCHS; (*): Hirschsprung disease; (\approx) NF-1].

 $r^2 = 0.82$)

the genetic determination of the disease and is similar to that found in HSCR patients [42,43,44,45].

As for the sequencing of NOS1 in IHPS, the results were mixed. Although Saur et al. have demonstrated not only a decrease in the expression of NOS1 in IHPS patients (particularly in the alternativesplicing variant NOS1c) but also a strong association of the exon 1c promoter variant c.-84g>a with the IHPS phenotype in 16 patients, in which carriers of the c.-84A SNP (rs41279104) had an increased risk for development of IHPS [46], we have only detected a total of 19 variants of the NOS1 gene in our population (Table 2). From these, 2 polymorphisms have not been previously observed [c.3827-42_3827-43del_insTA and c.*1805 c>g in the 3'-untranslated region (3'UTR)]. The missense mutation in codon 394 (c.1181A>C, p.D394A) was present in one patient and in one control, while the other mutations were silent. We have observed a marginally significant, more frequent occurrence of the polymorphisms in IHPS patients for the variants c.-460g>a (p=0.057); c.2706C>T (p=0.064) and c.3321G>A (p=0.058). The polymorph heterozygous or homozygous variant c.3258T in the silent mutation p.D1086D was present in 39% of the IHPS patients but only in 4% of the controls, thus significantly more frequent in affected individuals (p<0.001 vs. controls). Additionally, this variant was seen often in patients with a positive family history for IHPS (see Figure 5). There was no deviation from the Hardy-Weinberg equilibrium in any of the polymorphisms or mutations, yet the linkage disequilibrium analysis showed a significant correlation for the variants located in exon 18 [c.2706C>T (rs1047735) <=> c.2823+15a>g, (rs2291908)], r2 = 0.82). A diagram of the location of the variants found is shown in (Figure 5) (Table 2).



Other previously described mutations and polymorphisms were absent both in the control and in the patient populations. Due to the relatively small number of patients, and a single significant association in a variant, an analysis of haplotypes for gene-gene interactions was not performed. We did not observe any allelic loss when comparing the results of the tissue DNA analysis with the blood DNA sequencing from patients in cases with heterozygous variants or mutations Citation: Serra A, Henne-Bruns D (2015) Current Role of Molecular Genetics in Pediatric Surgery. Mol Biol 4: 133. doi:10.4172/2168-9547.1000133

