UNIVERSITA' DEGLI STUDI DEL PIEMONTE ORIENTALE

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1) SEARCH FOR GENES INVOLVED IN ISS

2) SEARCH FOR NOVEL GENES INVOLVED IN IGHD THROUGH WHOLE-EXOME SEQUENCING

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Genetic determination of Height

Height is a highly heritable and easily measurable complex continuous trait determined by a multitude of genetic, hormonal, nutritional and other environmental determinants. Resemblances in height between relatives suggest that 80% of height variation is under genetic control with the rest controlled by environmental factors such as diet and disease exposure ([1]McEvoy BP et al 2009)

In the past, several linkage genome scans have attempted to identify genetic factors involved in the determination of human height, leading to suggestive or significant linkage on many chromosomes. However, no region was consistently highlighted across scans and no gene was definitively associated to the determination of normal stature.

Recently several Genome-wide association study (GWAS) identified a subset of about 180 variations in the human genome that contribute to the determination of height. These variants, however, have small effect sizes and collectively explain approximately 10% of the heritability of height. A recent study ([2]Chan Y. et al 2011) indicated that these 180 common variants are robustly associated with stature when studied as a quantitative trait in the general population, whereas a similar strong association is not present at the extreme short tails of the height distribution. The polygenic model starts to break down in the extreme short individuals.

Short stature has been defined as height <-2 Standard Deviation (SD) below the mean for a given age, sex and population group and represents the most common cause for paediatric consultation.

In presence of “proportionate short stature”, the measurement of serum GH concentration is used to categorize two main classes of growth disorders: short stature caused by GH deficiency (GHD), and idiopathic short stature (ISS).

Growth hormone deficiency (GHD) encompasses a group of different aetiological disorders. It may occur in isolation (IGHD), or associated with other anterior and posterior pituitary hormone deficiencies. The condition may be sporadic or familial. The incidence is between 1 in 4000 and 1 in 10 000 live births ([3]Rona R.J. et al 1977), with the majority of cases being idiopathic in origin. Familial cases account for 5–20% of cases, suggesting a genetic aetiology (autosomal dominant or recessive or X-linked) for the condition.

ISS describes a heterogeneous group of children consisting of many presently unidentified causes of short stature ([4]Cohen P. et al 2008), that presents the following criteria: normal size for gestational age at birth, normal body proportions, no evidence of endocrine deficiency, no evidence of chronic organic disease, no psychiatric disease or severe emotional disturbance and

normal food intake. The most frequent genetic cause of ISS is represented by mutations and deletions in the Short Stature Homeobox gene (SHOX), whose heterozygous deletion is responsible for the short stature seen in Léri–Weill dyschondrosteosis and Turner Syndrome. Different studies reported mutations and deletions of this gene and the downstream enhancer region in 7–15% of patients diagnosed with ISS ([5]Schneider K.U. et al 2005, [6]Benito-Sanz S. et al 2011, [7]Sabherwal N. et al 2007).

Thus only a minority of the short stature patients received a precise diagnosis with the identification of the genetic defect.

1.Search for genes involved in ISS

1.1 IDENTIFICATION OF SHOX MUTATIONS AND DELETIONS IN ISS PATIENTS.

The Short Stature Homeobox containing gene (SHOX), is located on the very tip of the short arms of both sex chromosomes X and Y within the telomeric part of pseudoautosomal region 1 (PAR1, Xp22.33 and Yp11.3) which comprises about 2.6 Mb ([8] Binder G., 2011). SHOX encodes for a paired-related homeodomain transcription factor. There are seven known SHOX exons encoding two different isoforms SHOXa and SHOXb that employ different 3′ terminal exons (exon 6a or 6b). The SHOX gene contains 2 recognized motifs: a homeodomain, which is responsible for the DNA binding, encoded by exons 3 and 4 and a C-terminal-located OAR domain which is relevant for its transactivational activity. The homeodomain transcription factor encoded by this gene is expressed during fetal life in the development of bone tissue in the distal humerus, radius, ulna, wrist, similar bones in the legs, first and second pharyngeal arches ([9]Oliveira C.S. et al., 2011). The protein is specifically expressed in the growth plate of hypertrophic chondrocytes and seems to have a main role in the regulation of the differentiation and proliferation of these cells.

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The aim of this work the search for point mutations and deletions in a large cohort of patients with mild and severe ISS.

1.1.1 Materials and methods

Ninety-five ISS patients, 85 sporadic and 10 familial, have already been screened for the presence of SHOX mutations and deletion. Thirthy-nine had a severe phenotype with height between -3,9 and -2 SDS and 56 had a mild phenotype between -1,9 and -0,9 SDS. The mean age of the patients at the time of diagnosis was 8.3±3.9. We screened the gene through direct sequencing of all the exons and intron-exons boundaries. The search for deletions of the whole SHOX gene, of single exons and of the enhancer region has been performed by Multiple Ligation-dependent Probe Amplification assay (MLPA, MRC-Holland kit-P018-F1).

1.1.2 Results

All the exons and the splicing junctions of SHOX gene were sequenced in 95 short stature patients. We detected 3 exonic variation: Stop226Arg, Asp58Glu and Leu290Leu (Table 1). This latter mutation had been previously reported in a SHOX database with a frequency of 0,47% (http://www.hd-lovd.uni-hd.de). The deleterious nature of this variant has to be demonstrated.

The MLPA revealed 4 deletion/duplication in our cohort of patients of different size (Table 1).

Mutations	Patient	SDS
c.676 T>C (Stop226Arg)	1	-1,7
c.174 C>A (Asp58Glu)	1	-2,8
c.870 G>A (Leu290Leu)	2	-2,4
		-2,2
Duplication	1	-1,3
Enhancer: heterozygous deletion (SHOX-area probe 05645-L05099 and probe 05646-L15507)	2	-1,8
		-1,8
Enhancer: homozygous deletion (SHOX-area probe 09335-L19679)	1	-1,5
Enhancer: heterozygous deletion	1	-1,9

Table 1: SHOX variants detected by sequence analysis and MPLA in 95 ISS patients.

