

Associazione Italiana Sindrome di Shwachman-Diamond (AISS)
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Shwachman-Diamond Syndrome Italian Research Grant

Maximum Amount euro 10.000,00

Firm Deadline for Receipt of Applications: 31 December 2016

Eligibility: Persons applying for these grants if not in a faculty position need to provide a declaration by a supervisor with a position in the department (not a training position) and with authority to hold an independent research grant.

Terms of Support: Support may be provided for one (1) year in an amount not to exceed E 10,000. Indirect costs are permitted and are not to exceed 10% of the total costs.

The AISS will provide preference to those applications in which funds are used for supplies, equipment, technicians and other expenses and not for support of the salary of the PI or co-PIs.

Review: All applications will be reviewed by the AISS Scientific Committee (AISS-SC) or its designees.

Application: The application contains two sections.

Section 1, forms attached. The applicant and co-applicants must also include a current curriculum vitae. Section 2: Research Plan, divided as indicated below. Parts A through D should not exceed 6 pages, using a font no smaller than 10 point.

- Part A. Specific aims
- Part B. Significance and background
- Part C. Preliminary studies
- Part D. Experimental design and methods
- Part E. References (not included in the 6 page limit)
- Part F. Relevance of the research to Shwachman-Diamond Syndrome
- Part G. For junior faculty separate letter from supervisor or department head confirming commitment to project, and to provision of space and facilities
- Part K. If human subjects and animals are involved, a statement by the PI or supervisor overseeing human or animal studies is compulsory. If considered as necessary by the AISS-SC, more information about ethical committee study approval may be asked.

Submission by email to the AISS: aiss@shwachman.it

1. Title of Proposal:

Deletions of the long arm of chromosome 20 in bone marrow encompassing the *EIF6* gene in Shwachman-Diamond Syndrome (SDS): benign prognosis mechanisms evaluated by cytogenetic, aCGH and expression arrays approaches

2. Applicant Information:

Name: Roberto VALLI

Title and Degree(s):

Researcher in Medical Genetics, University of Insubria. Biological sciences degree. Ph.D in Congenital and Acquired Degenerative Diseases

Work Address:

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3. Applicant Curriculum Vitae: beginning on the next page, with 2 page limit. This will form application pages 3 and 4.

Curriculum Vitae

- Born in Varese (Italy) on February 8th, 1973
- In 2001 he graduates in Biological Sciences with a score of 110/110 summa cum laude dissenting an experimental thesis work titled "Cytogenetic characterization of Chromosome 10 breakpoint's in the t(10;21)(q23.1;q11.2) translocation associated with a patient affected by a rare form of congenital heart disease"
- In December 2001 he starts the PhD course in "Congenital and Acquired Degenerative Diseases" of the University of Insubria, working in the Medical Genetic lab of the Clinical and Experimental Biomedical Sciences Department in Varese.
- Since 2002 is member of the Italian's Human Genetics Society (S.I.G.U.)
- During the PhD studies, he works for a period in the Biology and Genetics labs of the D.A.P.E.G. Department of the University of Bari (Italy)
- In 2005 he doctorates dissenting the experimental works titled "Molecular and Citogenetical Characterization of familiar t(12;15) translocation".
- In November 2005 he starts the Post-doc on the "Mechanism of origin and molecular definition of structural chromosomal anomalies" working in the Medical Genetic lab of the Clinical and Experimental Biomedical Sciences Department in Varese.
- In December 2005 he achieved the qualification to practise the Biologist profession, passing the related Italian examination.
- Since 2001 he carries out integrative didactics, by substituting the official professors in the courses of "Genetics and Biology" and "Medical Genetics" for the degree courses of the Faculty of Medicines and Surgery of the University of Insubria.
- In October 2008 he becomes a researcher in Medical Genetics for the Medical and Surgery Faculty of the University of Insubria and works in the Medical Genetic lab of the Clinical and Experimental Biomedical Sciences Department in Varese.
- Since 2009 he is teacher in general biology or medical genetics in 6 degree courses of the Insubria's School of Medicine (formerly Faculty of Medicine and Surgery)
- Since 2014 is member of the European Cytogenetic Association

Research Interests

The principal research lines in field of Medical Genetics were and still are:

- Studies on karyotype/phenotype correlation in constitutional and acquired chromosome anomalies through the use of cytogenetic, molecular cytogenetics, array comparative genomic hybridization (aCGH) and molecular genetics tools.
- Studies on the chromosome variability in leukaemia and, in particular, in myelodysplasia and myeloproliferative disorders at the diagnosis, during the course of disease, and after bone marrow transplantation.
- Studies on families with Mendelian diseases associated to myeloproliferative disorders
- Studied on Shwachman-Diamond Syndrome, and other inherited bone marrow failure syndromes predisposing to myelodysplastic syndromes and myeloproliferative disorders.