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	(SNP Ref.)	Genotype Distribution						St	Statistical Analysis		
Polymorphism		Control		IHPS			IHPS vs. Controls				
		WW (%)	Wp (%)	рр (%)	WW (%)	Wp (%)	рр (%)	p	r ²		
c460g>a	<u>rs9658258</u>	47 (100)	0 (0)	0 (0)	48 (92)	4 (8)	0 (0)	0.057 [§]	n.m.		
c.1181 A>C (p.D394A)	<u>rs9658356</u>	47 (98)	1 (2)	0 (0)	52 (98)	1 (2)	0 (0)	1.00§	0.06 (c.1664+39 delG)		
c.1291-15 c>g	<u>rs2293051</u>	18 (38)	22 (47)	7 (15)	22 (42)	26 (50)	4 (8)	0.445	0.36 (c.1664+39 delG)		
c.1664+39 delG	<u>rs3215291</u>	18 (37)	20 (42)	10 (21)	17 (43)	24 (46)	11 (21)	0.787	0.36 (c.1291-15c>g)		
c.1664+531 g>a	<u>rs4766841</u>	36 (69)	16 (31)	0 (0)	39 (78)	11 (22)	0 (0)	0.182§	0.34 (c.1664+39 delG)		
c.2202 T>C (p.I734I)	<u>rs2293054</u>	39 (75)	13 (25)	0 (0)	42 (81)	10 (19)	0 (0)	0.638	0.04 (c.*865c>t)		
c.2137-105 c>t	<u>rs2293055</u>	5 (9)	26 (50)	21 (41)	4 (7)	22 (42)	27 (51)	0.762	0.12 (c.1291-15c>g) 0.12 (c.3258 C>T)		
c.2706 C>T (p.H902H)	<u>rs1047735</u>	21 (45)	22 (47)	4 (8)	17 (33)	24 (46)	11 (21)	0.064	0.82 (c.2823+15a>g)		
c.2823+15 a>g	<u>rs2291908</u>	23 (49)	20 (42)	4 (9)	23 (44)	26 (50)	3 (6)	0.753	<u>0.82</u> (c.2706 C>T)		
c.3235+31 a>g	<u>rs1875140</u>	28 (53)	20 (37)	5 (10)	24 (47)	24 (47)	3 (6)	1.00	0.21 (c.3258 C>T)		
c.3258 C>T (p.D1086D)	<u>rs3741475</u>	50 (96)	2 (4)	0 (0)	32 (61)	17 (33)	3 (6)	< 0.001*	0.21 (c.3235+31a>g)		
c.3321 G>A (p.T1107T)	<u>rs78855995</u>	48 (100)	0 (0)	0 (0)	45 (90)	5 (10)	0 (0)	0.058 [§]	n.m.		
c.3588 T>C (p.T1196T)	<u>rs9658501</u>	24 (46)	17 (33)	11 (21)	23 (44)	17 (33)	12 (23)	0.800	0.20 (c.*203a>g)		
c.3827-42_3827-43 del ins TA	n.m.	46 (96)	2 (4)	0 (0)	50 (96)	2 (4)	0 (0)	0.746	0.04 (c.1664+39delG) 0.04 (c.2706C>T) 0.04 (c.2823+15a>g)		
c.*203 a>g	n.m.	19 (36)	24 (45)	10 (19)	17 (32)	27 (51)	9 (17)	0.478	0.20 (c.3588T>C)		
c.*276 c>t	rs2682826	45 (86)	6 (12)	1 (2)	41 (79)	8 (15)	3 (6)	0.262	0.03 (c.*1688a>c)		
c.*865 c>t	<u>rs41511544</u>	44 (86)	7 (14)	0 (0)	47 (92)	4 (8)	0 (0)	0.340§	0.04 (c.2202T>C) 0.04 (c.2137-105c>t) 0.04 (c.*203a>g) 0.04 (c.*1805c>g)		
c.*1688 a>c	<u>rs7132722</u>	46 (96)	2 (4)	0 (0)	45 (86)	7 (14)	0 (0)	0.324§	0.09 (c.3588 T>C)		
c.*1805 c>g	n.m.	44 (85)	6 (11)	2 (4)	41 (80)	8 (16)	2 (4)	1.00	0.13 (c.3235+31a>g)		

Table 2: Genotype distributions (percentages in parenthesis) of *NOS1* polymorphisms in 53 hypertrophic pyloric stenosis patients (IHPS) and in 48 controls; the wild-type alleles (W) have been underlined as opposed to varient alleles (p). Genotype statistics present exact p-values for the comparision between controls and IHPS patients. In those cases where the analysis was not possible, p-values were calculated based on a dominant genetic model (§). The corresponding amino acid variants for each mutation are shown in parenthesis. Note that all mutations were silent, with the exception of c.1181A>C, which leads to an amino acid change. We have observed a marginally significant occurrence of the polymorph allele in hetero- or homozygosis in 3 variants (c.-460g>a; c.2706C>T and c.3321G>A), and a significantly higher occurrence of the variant allele in the silent mutation c.3258C>T (p < 0.001). There was no deviation from the Hardy-Weinberg equilibrium in any of the polymorphisms or mutations. The linkage disequilibrium analysis (r2) is shown as highest correlation coefficient against all other variants. Note a correlation between the markers on exon 18 (c.2706C>T vs c.2823+15a>g, r2 = 0.82). N.m. = no matches found.

(data not shown). Since these results also imply that there was no loss-of-heterozygosity (LOH), and assuming that the chromosome 12q24.2-q24.31 does not constitute a "hot-spot" for LOH, the comparison of the results between the 8 patients with both blood and tissue DNA probes allows us to extrapolate the absence of LOH for the remaining 45 cases in which only blood DNA probes were available. (Figure 6).

In conclusion, our data supports only an adjuvant role for *NOS1* variants in IHPS (at best), since we did not find a conclusive and definitive association of the polymorphisms with the IHPS phenotype. However, we did show that *NOS1* variants have a significant albeit weak statistical association with the phenotype, thus precluding the total exclusion of an association [47].