1.1.3 Discussion

Routine diagnostic testing of the SHOX patients allowed the detection of different mutations (Table 1). We detected point mutations in 3 patients with severe phenotype with a frequency of 7,7%. In 6 patients with mild phenotype, we identified mutations with a frequency 10,71%. Interestingly, our preliminary data have shown that SHOX mutations are frequent in patients with mild short stature, diagnosticated in prepubertal age. In conclusion, this study suggests the relevance of SHOX screening not only in patients with severe ISS but also in the mild short stature patients.

1.2 MUTATION SCREENING AND COPY NUMBER ANALYSIS OF HMGA2 GENE IN A COHORT OF ISS PATIENTS

Among the candidate genes the high-mobility group A2 gene (HMGA2, NM_003483) could be considered a suitable candidate for the presence of point mutations and deletions in the cases where variations in the known SHOX gene have been previously excluded. This is the first study based on HMGA2 sequence analysis in a large cohort of sporadic and familial ISS patients.

HMGA2, earlier known as HMGIC, encodes a chromatine-associated protein that belongs to the non-histone chromosomal high-mobility group (HMG) protein family. HMGA2 is located on chromosome 12q14 and contains 3 AT-hook motifs encoded by the first 3 exons, involved in DNA binding; a 11-amino acid sequence characteristic of HMGA2 and absent in the other family proteins (linker domain) encoded by exon 4 and part of exon 5 and an acidic C-terminal domain encoded by the fifth exon ([10]Mari F. et al. 2009).

HMGA2 has several isoforms; HMGA2a is the major isoform, with proven transcription factor function. The other five splice variants (b–f) have been identified by Hauke et al.: they lack exons 4 and 5 which are replaced by sequences derived from part of intron 3. The expression of these isoforms has been detected in several normal and tumor tissues and also in cultured cells ([11]Hauke S. et al 2005); however, the function of these isoforms is currently unknown. There are several line of evidence that indicate HMGA2 gene as possible candidate gene playing a role in regulating human growth

There are several line of evidences that indicate HMGA2 as a possible candidate gene playing a role in regulating human growth.

Previous GWAS studies independently confirmed that 3 SNPs (rs1042725, rs7968682 and rs7968902) in the high mobility group-A2 gene (HMGA2) are common variants influencing human height across different populations ([12]Takeshita H. et al. 2011). All of these three SNPs are located within the 3′-UTR region of HMGA2, which may directly or indirectly influence the mRNA stability of HMGA2 ([13]Yang T.L. et al. 2010).

Moreover the gene was considered as a strong biologic candidate for height because loss of function of HMGA2 in the mouse results in the pygmy phenotype that combines pre and postnatal growth failure, with resistance to the adipogenic effect of overfeeding ([10]Mari F. et al. 2009).

In humans CGH-Array results showed that patients with a microdeletions on the chromosomal region 12q14 of different size have as common features short stature. The Critical Region in patients with severe short stature include the HMGA2 gene (Figure 1, [14] Fadel Alyaqoub et al.

2012). Interestingly, it has been identified a submicroscopic deletion disrupting HMGA2 in a boy with idiopathic proportionate short stature, without any other abnormalities. It has been found that the deletion co-segregated perfectly with reduced adult height in the extended family of the boy ([15]Buysse K. et al.2009).

All these data provide evidence that a heterozygous deletion of HMGA2 could causes growth failure.

1.2.1 Materials and methods

One hundred-four sporadic and familial patients with Idiopathic Short Stature were recruited by the Unit of Pediatrics of Department of Health Sciences of Novara: 48 males and 56 females, with a height between -3,8 and -2 SDS (Standard Deviation Score).

A written informed consent was obtained from the patient's parents since they were all aged less than 18. Genomic DNA of short stature patients was extracted from the blood samples by standard salting-out method ([16] Miller et al.,1988).

Sequence analysis

Primers were designed to amplify all 5 coding exons and the 5'- and 3'-flanking regions, using NCBI database (http://www.ncbi.nih.gov/), Primer3 Input (Version 0.4.0, http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi/). The primer pair specificity was controlled with the Basic local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

The PCR reaction was carried out with the GoTaq Flexi DNA polymerase (Promega) in a 15 ul reaction volume, at an annealing temperature of 58°C for exon 2 and 4 and 62℃ for exon 1,3, 5.

The PCR products were purified using Affymerix ExoSAP-IT enzymatic PCR clean up system (37℃ 15 min and 80℃ 15 min) and directly sequence d with Big Dye Terminator kit (Applied Biosystems) and automatic sequencer ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Multiplex ligation-dependent probe amplification

Multiplex ligation-dependent probe amplification (MLPA) for all exons of HMGA2 gene was performed by means of P323 commercial kit according to the manufacturer's protocol (MRC Holland, Amsterdam, The Netherlands).

1.2.2 Results

All the exons and the splicing junctions of the novel candidate gene HMGA2 were sequenced in 104 short stature patients. In one patient we identified a synonymous heterozygous exonic variation, namely Ser105Ser (exon 5, rs 201860923, Table 2) already reported in the Exome Variant Server, with 0,07% of frequency (http://evs.gs.washington.edu/EVS/).

Additionally, we identified several known polymorphisms in HMGA2, all outside the coding regions (Table 2).

Exon	**Rs number**	**Allele**	**MAF**			
			ISS	dbSNP	EA	AA
3	60081000	T>**C**	1,90%	4,9%	0,23%	13,4%
3	73115423	T>**A**	5,8%	1,6%	2,46%	0,47%
3	3834468	-/**G**	9,6%	8,3%	8,93%	8,4%
5	57800850	C>**T**	0,96%	3,9%	0,07%	12,76%
5	201860923	T>**C**	0,96%	C=0,000/1	0,07%	0,15%

Table 2: HMGA2 variants detected by sequencing in 104 ISS patients. MAF, minor allele frequency; NA, not available. The minor allele is indicated in bold. EA, European American population; AA, African American population data reported in the Exome Variant Server.

As shown in the table 2, there are no statistically significant differences between the variant frequencies detected in our ISS patients and the frequencies of the European American population.

We performed a multiplex ligation probe amplification (MLPA) to detect deletions and duplications of the whole HMGA2 gene and of single exons. In 104 patients we didn't detect any copy number variations.