- Studies on chromosome anomalies with gene effects leading to peripheral cytopenias (uni-, bi-, and tri- linear) and/or bone marrow aplasia/hypoplasia.
- Studies on the sensitivity of the array comparative genomic hybridization (aCGH) and its implications in acquired chromosome imbalances.
- Study of the correlation between different batch of HeLa cells and transcriptional effects by the use of aCGH, real-time PCR and whole transcriptome arrays.

All research works is carried out by means of conventional and molecular cytogenetic methods, and of molecular techniques, besides basic cell biology methods, as different cell culture techniques: the most relevant and informative methods used in relation to projects goals include Fluorescent In Situ Hybridization (FISH) and Multipainting, and by microarray based comparative genomic hybridization (aCGH) or whole transcriptome microarrays; Quantitative real.time PCR for expression studies.

Teaching experiences

The teaching experiences (since 2009) includes:

- Course of General Biology and Medical Genetics in the Course for degree in Cardio-circulatory Perfusion, at the University of Insubria
- Course of General Biology and Medical Genetics in the Course for degree in Dental Hygiene, at the University of Insubria
- Course of General Biology in the Course for degree in Laboratory techniques, at the University of Insubria
- Course of General Biology in the Course for degree in Radiotherapy and Radiological Techniques, at the University of Insubria
- Course of Medical Genetics in the Course for degree in Obstetrician, at the University of Insubria
- Course of Medical Genetics in the Course for degree in Professional Education, at the University of Insubria
- Specific lessons of the course of General Biology and Medical Genetics in the Course for degree in Medicine and Surgery, at the University of Insubria.

4. Applicant's Commitment as Investigator of the Project:

I agree as the applicant to accept responsibility for the scientific management of this project as outlined in this application. I further agree to submit a report at the end of the granting period.

5. Applicant's Affirmation:

I certify that the investigations involving human subjects to be carried out in the application will have approval of the applicant's Institutional Ethical Committee

Approvals from the Institutional Ethical Committee must be included with the application.

6. Research Results:

Results of research may be made available to the public through appropriate scientific channels.

All publications will bear the statement:

"THIS WORK WAS SUPPORTED BY A GRANT FROM ASSOCIAZIONE ITALIANA SINDROME DI SHWACHMAN (AISS)"



Signature of Applicant

Varese, 20/12/2016

7. Applicant's Institution Certification and Commitment:

I certify that the statements herein and the Applicant's Affirmation are true, complete and accurate to the best of my knowledge and I agree to accept responsibility for the fiscal management of this project as outlined in this application. I further agree to commit this institution to comply with the Associazione Italiana Sindrome di Shwachman-Diamond (AISS) terms and conditions if a grant is awarded as a result of this application.

Name of Institution Official: Anna Maria Grandi, professor

Title: Director of the Department of Clinical and Experimental Medicine

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Varese, 20/12/2016

DIPARTIMENTO DI MEDICINA CLINICA E SPERIMENTALE
IL DIRETTORE
(Prof. Anna Maria Grandi)

Anna Maria Grandi
Director of Department of Clinical and Experimental Medicine
(signature)



ABSTRACT OF RESEARCH PLAN

Within the space provided, summarize the long-term objectives, scientific aims and methodology of the proposal.

TITLE: Deletions of the long arm of chromosome 20 in bone marrow encompassing the *EIF6* gene in Shwachman-Diamond Syndrome (SDS): benign prognosis mechanisms evaluated by cytogenetic, aCGH and expression arrays approaches

Scientific aims

The present project will focus on the interstitial deletions of the long arm of the chromosome 20, del(20)(q), which are found in the bone marrow (BM) of many patients by long-lasting cytogenetic monitoring and were shown to imply a benign prognostic role as to the development of myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML). We previously reported that all the patients carrying the del(20)(q) always lose the *EIF6* gene, located at 20q11.22. The *EIF6* gene is involved in the same pathway of *SBDS* gene and we postulated that its haploinsufficiency, due to the del(20)(q) deletion in SDS patients, could be related to a better "fitness" of the BM clone carrying the anomaly through a dosage mechanism. Our goal is to better investigate this mechanism of protection from MDS/AML by the use of routine cytogenetics, array-based Comparative Genomic Hybridization (aCGH) and whole genome expression analysis studied by array methods.