Analyses of genomic rearrangements

All 80 HSCR and 38 CCHS patients had undergone complete sequence analysis of the *RET* proto-oncogene, which revealed 22 different mutations in 21 patients, including 2 nonsense, 13 missense, 3 splice-site and 4 silent mutations. In addition, several polymorphisms located in the coding or the intronic region of *RET* have been detected. Two HSCR patients harbored two mutations each - one missense and one silent mutation. Two non-consanguineous HSCR patients harbored the same missense mutation in c.2618 G>A (exon 15) [18]. Interestingly, we did not identify any deletion or amplification in these four genes in all patients, but in two cases we found a less than 50% reduction of the MLPA peak in exons 2 and 5 (Figure 7). Both patients harbored a single base pair substitution in the heterozygous state, which is located

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Figure 6: Schematic representation of families with IHPS (in black). The index-patient in each family has been analyzed for NOS1 mutations, shown in the respective adjacent tables. The variants were either heterozygous (Wp) or homozygous (pp). Note the predominance of the disease in males and the complex pattern of inheritance, as well as the occurrence of multiple polymorphisms in each patient. The silent mutation c.3258C was present in most of the patients, particularly those with affected family members.



in the binding region of the respective MLPA probe: c.159 C>T (exon 2) and c.1013 C>T (exon 5). All other mutations located in probe-binding regions such as c.1825 T>A (exon 10), c.1858 T>C (exon 10), c.2338 A>T (exon 13), c.2618 G>A (exon 15) in two patients, c.2734 G>A (exon 16) and the exon 13 c.2307 T>G variant did not show a detectable

influence on peak height. Additionally, we found two new RET variants not previously described in two patients with long-segment sporadic HSCR, namely a polymorphism in intron 9 (IVS9 +1G>A) located at a splice site and a nonsense mutation in exon 2 (c.110 G>A).

Although the MLPA assessment of rearrangements in the RET



proto-oncogene and in 3 other associated genes did not show any variants in 80 sporadic HSCR patients, we were able to conclude that genomic rearrangements in RET are indeed rare and were not responsible for the HSCR phenotype in individuals without identifiable germline RET variants, another important step in elucidating the origin of HSCR. [48].

Since we already investigated patients with HSCR, we decided to

look into those patients with CCHS alone and CCHS/HSCR to verify if in this subset of HSCR patients genomic rearrangements were present. Although the main genetic variants detected in CCHS patients have been mutations (deletions or expansions) in PHOX2b, mostly in the polyalanine tract in exon 3 [17,49,50,51], 14 non-polyalanine repeatexpansion mutations have also been found in 184 CCHS patients and were linked to a more severe phenotype, with increased prevalence of continuous ventilatory dependence, HSCR and neural crest tumors such as NB [52]. Compound effects of PHOX2b and RET variants in CCHS and CCHS/HSCR patients have also been demonstrated, showing that PHOX2b is the main responsible for CCHS, yet PHOX2b and RET contribute to the combined CCHS/HSCR phenotype [43]. Accordingly, a study by Sasaki et al. looking into variants of RET and associated genes (GDNF, GFRA1, PHOX2a, PHOX2b, HASH-1, EDN1, EDN3, EDNRB, and BDNF) in seven patients with isolated CCHS and three patients with HSCR showed several mutations of RET, GFRA1, PHOX2a, and HASH-1 genes in patients with or without mutations of the PHOX2b gene [53], while RET has also been associated with the CCHS phenotype (independently from PHOX2b) in two further studies [54,55]. Hence, microdeletions or amplifications in sensitive areas of RET may account for some of the CCHS/HSCR cases in which RET germline variants have not been identified. Again we tested this hypothesis by MLPA analyses of CCHS and CCHS/HSCR patients and again we did not identify any deletion or amplification in any of the studied samples. In patients who harbored the single base pair substitution c.2307 T >G (RET exon 13) in either a heterozygous or homozygous state, located in the binding region of the SALSA probe RET 5507-L4930, no reduction in peak size was observed, similarly to the previous data on HSCR patients in which other mutations located in probe-binding regions did not show a detectable influence on peak height [49]. In a previous analyses of the same samples with capillary DNA sequencing, two missense RET mutations (c.2522 C>T and c.2371 A>T) in two CCHS/HSCR patients and one variant located in intron

12 (IVS12 + 54 c>a) in one patient with CCHS alone were identified, which were not observed in over 100 healthy controls [43]. Additionally, the analysis of PHOX2b variants in CCHS and CCHS/HSCR patients confirmed the preponderance of PHOX2b germline mutations on the etiology of CCHS, as 12 different germline mutations were found in 28 CCHS or CCHS/HSCR patients (73.7%). Although no rearrangements were found, we were able to confirm that genomic rearrangements in RET are rare and exclude their role in the HSCR phenotype or for a "HSCR trait" in CCHS individuals without identifiable germline RET variants in our group of patients [21].