In our cohort of ISS patients sequencing and copy number variation analysis did not show any pathological variants in HMGA2, suggesting that mutations in this gene are not a frequent cause of ISS.

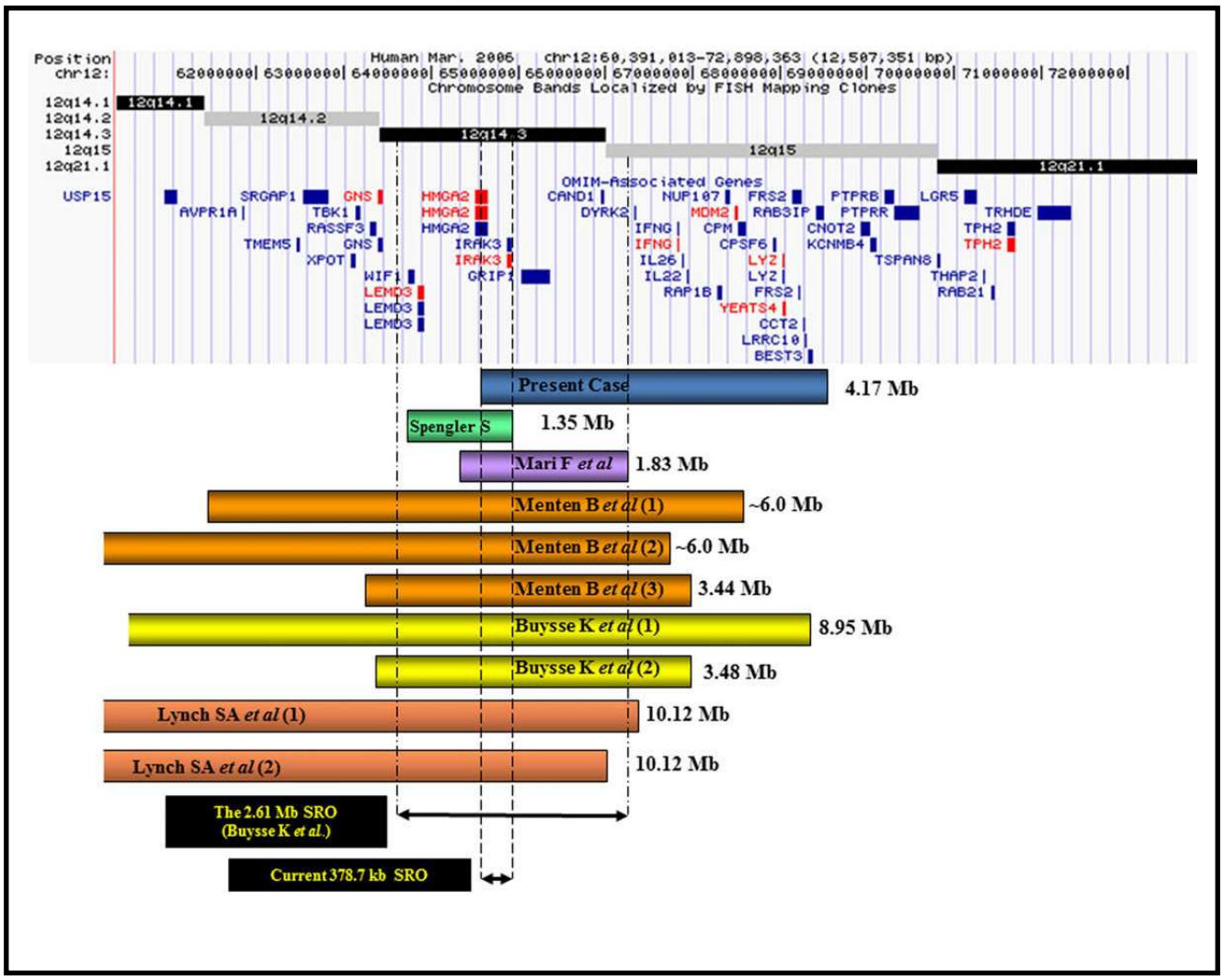

Figure 1. Comparison of all reported 12q14 microdeletions detected by array-CGH, along with the initial and current smallest region of overlap ([14] Fadel Alyaqoub et al. 2012).

2.Search for novel genes involved in IGHD through Whole-Exome sequencing

Whole-Exome sequencing (WES) has, in the last three years, proven its potential in the discovery of novel Mendelian disease genes ([17]Bamshad et al, 2011), as well as in the diagnosis of rare conditions ([18]Choi et al, 2009; [19]Worthey et al, 2011), or of known genetic conditions with an unusual phenotypic presentation ([20]Majewski et al, 2011).

IGHD is characterized by short stature of less than -2SDS and low levels of Growth Hormone after the stimulation test. Mutations in the gene encoding the GH (*GH1*) and Growth Hormone Releasing Hormone Receptor (*GHRHR*) have been identified in only 10-30% of familial cases of IGHD. It is conceivable that other genes are involved in monogenic IGHD ([21]Mullis PE, 2005).

We collected 10 IGHD familial cases (Figure 2) that have been already screened for the presence of mutations in specific known genes involved in the disease (GH1, GHRHR), in which mutations were excluded.

In order to identify novel genes involved in IGHD, we will perform whole-exome sequencing on the affected members of the 10 families, in which the genetic causes of short stature remained unidentified by conventional approaches.

To date we sequenced through the whole exome and targeted sequencing experiments the affected member of family #1 and #2 (Figure 2, red circle).

A family-based sequencing approach should actually allow an easier filtering and prioritization of the variants identified by massive parallel sequencing ([22]Robinson et al, 2011; [17]Bamshad et al, 2011), by focusing on those segregating with the condition.