Methodology

We perform cytogenetic investigations since 1999 in a cohort of 91 Italian patients with SDS. Up to now, 18 patients have acquired a deletion of the long arm of chromosome 20, del(20)(q) in the BM. In 10 cases, it was possible to apply aCGH analysis and *EIF6* gene was lost in all patients.

The material of the present project includes bone marrow specimens for routine and molecular cytogenetic analysis, and DNA and RNA from BM. The aCGH system which will be used is the 244K genome-wide system (Agilent Technologies Inc., Santa Clara, CA, USA) applied and analysed according to manufacturer's instruction and software. Results will be validated by FISH with the probes indicated as informative by aCGH.

Whole genome expression analysis by arrays will be performed with the RNA from BM of patients with the 8x60K human whole transcriptome array (Agilent Technologies Inc., Santa Clara, CA, USA) according to manufacturer's instructions and data will be analysed by the use of R-software with suitable packages. A pool of genes which result to be up/down regulated will be validated by the use of standard real time PCR thermocycler ABI 7000 (Applied Biosystems, Foster City, CA, USA) with suitable primers sets and SYBR green master-mix (Bio-Rad, Hercules, CA, USA). RNA from BM of normal donors will be used as normal control both in transcriptome arrays as in real-time PCR experiments.

Long-term objectives

We have already reported 6 patients with SDS in whom the del(20)(q) always encompasses the *EIF6* gene, at variance with the del(20)(q) in patients with MDS/AML in whom the deletion appears to be different from case to case and the loss of *EIF6* not always present. We have now found 4 new patients in which aCGH demonstrated the deletion of *EIF6*.

The present project will focus on the following long-term objectives:

1 - We plan to follow-up the patients that carry the del(20)(q) clone and to find out new cases to be evaluated, by routine cytogenetic monitoring.

2 – In the patients with a BM clone with the del(20)(q) above the detection limits of a-CGH (8-10%), this analysis will be performed in order to characterize precisely the deletion and demonstrate the peculiar *EIF6* loss already found in all other cases.

3 - RNA from BM of patients with *EIF6* loss (demonstrated by aCGH and FISH) will be collected and whole transcriptome analysis by arrays will be performed. We expect to validate our preliminary transcriptome array data in three patients with the del(20)(q) at various clonal percentage. In these three patients, the dendrogram analysis of whole transcriptome and hematopoietic genes pathways showed that at higher percentage of del(20)(q) the overall expression pattern is more similar to normal donor subjects than in patients with low percentage of del(20)(q).

BUDGET

List below a budget by categories for the support. The review committee will carefully consider the appropriateness of your budget. It must be well defined, justified, and realistic to complete the work proposed. The first column defines the total expenses that are expected to be necessary to realistically complete the project. The second column indicates the expenses requested from the AISS. Applicants will not be penalized in funding considerations for requiring additional funds beyond what is requested from the Foundation(AISS); however, the true costs of the project must be acknowledged. [This and the section on page 1 re: Other Funding need to be consistent]

EURO Amount Requested for:

	TOTAL COSTS REQUIRED TO COMPLETE PROJECT:	COSTS REQUESTED FROM AISS:(not to exceed E 10,000)
Personnel (including fringe benefits): PI: Name: Co-I Name: Additional personnel (identify role): Name:	-	-
Equipment:	-	-
Supplies:	€ 27,530	€ 10,000
Other Expenses:	-	-
Indirect Costs (not to exceed 10% of total)	-	-
TOTAL COSTS:	€ 27,530	€ 10,000

Justification: Define and justify expenses in each category. Explain the role of each of the individuals named in the Personnel section. The justification must include an explanation of what each category contributes to the project. Also explain any marked differences between the first- and second-year expenses in a particular category. The AISS will provide preference to those applications in which funds are used for supplies, equipment, technicians and other expenses and not for support of the salary of the PI or co-PIs. The AISS-SC may ask for further expense details.

Personnel and equipment for this research project are already available.