Comparative Genomic Hybridization and copy number variation analyses

The initial chromosomal analysis of the DSD patient revealed a 46,XX female karyotype with spontaneous chromosome instability in the majority of metaphases, consistent with FA, which was confirmed by a high spontaneous breakage index of 0.33 breaks per metaphase (normal rate ≤ 0.02) and excessively increased chromosome breakage after induction of cultured cells with mitomycin C, in which there were more than 10 breaks in 100% of the metaphases following exposure to 50ng/ml (normal rate ≤ 0.03 per metaphase) and 100ng/ml (normal rate ≤ 0.06 breaks per metaphase). This unusually high breakage rate suggested that the patient belonged to either the FA subtype D1 or N, and sequencing of the 13 exons of the PALB2/FANCN gene revealed a novel homozygous frameshift mutation c.1676_1677delinsG (c.1676_ c1677delAAinsG), which leads to a premature stop codon and protein truncation (pGln526ArgfsX1). Father, mother and brother of the patient were heterozygous for this mutation (figure 8). In addition, the patient was heterozygous for the common polymorphism in the 5'-UTR of WT1, c1-7G>T. The NB revealed 2 to 10 signals pro nucleus for both the control region and MYCN, reflecting aneuploidy. Some cell nuclei showed a small excess (up to 4-fold) of MYCN signals over control signals, suggesting the possibility that subclones with MYCN



Figure 9: Chromosomal projection of array comparative genomic hybridization (aCGH) results from the patient's WT (A) and NB (B), indicating deletions (green bars) and chromatin gains or amplifications (red bars). Of particular interest are the shared gainsor amplifications (circles) in the chromosomal regions 11p15.5 and 17q21.2-q25.3, "hot-spot" areas for genes associated with WT and NB development.

amplification had emerged during tumor progression. Screening for chromosome 1p deletions in the NB was only possible in few cell nuclei (n=61).

Finally, array- comparative genomic hybridization (aCGH) revealed a highly significant, complex pattern of amplifications or gains and deletions. These included: Amplification 11p15.5 (with target genes for NB, WT), Amplification 17q21.31-25.3 (also target genes for NB, WT); Chromatid gain 2p21-p25.2 (target genes for NB); Chromatid gain 7q11.23-q36.3 (target genes for NB); and Deletion 17p12-p1 3.3 (target genes for WT) (Figure 9).

Based on these data we were able to conclude that genomic alterations in terms of CNV, with overlapping amplifications in 11p15.5 and 17q21.31-25.3 in both tumors, including key genes for WT/NB development, were associated with the simultaneous development of WT and NB in this patient with FA-N, incomplete VL and extreme chromosomal instability. Consequently, genomic regions of shared amplification among "embryonal" type cancers may play a significant role in the biology of these tumors [56,57].

Conclusion

The development of biomedical sciences has allowed a much higher level of integration between basic research and clinical practice. Accordingly, the pediatric surgery specialty has increasingly utilized knowledge obtained in genetic research for tackling some of the still unanswered questions remaining in this area. In this short review, we demonstrated that a plethora of genetic variants are intimately associated with tumoral diseases or even common routine pediatric illnesses. Alternatively, in some other diseases, putative associations can and have been refuted through genetic investigation. Overall, we believe that the cases demonstrated have proven beyond any doubt the importance of molecular genetic research for the development of pediatric surgery, emphasizing that the application of molecular genetic techniques and the development of a "genetically oriented" thinking for diagnostic and therapy strategies will certainly broaden the expertise and knowledge of pediatric surgeons, ultimately resulting in a better quality of care and higher rate of success for pediatric patients.