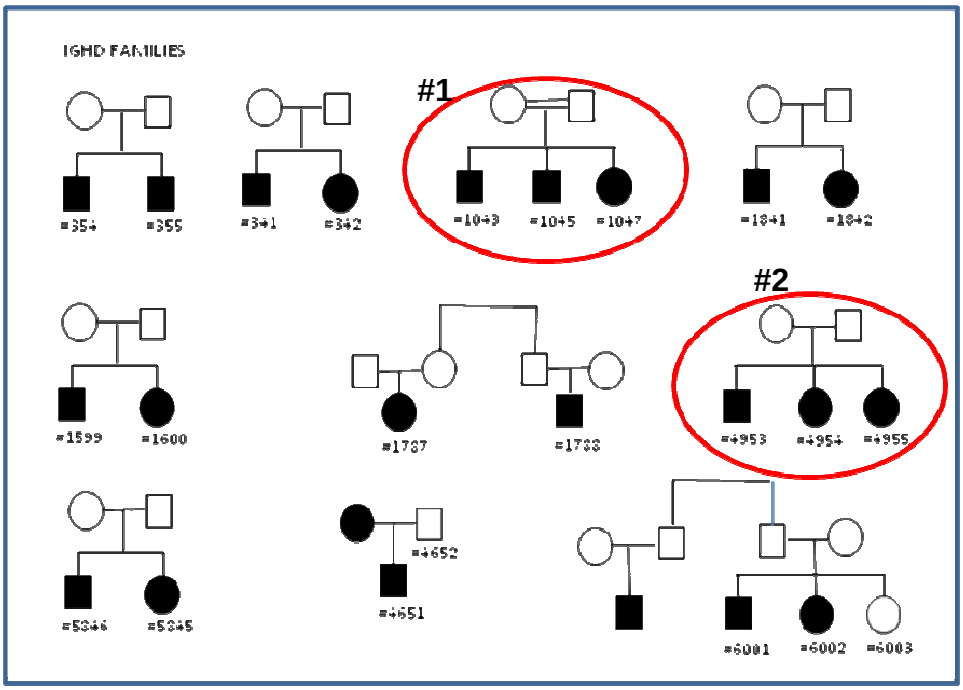

Figure 2. Pedigree of the patients that will be studied through whole exome sequencing. Family 1 and family 2 have been sequenced up to now.

2.1 Materials and methods

The Whole Exome Sequencing have been performed in the laboratory of PAVIA were a Genome Analyzer IIx, a new generation desktop sequencer MiSeq (both from Illumina, San Diego, CA, USA) and the appropriate informatics infrastructures are available.

Family 1: a Turkish family with three affected siblings born to consanguineous parents was considered for the present study (figure 3). The affected siblings resulted negative for the presence of mutation in the GH1 and GHRHR genes . The WES was carried out in one of the three siblings (II-2) as the DNA of the other two was not enough for this analysis.

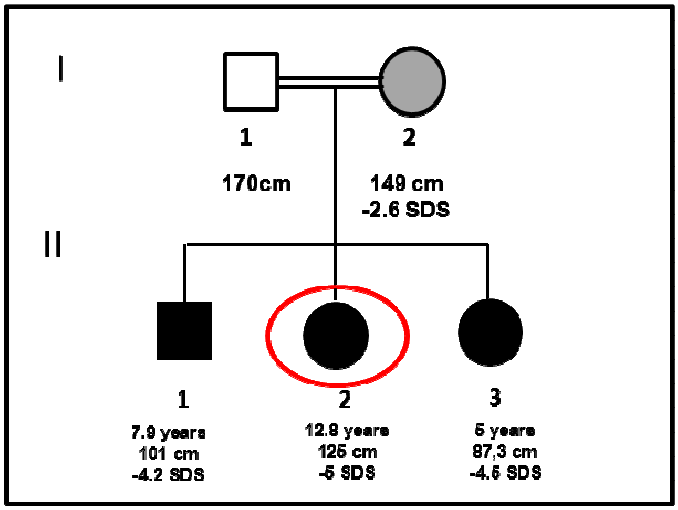

Figure 3: Pedigree of family 1, studied through WES

Whole genome amplification kit (illustra, Genomiphi V2, UK) was used to amplify the DNA of the other two siblings who were not analysed by WES.

Sanger sequencing was performed to confirm the prioritized variants identified by WES and for the segregation analysis in all the three siblings and parents.

Family 2: an Italian family with four siblings of which three affected, was considered for the present study (figure 4). The affected siblings resulted negative for the presence of mutation in the GH1 and GHRHR genes. Recently we knew that there is a fourth sister (in blank), without GH deficiency. We are waiting for the test results of the parents (in grey).

The Whole Exome Sequencing (WES) was carried out in all three siblings (II-1-2-3, red circle).

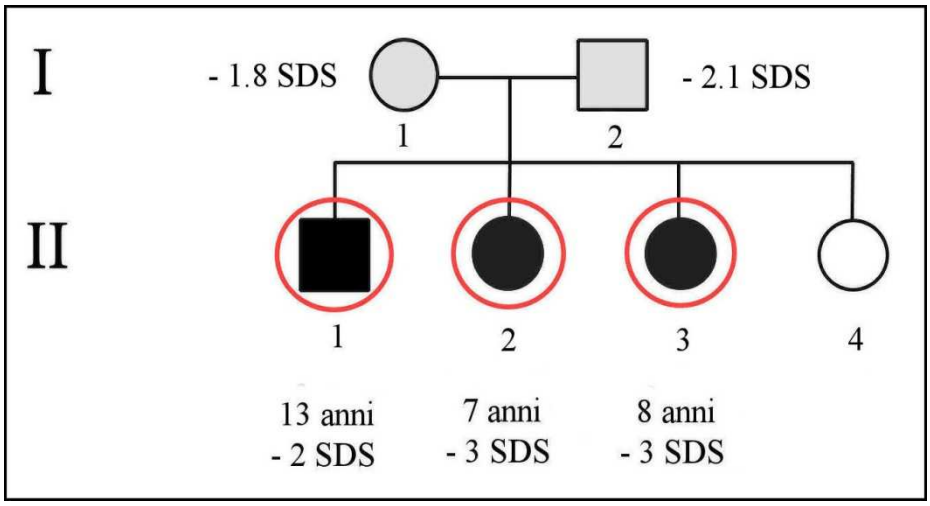

Figure 4: Pedigree of family 2, studied through WES

Sanger sequencing was performed to confirm the prioritized variants identified by WES and for the segregation analysis in all the three siblings and parents.