The entire group of researchers is involved in all parts of the research work.

The costs concern only supplies, and are in part covered by other grants already available.

- Each a-CGH test costs approximately € 670, including
 - o Array platform
 - o Labeling Kit
 - o 244K aCGH array slides and Gasket slides
 - o Washing solutions
 - o Software

No. aCGH test planned: 25 = 16,750

- Costs for cell cultures, conventional cytogenetics and FISH (when needed): approximately € 300 per case

Total foreseeable costs: 4,000

- Costs for real-time-PCR for the informative genes: approximately € 100 per 96 well plate of real time

Total foreseeable costs: € 1,500

- Whole transcriptome arrays: the format for this test is a multiple arrays. (8x60K) in which there are 8 cases for each array. The cost for each case/patient is approximately € 220, including:
 - o Array platform
 - o Labeling Kit
 - o 8x60K whole transcriptome arrays and gasket slides
 - o Washing solution
 - o Software

No. of Whole transcriptome arrays planned: 24 = 5,280

- Total supplies costs: € 27,530

Other Support for this Project:

Applicants are allowed to receive funding from other sources for parts of the project not funded by the AISS. Please, list all other funding sources.

- Università dell'Insubria FAR 2015 e 2016

Research Plan

Part A – Specific aims

The present project will focus on the deletion of the long arm of the chromosome 20, del(20)(q), a change frequently found in the bone marrow (BM) of patients with Shwachman-Diamond syndrome (SDS), and on the relationship of the concomitant loss of *EIF6* gene (located in the deleted region 20q11.22) with the relatively benign prognosis of patients with this anomaly. As the *EIF6* gene is involved in the pathway of *SBDS* gene, we postulated that its haploinsufficiency (due to the del(20)(q) deletion) could be related to a better “fitness” of the BM clone carrying the anomaly through a dosage mechanism. The results may be relevant for the study of pathogenic mechanisms leading to (or protecting from) Myelodysplastic syndrome (MDS) and Acute Myeloid Leukaemia (AML), with obvious practical implications for the best clinical management of the patients.

Part B – Significance and background

The proportion of patients with SDS who develop MDS and/or AML is not far from 30% (Dror, 2005), and evaluations which take to account the age of the patients estimate a risk of 19% at 20 years and of 36% at 30 years (Shimamura, 2006). Clonal chromosome changes, mainly involving the chromosomes 7 and 20, are often found in BM of SDS patients. The most frequent are: an isochromosome of the long arm of chromosome 7, i(7)(q10), and the deletion of the long arm of chromosome 20, del(20)(q) (Maserati et al, 2006). The relationship between these and other chromosome changes in the BM and the risk of MDS/AML is object of discussion (Dror et al, 2002; Dror, 2005). Since 1999 we collected a cohort of 91 Italian SDS patients that we routinely monitor by cytogenetics in order to early discover any cytogenetic acquired anomaly that could be related to a progression to MDS/AML. During these years, we showed that the acquisition of BM clonal chromosome anomalies is age-related, in parallel with the risk of MDS/AML (Maserati et al, 2009). We suggested a mutator effect of *SBDS* mutations leading to a specific type of karyotype instability which leads, in turn, to the clonal anomalies in the BM (Maserati et al, 2006). This view was supported by the evidence that *SBDS* protein promotes spindle stability and normal chromosome segregation (Austin et al, 2008): the defect in SDS cells may so explain the karyotype instability, possible through cytokinesis failure and tetraploidy, with subsequent chromosome changes. Comparative genomic hybridization on microarray (aCGH) provides a powerful tool to investigate unbalanced chromosome anomalies, and some results in SDS have been already reported (Maserati et al, 2009). The definition of aCGH is much higher than conventional cytogenetics and can detect cryptic or very small chromosome imbalances. In particular an exhaustive analysis by aCGH was performed on the BM of patients carrying the del(20)(q). In a recent work (Valli et al, 2013), we showed that 6/6 patients with the del(20)(q), always lost the *EIF6* gene, located at 20q11.22, although the deletion could be very different from patient to patient (4,14 Mb the smallest, 26,86 Mb the largest). Up to now, 4/4 further new patients analysed showed the same loss of *EIF6* (unpublished data). So, the loss of *EIF6* seems to present in every SDS patients with the del(20)(q). On the contrary, the del(20)(q) imbalance that can occur in patients with myeloproliferative neoplasms, may imply or not the loss of *EIF6* (Valli et al, 2013).