References

- Granata C, Battistini E, Toma P, Balducci T, Mattioli G, et al. (1997) Mucoepidermoid carcinoma of the bronchus: a case report and review of the literature. Pediatr Pulmonol 23: 226-232.
- Welsh JH, Maxson T, Jaksic T, Shahab I, Hicks J (1998) Tracheobronchial mucoepidermoid carcinoma in childhood and adolescence: case report and review of the literature. Int J Pediatr Otorhinolaryngol 45: 265-273.
- Shin SY, Yoo HW, Lee BH, Kim KS, Seo EJ (2012) Identification of the mechanism underlying a human chimera by SNP array analysis. Am J Med Genet A 158A: 2119-2123.
- 4. Tuna M, Knuutila S, Mills GB (2009) Uniparental disomy in cancer.
- Spix C, Pastore G, Sankila R, Stiller CA, Steliarova-Foucher E (2006) Neuroblastoma incidence and survival in European children (1978-1997): report from the Automated Childhood Cancer Information System project. Eur J Cancer 42: 2081-2091.
- Longo L, Tonini GP, Ceccherini I, Perri P (2005) Oligogenic inheritance in neuroblastoma. Cancer Lett 228: 65-69.
- Maris JM, Weiss MJ, Mosse Y, Hii G, Guo C, et al. (2002) Evidence for a hereditary neuroblastoma predisposition locus at chromosome 16p12-13. Cancer Res 62: 6651-6658.
- Trochet D, Bourdeaut F, Janoueix-Lerosey I, Deville A, de Pontual L, et al. (2004) Germline mutations of the paired-like homeobox 2B (PHOX2B) gene in neuroblastoma. Am J Hum Genet 74: 761-764.
- Abel RM, Bishop AE, Moscoso G, Spitz L, Polak JM (1998) The ontogeny of innervation of the human pylorus. J Pediatr Surg 33: 613-618.

- Abel RM, Bishop AE, Dore CJ, Spitz L, Polak JM (1998) A quantitative study of the morphological and histochemical changes within the nerves and muscle in infantile hypertrophic pyloric stenosis. J Pediatr Surg 33: 682-687.
- Amiel J, Attié T, Jan D, Pelet A, Edery P, et al. (1996) Heterozygous endothelin receptor B (EDNRB) mutations in isolated Hirschsprung disease. Hum Mol Genet 5: 355-357.
- Edery P, Lyonnet S, Mulligan LM, Pelet A, Dow E, Abel L, Holder S, Nihoul-Fékété C, Ponder BA, Munnich A. (1994). Mutations of the RET proto-oncogene in Hirschsprung's disease. Nature 367 (6461): 319-20.
- Fitze G, Cramer J, Ziegler A, Schierz M, Schreiber M, et al. (2002) Association between c135G/A genotype and RET proto-oncogene germline mutations and phenotype of Hirschsprung's disease. Lancet 359: 1200-1205.
- Garcia-Barcelo M, Ganster RW, Lui VC, Leon TY, So MT, et al. (2005) TTF-1 and RET promoter SNPs: regulation of RET transcription in Hirschsprung's disease. Hum Mol Genet 14: 191-204.
- 15. Amiel J, Sproat-Emison E, Garcia-Barcelo M, Lantieri F, Burzynski G, Borrego S, Pelet A, Arnold S, Miao X, Griseri P, Brooks AS, Antinolo G, de Pontual L, Clement-Ziza M, Munnich A, Kashuk C, West K, Wong KK, Lyonnet S, Chakravarti A, Tam PK, Ceccherini I, Hofstra RM, Fernandez R. (2008). Hirschsprung Disease Consortium. Hirschsprung disease, associated syndromes and genetics: a review. J Med Genet 45:1-14.
- Chattopadhyay P, Pakstis AJ, Mukherjee N, Iyengar S, Odunsi A, et al. (2003) Global survey of haplotype frequencies and linkage disequilibrium at the RET locus. Eur J Hum Genet 11: 760-769.
- Weese-Mayer DE, Berry-Kravis EM, Zhou L, Maher BS, Silvestri JM, Curran ME, Marazita ML. (2003). Idiopathic congenital central hypoventilation syndrome: analysis of genes pertinent to early autonomic nervous system embryologic development and identification of mutations in PHOX2b. Am J Med Genet A. 123A(3):267-78.
- Fitze G, Appelt H, König IR, Görgens H, Stein U, et al. (2003) Functional haplotypes of the RET proto-oncogene promoter are associated with Hirschsprung disease (HSCR). Hum Mol Genet 12: 3207-3214.
- Engenheiro E, Møller RS, Pinto M, Soares G, Nikanorova M, et al. (2008) Mowat-Wilson syndrome: an underdiagnosed syndrome? Clin Genet 73: 579-584.
- Neveling K, Endt D, Hoehn H, Schindler D (2009) Genotype-phenotype correlations in Fanconi anemia. Mutat Res 668: 73-91.
- 21. A Serra, H Görgens, K Alhadad, G Fitze , HK Schackert. (2010). Analysis of RET, ZFHX1B, EDN3 and GDNF genomic rearrangements in 38 patients with Central Congenital Hyperventilation Syndrome (using multiplex ligationdependent probe amplification). Ann Hum Genet. 74 (4): 369-374.
- Benn PA, Perle MA. (1992). Chromosome staining and banding techniques. In: Rooney DE, Czepulkowski BH (eds.) Human Cytogenetics, vol. I. Oxford University Press, New York, pp 95-96.
- 23. Mohr B, Bornhäuser M, Thiede C, Schäkel U, Schaich M, et al. (2000) Comparison of spectral karyotyping and conventional cytogenetics in 39 patients with acute myeloid leukemia and myelodysplastic syndrome. Leukemia 14: 1031-1038.
- Stollmann B, Fonatsch C, Havers W (1985) Persistent Epstein-Barr virus infection associated with monosomy 7 or chromosome 3 abnormality in childhood myeloproliferative disorders. Br J Haematol 60: 183-196.
- Liehr T, Heller A, Starke H, Rubtsov N, Trifonov V, et al. (2002) Microdissection based high resolution multicolor banding for all 24 human chromosomes. Int J Mol Med 9: 335-339.
- Wellek S (2004) Tests for establishing compatibility of an observed genotype distribution with Hardy-Weinberg equilibrium in the case of a biallelic locus. Biometrics 60: 694-703.
- Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, et al. (2002) Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 30: e57.
- Tonon G, Modi S, Wu L, Kubo A, Coxon AB, et al. (2003) t(11;19)(q21;p13) translocation in mucoepidermoid carcinoma creates a novel fusion product that disrupts a Notch signaling pathway. Nat Genet 33: 208-213.
- Conkright MD, Canettieri G, Screaton R, Guzman E, Miraglia L, et al. (2003) TORCs: transducers of regulated CREB activity. Mol Cell 12: 413-423.
- 30. lourgenko V, Zhang W, Mickanin C et al. (2003). Identification of a family