2.2 Results

Family1

WES experiment detected 17000 variants that were initially filtered and prioritized by focusing on the recessive model of inheritance (figure 5). The variants which were at the homozygous state were considered as the primary filtering criteria. They were considered on the basis of their presence and allele frequency in publicly available genomic variants databases such as dbSNp, 1000 Genome project (http://browser.1000genomes.org), Exome Variant Server database (http://snp.gs.washington.edu/EVS/) and as well as in uncorrelated samples included in our in-house database, filtering out those likely to be common in the general population (figure 5).

Fourty-three variants in 41 genes were short listed considering only the exonic variants which are absent in dbSNP or with a frequency less than 1% (Table 3). No obvious candidate genes were present among the selected genes. After sequencing all these variations in the 3 siblings and in the parents, two were present at the homozygous state in the children and at the heterozygous state in the parents: A71V in ELN (Elastin) and G1669S in MYH7B (Myosin Heavy Chain 7B). However, these two genes were identified to be involved in Cutis laxa (ELN) and Hypertrophic Cardiomyopathy (MYH7B) but not related to IGHD. Hence, these two genes could not be considered as the candidate genes for IGHD.

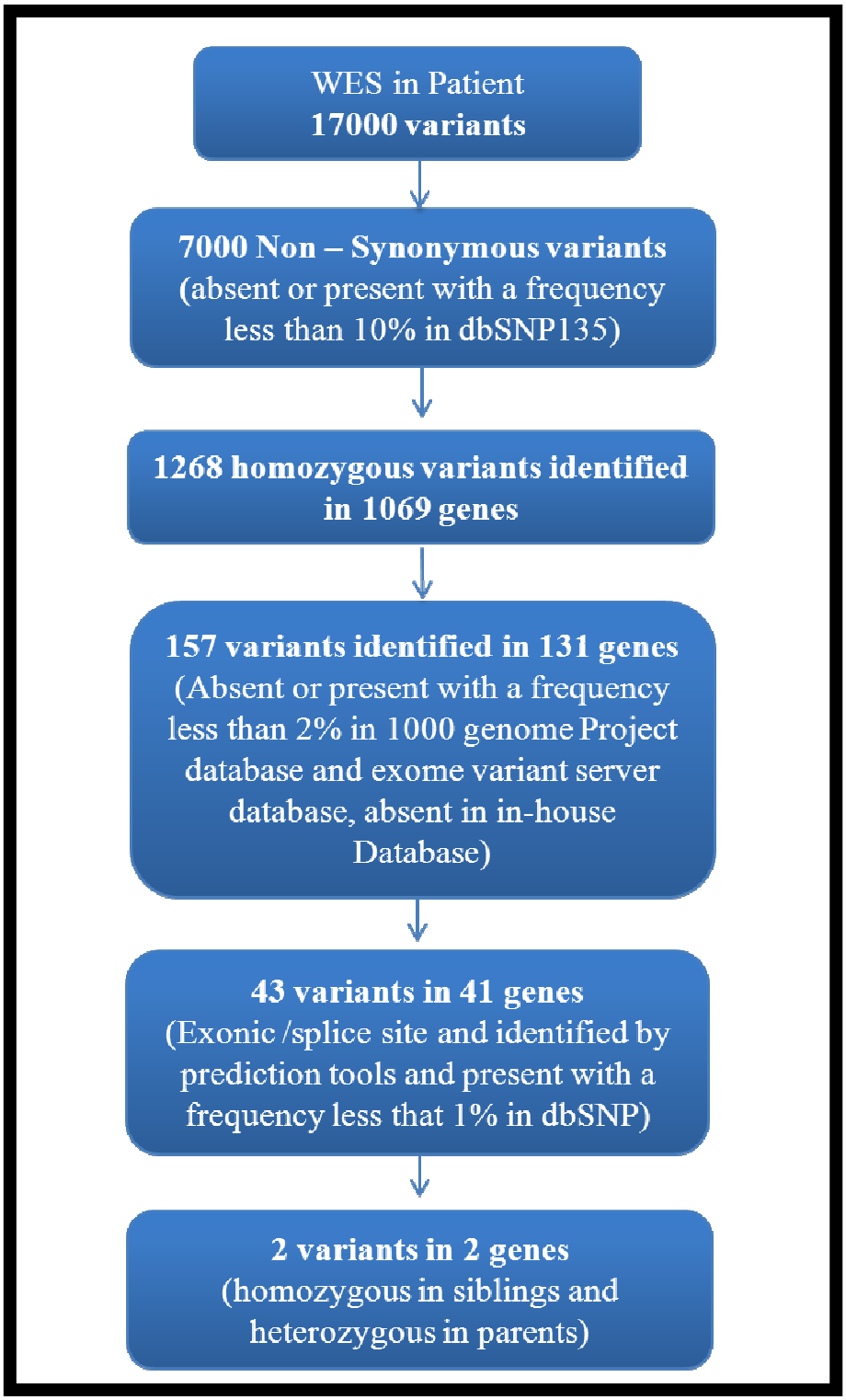

Fig. 5: filtering and prioritization of the variants identified by WES in one of the three siblings (II-2).