It is intriguing that the *EIF6* protein itself is the target of the *SBDS*/EFL1 GTPase action in the early ribosome assembly. It is necessary to remove *EIF6* factor from the pre-60S subunit to let the formation of the 80S ribosome and to start the translation (Finch et al, 2011). SDS patients have very low levels of *SBDS* protein in the BM leading to an abnormal accumulation of the pre-60S subunit. So, SDS is considered a ribosomopathy, as, at the end, the overall translation of specific tissues (BM *in primis*) is compromised (Finch et al, 2011). It is therefore possible to hypothesize a dosage-dependant

mechanism: the haploinsufficiency of *EIF6* (due the del(20)(q) in many SDS patients) causes a lower dosage of EIF6 protein which, together with SBDS low levels due to the *SBDS* mutations, lead to a better ribosome assembly in the clone carrying the anomaly and, in fact, to a better "fitness" of the clone itself and to a lower risk to develop MDS/AML. (Pressato et al, 2012. Valli et al, 2013).

The aim of this research project, in parallel with the standard long lasting cytogenetic analysis, is to perform aCGH analysis on all available patients with or without the del(20)(q) in order to verify the loss of *EIF6* gene. Little is known about the expression patterns of BM in SDS patients. Only Rujkijyanont et colleagues (Rujkijyanont et al, 2007 and 2009) performed whole transcriptome analysis of RNA from BM of SDS patients and normal donors putting in evidence the activation of leukaemogenesis pathways in SDS patients and disruption of ribosome biogenesis and RNA processing. More specifically, no data of whole transcriptome analysis of patients with the del(20)(q) is available. Recently we performed whole transcriptome array analysis on three patients carrying the del(20)(q) (Valli et al, 2016), and we found a clear direct correlation between the amount of cells with del(20)(q), in term of clonal percentage in BM, and the evidence of "restoring" a normal expression pattern. As available data are based only on three patients with the del(20)(q), the second point of the aims of the present project is to expand the whole transcriptome analysis to all the patients carrying the del(20)(q).

Part C – Preliminary studies

- 1) Up to now, by long lasting cytogenetic monitoring of all the patients of our cohort, we have found 18/91 SDS patients carrying del(20)(q). In particular:
 - 8 of them have been characterised by aCGH as the clonal amount of abnormal cells was sufficient (5 already reported in Valli et al, 2013)
 - 2 of them had a very small cryptic del(20)(q) only detectable by aCGH (one already reported in Valli et al, 2013)
- 2) We collected the RNA from BM of three patients with del(20)(q) with different clonal percentage of abnormal cells and two patients without the del(20)(q). We performed whole transcriptome array analysis comparing these subjects with RNA from BM of 5 normal control donors. These preliminary results were presented at the last International Shwachman-Diamond Conference in Verona (Valli et al, 2016). Our results are of interest: in these three patients the dendrogram analysis of whole transcriptome and haematopoietic genes shows that at higher percentage of del(20)(q), the overall expression pattern is more similar to the normal donors subjects than in patients with low percentage of del(20)(q), prompting to a sort of "rescue" of the ribosome assembly in the clones carrying the anomaly that, eventually, restore the expression patterns.

Part D – Experimental design and methods

The patients object of the study will be the ones of our cohort already under study, and all the SDS patients who will be detected during the project, with or without the del(20)(q). Control subjects will be selected among healthy BM donors for transplantation. An informed consent will be obtained by the patients or the patients' parents, as well as well by the control subjects.

The material for the project includes BM and peripheral blood in heparin or EDTA, for cytogenetic or molecular analysis respectively. BM samples will be collected also by the use of PAX gene tubes (Qiagen, Hilden, Germany) in order to stabilise RNA. DNA and RNA will be extracted by Qiagen columns kits. All informative techniques of cytogenetics and of molecular cytogenetics will be used, in particular Fluorescent In Situ Hybridization (FISH) with informative libraries and probes, with the same methods

and probes already described in previous publications (Maserati et al, 2006; Maserati et al, 2009; Pressato et al, 2012; Valli et al, 2013). The aCGH system used will be the 244K genome-wide system (Agilent Technologies Inc., Santa Clara, CA, USA), applied and analysed according to the manufacturer's instruction and software. All aCGH results will be confirmed, when necessary, by FISH with the probes indicated as informative by aCGH. Possible unknown Copy Number Variations will be confirmed on the peripheral blood of the patients and of their parents.