of cAMP response element-binding protein coactivators by genome-scale functional analysis in mammalian cells. Proc Natl Acad Sci USA 100: 12147-12152, 20037.

- Winnes M, Enlund F, Mark J, Stenman G (2006) The MECT1-MAML2 gene fusion and benign Warthin's tumor: is the MECT1-MAML2 gene fusion specific to mucuepidermoid carcinoma? J Mol Diagn 8: 394-395.
- Okabe M, Miyabe S, Nagatsuka H, Terada A, Hanai N, et al. (2006) MECT1-MAML2 fusion transcript defines a favorable subset of mucoepidermoid carcinoma. Clin Cancer Res 12: 3902-3907.
- Behboudi A, Enlund F, Winnes M, Andres Y et al. (2006). Molecular Classification Of Mucoepidermoid Carcinomas – Prognostic Significance Of The MECT1-MAML2 Fusion Oncogene. Genes, Chromosomes & Cancer 45: 470-481
- 34. Serra A, Schackert HK, Mohr B, Weise A, Liehr T, et al. (2007) t(11;19) (q21;p12~p13.11) and MECT1-MAML2 fusion transcript expression as a prognostic marker in infantile lung mucoepidermoid carcinoma. J Pediatr Surg 42: E23-29.
- 35. Alonso G, Pasqualini T, Busaniche J, Ruiz E, Chemes H (2007) True hermaphroditism in a phenotypic male without ambiguous genitalia: an unusual presentation at puberty. Horm Res 68: 261-264.
- Niu DM, Pan CC, Lin CY, Hwang B, Chung MY (2002) Mosaic or chimera? Revisiting an old hypothesis about the cause of the 46,XX/46,XY hermaphrodite. J Pediatr 140: 732-735.
- 37. Grati FR (2014) Chromosomal Mosaicism in Human Feto-Placental Development: Implications for Prenatal Diagnosis. J Clin Med 3: 809-837.
- Souter VL, Parisi MA, Nyholt DR, Kapur RP, Henders AK, et al. (2007) A case of true hermaphroditism reveals an unusual mechanism of twinning. Hum Genet 121: 179-185.
- Serra A, Denzer F, Hiort O, Barth TF, Henne-Bruns D, et al. (2015) Uniparental Disomy in Somatic Mosaicism 45,X/46,XY/46,XX Associated with Ambiguous Genitalia. Sex Dev 9: 136-143.
- Perri P, Bachetti T, Longo L, Matera I, Seri M, et al. (2005) PHOX2B mutations and genetic predisposition to neuroblastoma. Oncogene 24: 3050-3053.
- Raabe EH, Laudenslager M, Winter C, Wasserman N, Cole K, et al. (2008) Prevalence and functional consequence of PHOX2B mutations in neuroblastoma. Oncogene 27: 469-476.
- 42. Fitze G, König IR, Padditz E, Serra A, Schläfke M, Roesner D, Ziegler A, Schackert HK. (2008). A compound effect of PHOX2B germline mutations and RET proto-oncogene variants results in the Congenital Central Hypoventilation Syndrome combined with Hirschsprung Disease. Am J Hum Genet A. 146A(11):1486-9
- 43. Longo L, Panza E, Schena F, Seri M, Devoto M, et al. (2007) Genetic predisposition to familial neuroblastoma: identification of two novel genomic regions at 2p and 12p. Hum Hered 63: 205-211.
- 44. de Pontual L, Pelet A, Trochet D, Jaubert F, Espinosa-Parrilla Y, et al. (2006)