CHOMOSOME	POSITION	refUCSC	OBSERVED	UCSC GENE	REGION	CHANGE	AA CHANGE
1	36181813	G	C	C1orf216	exon	missense	A>G
1	152681689	G	GGGCTGCTGTAGCTCTGGC	LCE4A	exon	na	na
1	161172233	C	A	NDUFS2	exon	missense	P>T
1	27124130	C	T	PIGV	exon	missense	P>L
1	32647133	C	G	TXLNA	exon	missense	R>G
1	241886645	G	C	WDR64	exon	missense	K>N
1	247151487	C	T	ZNF695	exon	missense	M>I
2	39187217	G	T	LOC100271715	exon	missense	V>L
2	132201189	G	C	LOC401010	exon	missense	S>R
2	141232800	C	T	LRP1B	exon	missense	A>T
2	137928320	C	T	THSD7B	exon	missense	T>M
2	145157076	G	A	ZEB2	exon	missense	R>C
3	156870948	A	G	CCNL1	splice-site	na	na
3	160131347	A	G	SMC4	exon	missense	N>S
3	160132026	G	T	SMC4	exon	missense	R>S
5	1084074	G	A	SLC12A7	splice-site	na	na
7	73455561	C	T	ELN	exon	missense	A>V
7	6547902	G	A	GRID2IP	exon	missense	P>L
7	72721449	C	T	NSUN5	exon	missense	D>N
7	148907686	C	T	ZNF282	exon	missense	P>L
11	116692393	G	T	APOA4	exon	missense	N>K
11	13398206	G	A	ARNTL	exon	missense	G>R
11	76255829	C	T	C11orf30	exon	missense	S>L
11	113852013	C	T	HTR3A	exon	missense	P>L
11	118922659	C	T	HYOU1	exon	missense	E>K
11	118376925	A	G	MLL	exon	missense	I>V
11	78449583	G	A	ODZ4	exon	missense	T>I
11	397331	G	C	PKP3	exon	missense	R>P
11	117066651	G	A	SIDT2	exon	missense	R>Q
12	121454148	C	T	C12orf43	exon	missense	G>R
12	123003416	C	T	RSRC2	exon	missense	R>K
12	119568596	G	A	SRRM4	exon	missense	S>N
15	63982745	C	A	HERC1	exon	missense	A>S
15	64025223	T	C	HERC1	exon	missense	N>S
17	6663894	G	A	XAF1	exon	missense	R>H
18	77171480	T	C	NFATC1	exon	missense	L>P
19	49303352	G	T	BCAT2	exon	missense	F>L
19	2341192	TG	T	KIAA1532	exon	na	na
19	50951640	G	A	MYBPC2	exon	missense	V>I
19	15580658	G	A	PGLYRP2	exon	missense	R>W
19	58213743	G	A	ZNF154	exon	nonsense	R>STOP
19	12462839	G	A	ZNF442	exon	missense	P>L
20	33588193	G	A	MYH7B	exon	missense	G>S

Table 3: Candidate genes identified by focusing on the recessive model of inheritance

Family2

WES experiment detected 22000 variants in each patients, according to literature data ([15]Bamshad et al., 2011), that were initially filtered and prioritized by focusing on the autosomal dominant model of inheritance (figure 6). The variants which were at the heterozygous states, shared by all 3 affected individuals were considered as the primary filtering criteria. They were considered on the basis of their presence and allele frequency in publicly available genomic variants databases such as dbSNp, 1000 Genome project (http://browser.1000genomes.org), Exome Variant Server database (http://snp.gs.washington.edu/EVS/) and as well as in uncorrelated samples included in our in-house database, filtering out those likely to be common in the general population (figure 6).

Ninety-eight rare variants in 98 genes were short listed considering only the heterozygous variants, shared by all 3 patients, of which 48 exonic, 14 intronic and 36 found in 3'/5' UTR (Table 4). We started considering the exonic variant filtering out those predicted non-disease causal variants by the software Polyphen 2 (http://genetics.bwh.harvard.edu/pph2/index.shtml). In this way we reduced the number of potential candidate variants at 23.
The current strongest candidate gene from the literature is EGR1 (Early Growth Response 1) that encodes for early growth response protein 1, that is expressed in anterior pituitary gland ([23]Topilko et al., 1998). The exome data reveal a 24-bp deletion of 8 aminoacids: Tyr, Pro, Ser, Pro, Val,Ala, Thr, Ser (Figure 7).
At the time of the analysis the deletion was not present in publicly available genomic variants databases. Later it has been reported in Exome Variant Server database with a frequency of 0,4% in 12500 individuals.
The deletion was validated in the 3 probands by Sanger sequencing that was also be used to test the segregation in other family members. It has been found that they inherited deletion from mother.

Moreover, by alleles separation on gel electrophoresis, the deletion was tested in a group of 404 normal stature individuals, matched for age, sex and geographical origin and the deletion was not found. Then we analyzed 234 sporadic cases, of which 148 IGHD and 86 CPHD to better evaluated a possible pathogenetic role of the variation. We detected the deletion in 1 IGHD patient.

To examine in depth the role of EGR1 in the pathology, we sequenced the 2 exons of the gene in 50 IGHD patients. We didn’t detect any pathological variants.