Whole transcriptome analysis will be performed on the RNA from BM of patients with the 8x60K human whole transcriptome array (Agilent Technologies Inc., Santa Clara, CA, USA) according to manufacturer's instructions. Data will be analysed by the use of R-software with suitable packages. *EIF6* expression levels, as well as informative pools of up/down regulated genes, will be validated by the use of standard real time PCR thermocycler ABI 7000 (Applied Biosystems, Foster City, CA, USA) with suitable primers sets and SYBR green master-mix (Bio-Rad, Hercules, CA, USA). RNA from BM of normal donors will be used as normal control both in transcriptome arrays as in real-time PCR experiments.

Part E – References

- Austin KM, Gupta ML Jr, Coats SA, Tulpule A, Mostoslavsky G, Balazs AB, Mulligan RC, Daley G, Pellman D, Shimamura A. Mitotic spindle destabilization and genomic instability in Shwachman-Diamond syndrome. *J Clin Invest.* 2008 Apr;118(4):1511-8
- Dror Y. Shwachman-Diamond syndrome. *Pediatr Blood Cancer.* 2005 Dec;45(7):892-901
- Dror Y, Durie P, Ginzberg H, Herman R, Banerjee A, Champagne M, Shannon K, Malkin D, Freedman MH. Clonal evolution in marrows of patients with Shwachman-Diamond syndrome: a prospective 5-year follow-up study. *Exp Hematol.* 2002 Jul;30(7):659-69
- Finch AJ, Hilcenko C, Basse N, Drynan LF, Goyenechea B, Menne TF, González Fernández A, Simpson P, D'Santos CS, Arends MJ, Donadieu J, Bellanné-Chantelot C, Costanzo M, Boone C, McKenzie AN, Freund SM, Warren AJ. Uncoupling of GTP hydrolysis from eIF6 release on the ribosome causes Shwachman-Diamond syndrome. *Genes Dev.* 2011 May 1;25(9):917-29
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- Pressato B, Valli R, Marletta C, Mare L, Montalbano G, Lo Curto F, Pasquali F, Maserati E. Deletion of chromosome 20 in bone marrow of patients with Shwachman-Diamond syndrome, loss of the EIF6 gene and benign prognosis. *Br J Haematol.* 2012 May;157(4):503-5
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- Shimamura A. Shwachman-Diamond syndrome. *Semin Hematol.* 2006 Jul;43(3):178-88.

Valli R, Pressato B, Marletta C, Mare L, Montalbano G, Curto FL, Pasquali F, Maserati E. Different loss of material in recurrent chromosome 20 interstitial deletions in Shwachman-Diamond syndrome and in myeloid neoplasms. *Mol Cytogenet.* 2013 Dec 12;6(1)

Valli R, Fabbri M, De Paoli E, Montalbano G, Minelli A, Nacci L, Frattini A, Pasquali F, Maserati E - Expression Studies in Bone Marrow of patients with Shwachman-Diamond Syndrome Carrying a Chromosome 20 long arms deletion, 8th international Congress on Swachman-Diamond Syndrome, Verona, April 17-20, 2016, abstracts, p. 33

Part F. Relevance of the research to Shwachman_Diamond Syndrome

The results of the research will be both of theoretical and practical relevance. In particular:

1. The results of aCGH will have a pivotal role in the precise definition of acquired unbalanced chromosome anomalies detected by chromosome analysis and FISH, especially for the cases that carry the del(20)(q) that will be further analysed by the use of whole transcriptome arrays and real-time PCR.
2. Cryptic unbalanced chromosome anomalies, undetected by conventional cytogenetics, may be found by aCGH (as was in two patients of our cohort already reported in the literature and in a new case mentioned above) and should be discussed especially in relationship with the possible cryptic deletion of the chromosome 20 involving the loss of the *EIF6* gene
3. aCGH tool, will have significance relevance (integrated with routine cytogenetics) in the precise detection of clonal anomalies (del(20)(q) *in primis*) and possible pathogenetic significance will be analysed.
4. Whole transcriptome analysis and the expression studies in SDS patients with and without the del(20)(q) and loss of EIF6, will shed light on pathogenetic mechanisms in SDS related to benign prognosis as to progression towards MDS/AML.

Parts G, K

Not applicable