Mutations of the RET gene in isolated and syndromic Hirschsprung's disease in human disclose major and modifier alleles at a single locus. J Med Genet 43: 419-423.

- 45. Serra A, Häberle B, König IR, Kappler R, Suttorp M, et al. (2008) Rare occurrence of PHOX2b mutations in sporadic neuroblastomas. J Pediatr Hematol Oncol 30: 728-732.
- 46. Saur D, Vanderwinden JM, Seidler B, Schmid RM, De Laet MH, Allescher HD. (2004). Single-nucleotide promoter polymorphism alters transcription of neuronal nitric oxide synthase exon 1c in infantile hypertrophic pyloric stenosis. Proc. Nat. Acad. Sci. 101: 1662-1667.
- Serra A, Schuhardt K, Genuneit J, Schackert-Görgens H, Leriche C, Schackert HK, Fitze G. (2011). Genomic variants in the coding region of NOS1 in infantile hypertrophic pyloric stenosis. J Pediatr Surg. 46(10):1903-8.
- 48. Serra A, Görgens H, Alhadad K, Ziegler A, Fitze G, et al. (2009) Analysis of RET, ZEB2, EDN3 and GDNF genomic rearrangements in 80 patients with Hirschsprung disease (using multiplex ligation-dependent probe amplification). Ann Hum Genet 73: 147-151.
- 49. Amiel J, Laudier B, Attié-Bitach T, Trang H, de Pontual L, et al. (2003) Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. Nat Genet 33: 459-461.
- Gaultier C, Dauger S, Simonneau M, Gallego J (2003) Genes modulating chemical breathing control: lessons from mutant animals. Respir Physiol Neurobiol 136: 105-114.
- Amiel J, Laudier B, Attié-Bitach T, Trang H, de Pontual L, et al. (2003) Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. Nat Genet 33: 459-461.
- Berry-Kravis EM, Zhou L, Rand CM, Weese-Mayer DE (2006) Congenital central hypoventilation syndrome: PHOX2B mutations and phenotype. Am J Respir Crit Care Med 174: 1139-1144.
- Sasaki A, Kanai M, Kijima K, Akaba K, Hashimoto M, et al. (2003) Molecular analysis of congenital central hypoventilation syndrome. Hum Genet 114: 22-26.
- 54. Kanai M, Numakura C, Sasaki A, Shirahata E, Akaba K, et al. (2002) Congenital central hypoventilation syndrome: a novel mutation of the RET gene in an isolated case. Tohoku J Exp Med 196: 241-246.
- 55. Sakai T, Wakizaka A, Nirasawa Y (2001) Congenital central hypoventilation syndrome associated with Hirschsprung's disease: mutation analysis of the RET and endothelin-signaling pathways. Eur J Pediatr Surg 11: 335-337.
- Bissig H, Staehelin F, Tolnay M, Avoledo P, Richter J, et al. (2002) Cooccurrence of neuroblastoma and nephroblastoma in an infant with Fanconi's anemia. Hum Pathol 33: 1047-1051.
- Serra A, Eirich K, Winkler AK, Mrasek K, Göhring G, et al. (2012) Shared Copy Number Variation in Simultaneous Nephroblastoma and Neuroblastoma due to Fanconi Anemia. Mol Syndromol 3: 120-130.

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