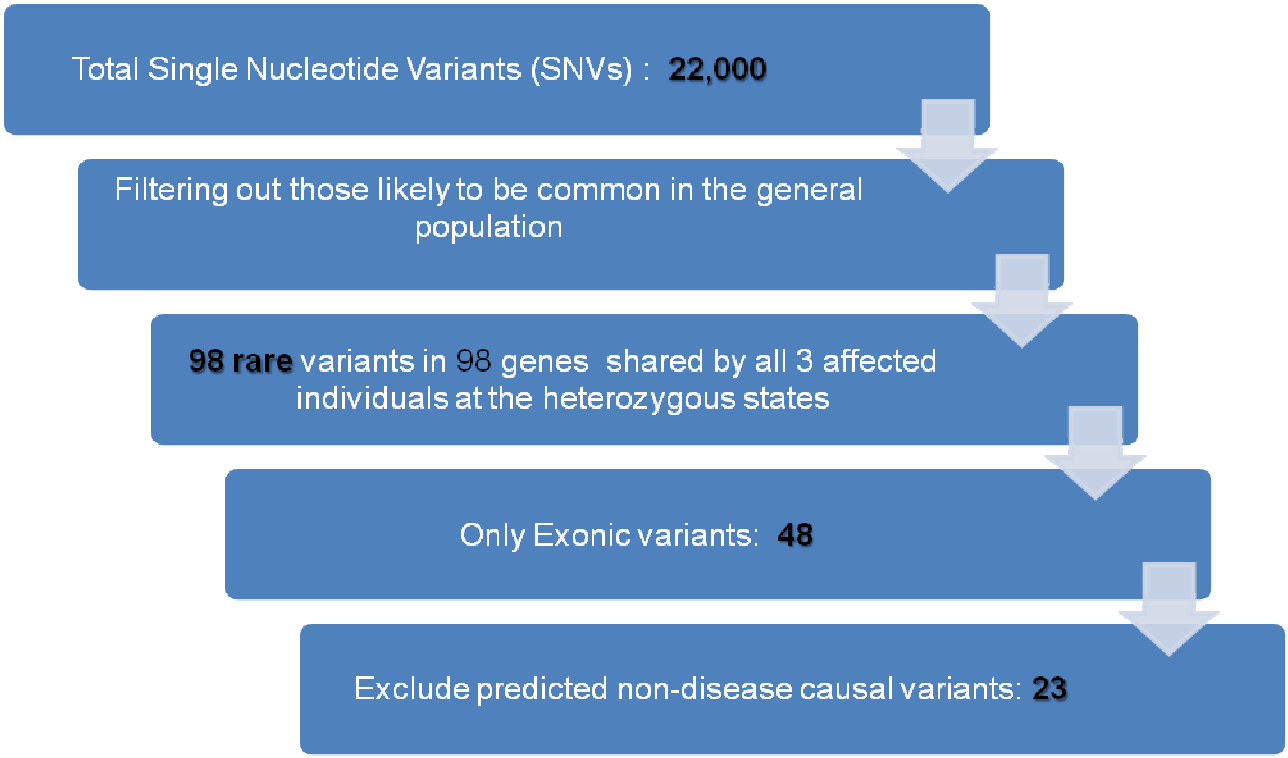

Figure 6: filtering and prioritization of the variants identified by WES, carried out in the 3 affected siblings.

Variations	Family #1
3'/5' UTR	36
Intronic	14
Splice-site	0
Exonic	44 Missense, 1 Nonsense, 3 del/ins

Table 4: heterozygous variants, shared by all 3 patients.

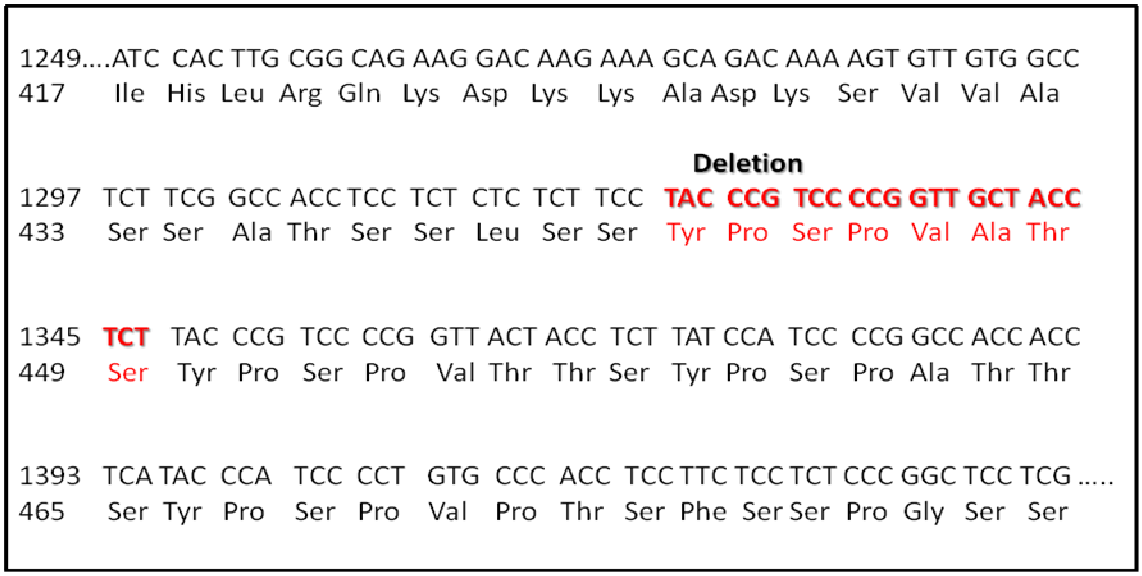

Figure 7: 24-bp deletion of 8 aminoacids detected by WES.

2.3 Discussion

Family 1

A consanguineous Turkish family was studied in order to identify novel disease candidate genes involved in IGHD through WES.

The consanguinity of the parents strongly suggested an austosomal recessive model of inheritance of the disease, caused by a mutation at the homozygous state. The homozygous variants identified in the affected member (II-2) analysed by the WES were filtered and reduced to two potential candidate genes ELN and MYH7B respectively. However these genes are not involved in any of the biological pathways related to IGHD.

Other models of inheritances, although less likely, could be taken into account. Autosomal dominant and X-linked dominant models of inheritance, might be considered due to the short height (-2.6 SDS) of the mother who was not assayed for the GH deficiency. By altering the filtering strategies based on these hypotheses, IGF2 (autosomal dominant) and ESX1 (X-linked dominant) were identified as strong candidate genes with heterozygous variations.

Interestingly, these genes are involved in the pathways influencing fetal growth. Further confirmation of the variants by sanger sequencing in the other family members as well as in other patients has to be performed.

Family 2

An Italian family was studied in order to identify novel disease candidate genes involved in IGHD through WES and was initially considered an autosomal dominant model of inheritance.

The 3 affected siblings resulted negative for the presence of mutation in the GH1 and GHRHR genes. Recently we knew that there is a fourth sister without GH deficiency and we are waiting for the results of GH response to pharmacological stimulation in the parents.

The current strongest candidate gene from the literature is EGR1, a transcription factors involved in the development of the anterior pituitary gland.

The deletion was validated in the 3 probands by Sanger sequencing and we identified that the they inherited deletion from mother even if the father presents a lower SDS value.

Moreover, the deletion was found in 1 IGHD patient but not in a group of 404 normal stature individuals. To better examine the role of EGR1 in the pathology, we sequenced the 2 exons of the gene in 50 IGHD patients, previously analyzed by gel electrophoresis and resulted negative for the presence of the deletion. We didn't detect any pathological variants. We are planning to expand the number of controls and patients.

However in this family we can't exclude the possibility of a digenic inheritance: one inherited from the mother (EGR1) and the other from the father, which is not yet identified.

3. REFERENCES

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SEMINARS

- 7 December 2012: “The role of the Nfr2 transcription factor in aging and stem cell differentiation “. Prof. Dirk Bohmann
- 29 January 2013: “ HELPs: biomimetic polypeptides for biomedical applications”. Dr. Antonella Bandiera
- 15 February 2013: “Mechanisms of chronic inflammation and immunoregulation in infections and tumors”. Prof. Vincenzo Barnaba
- 14 March 2013: “ Long non-coding RNAs and atypical protein-coding genes as new players in the regulation of neuronal functions”. Dr. Silvia Zucchelli
- “Molecular interactions of histone modification and de novo DNA methylation”
- 15 April 2013: “Molecular interactions of histone modification and de novo DNA methylation”. Dr. Kyung-Min Noh, PhD
- 17 April 2013: “mRNA & Genome Editing”. Dr. Angelo Lombardo
- 22 April 2013: “Glioblastoma stem cell biology and its implications in cancer therapy”. Dr. Giuliana Pelicci
- 3 May 2013: “Carbapenemases: a last frontier for beta-lactam antibiotics?” Prof. Giuseppe Cornaglia.
- 6 May 2013: “Autophagy and human disease; model Cystic Fibrosis”. Prof. Luigi Maiuri
- 7 May 2013: “Experimental and Clinical Applications of polychromatic cytometry and multi-purpose”. Prof. Andrea Cossarizza.
- 31 May 2013: “Cancer in the post-transplant: emerging issues and new opportunities to care”. Prof. Emanuela Vaccher
- 6 June 2013: ”Skin cancer in vivo models, what they have and can tell us”. Dr. Girish Patel
- 13 June 2013: “Cytotoxic potential of plasmacytoid dendritic cells in autoimmune diseases - Prof. Silvano SOZZANI

Congresses/courses/workshops

- Novara, November 2012/April 2013 Scientific English course, Level B1, ABESCHOOL
- Pavia 5-7 June 2013, SIBBM 2013:" Revisiting the Central Dogma: Emerging New Concepts in Replication, Transcription, and Translation".
- Sorrento, 21-24 November 2012 XV CONGRESSO NAZIONALE SIGU (SOCIETÀ' ITALIANA DI GENETICA UMANA)
  Post congress course:" NEXT GENERATION SEQUENCING: ISTRUZIONI PER L' USO"
- Novara, March 21-22 2013 "Auto antibodies workshop "
- Novara, 12 September, 2013: Illumina Seminar "Using New Technologies to Study the Genetics of Disease"
- Rome, 25-27 September 2013 XVI CONGRESSO NAZIONALE SIGU (SOCIETÀ' ITALIANA DI GENETICA UMANA)

MASTER CLASSES

- 27 November - 5 December 2012: "DROSOPHILA AS A MODEL SYSTEM FOR BIOMEDICAL RESEARCH", Prof. Dirk Bohmann
- June 2013: "Single cell and single particle analysis for biomedical, diagnostics and medical discovery". Prof. Steve Graves
- September 2013: "Genetics & Molecular Medicine". Prof. Steve Ellis

ABSTRACTS:

1) “IDENTIFICATION OF NOVEL EXON SPLICE ENHANCERS (ESEs) IN THE GROWTH HORMONE (GH) GENE MUTATED IN ISOLATED GH DEFICIENCY (IGHD) PATIENTS”

D. Babu, I. Fusco., S. Mellone, M. Godi, A. Petri, F. Prodam, S.Bellone, P. Momigliano-Richiardi, G. Bona and M. Giordano**.** Department of Health Sciences, University of Eastern Piedmont, Novara, Italy. **Annual meeting of American Society for Human Genetics, November 6-8, 2012.**

2) IDENTIFICATION OF NOVEL EXON SPLICE ENHANCER AND PROMOTER VARIATIONS IN THE GROWTH HORMONE GENE CONTRIBUTING TO SPORADIC ISOLATED GH DEFICIENCY. D. Babu, I. Fusco., S. Mellone, M. Godi, A. Petri, F. Prodam, S.Bellone, P. Momigliano-Richiardi, G. Bona and M. Giordano**.** Department of Health Sciences, University of Eastern Piedmont, Novara, Italy. **Annual Meeting, Società Italiana di Genetica Umana, November 21-24, 2012. Pubblication of the Article is in progress**

3) SEARCH FOR NOVEL GENES INVOLVED IN ISOLATED GROWTH HORMONE DEFICIENCY (IGHD) THROUGH WHOLE-EXOME SEQUENCING IN A CONSANGUINEOUS TURKISH FAMILY. Annalisa Vetro[1], Simona Mellone[2], Ileana Fusco[2], Barbara Fre[2], Deepak Babu[2], Gianni Bona[2], Orsetta Zuffardi[1], Mara Giordano[2]. 1-Dipartimento di Medicina Molecolare, Università degli Studi di Pavia. 2-Laboratorio di Genetica Umana, Dipartimento di Scienze Mediche, Università del Piemonte Orientale, Novara. **Annual Meeting, Società Italiana di Genetica Umana, September 25-28, 2013**

4) INCIDENCE OF *SHOX1* MUTATIONS IN PATIENTS WITH SHORT STATURE AND VARIABLE GROWTH RETARDATION RATE. Ileana Fusco[1], Simona Mellone[1], Deepak Babu[1], Ranjith Muniswamy[1], Flavia Prodam[2], Simonetta Bellone[2], Antonella Petri[2], Gianni Bona[2] and Mara Giordano[1].1- Laboratorio di Genetica Umana, Dipartimento di Scienze Mediche, Università del Piemonte Orientale, Novara 2-Università del Piemonte Orientale, Dipartimento di Scienze Mediche, Unità di Pediatria, Novara **Annual Meeting, Società Italiana di Genetica Umana, September 25-28, 2013.**

SUBMITTED ARTICLES:

IDENTIFICATION OF NOVEL EXON SPLICE ENHANCER AND PROMOTER VARIATIONS IN THE GROWTH HORMONE GENE CONTRIBUTING TO SPORADIC ISOLATED GH DEFICIENCY. D. Babu, I. Fusco., S. Mellone, M. Godi, A. Petri, F. Prodam, S.Bellone, P. Momigliano-Richiardi, G. Bona and M. Giordano. Submitted to Journal of Clinical Endocrinology & Metabolism

MUTATION SCREENING AND COPY NUMBER ANALYSIS OF HMGA2 GENE IN A COHORT OF ITALIAN ISS PATIENTS. I.Fusco , S. Mellone , B. Fre , D.Babu , R. Muniswamy, F. Prodam, S. Bellone, G.Bona and M. Giordano . Submitted to Hormone Reaseach